Program Announcement (PA)

2020 MSTS Mentored Research and Scholar Development Program Award

Release Date:	August 14, 2020
Letter of Interest/Inquiry:	October 15, 2020 (Not Mandatory)
Grant Receipt Date:	January 15, 2021
Peer Review Date:	January-February, 2021
Executive Committee Review:	March/April, 2021
Earliest Award Start Date:	May 1, 2021
Award Amount:	up to $25,000.00
Award Number:	MSTS-MRSDPA2
Award Duration:	One year (May 2021-Apr, 2022) No-Cost-Extension to Second Year is allowed.
Submission of Applications:	Applications in a PDF format; info@msts.org;
Inquiries:	Francis Y. Lee, M.D., Research Committee Chair, francis.lee@yale.edu

I. Program Description

MSTS is enthusiastic about nurturing a culture of discovery and fostering mentorship for candidate, associate, and young members of our society. To this end, MSTS Executive Committee and Presidential Line supported establishment of a new MSTS Mentored Research and Scholarly Development Program Award. This award is a combined mentoring and research scholarly career development program rather than funding a single research project. The research fund was raised by Sarcoma Strong Foundation (President: Matthew DiCaprio, M.D.) The purpose of the new program award is to foster mentorship, to enhance collaborative research, and to facilitate career advancement for young MSTS Candidate/Associate/Full Members.

1. Eligibility
- Candidate Members, Associate Members, and Members of the MSTS within 6 Years of first faculty appointment
- Orthopaedic Surgeons with successful completion of Orthopaedic Oncology Fellowship Training
- Those who have specific plans to submit external research grants within 2 years of the initial award date

2. Ineligibility
- More than 6 years of Faculty Appointment
- Those who received OREF Grants larger than $50,000, Institutional K12, or NIH R03/R21/K08/R01 Grants
- Those who do not have specific plans to submit Foundation, NIH, or DOD Grants within 2 years of the initial award date
- Those who do not have mentors or mentored research plans

3. Program Contents
The Award has 3 mandatory components.
i) Mentorship (Mentorship by Established Mentors at the home and outside institutions): The goal is to learn about impactful mechanistic research or meaningful clinical trials through collaboration with highly effective mentors. It is encouraged to have a mentoring committee consisting of Principal Mentor and Co-Mentors who are established scholars with track records in receiving major federal grants (NIH, NCI, DOD, VA Grants), private foundations (American Cancer Society), and Clinical Trial Leaders of Cancer Networks (SWOG, COG). Principal Mentor and Co-Mentors do not have to be orthopaedic surgeons.
Note) Please see Appendix for Preliminary Mentor List.

ii) Mandatory Attendance at Grant Writing Workshops and Hard-Core Research Training Workshops: The goal is to learn about building sustainable research programs. Continued research funding is critical for scholarly advancement and impactful research. It will be important to secure a few days to attend regional and national grant writing workshops sponsored by American Bone and Joint Decade, AAOS/ORS/OREF, AAOS

ICL Courses, ASBMR Grant Writing Workshop, and others. It is also recommended to attend Research Training Workshops sponsored by American Association for Cancer Research (AACR), NCI, Cold Spring Harbor Laboratories, Gordon Research Conference, and others.

American Association of Cancer Research Educational Courses and Workshops: http://www.aacr.org/MEETINGS/PAGES/EVENTLISTING.ASPX#.Wq7Ee2aZO8U

Cold Spring Harbor Educational Courses: https://meetings.cshl.edu

Gordon Research Conference: https://www.grc.org

NCI Cancer Symposiums

iii) Guided Mentored Research/Research Training Activities:

a. Objective: The goal is to generate thoughtful Preliminary Data for future grant applications for more impactful research under the guidance of established mentors. Without Preliminary Data, it is almost impossible to obtain large-amount grants. Through this mentored research program, a candidate should be able to acquire rigorous scholarly skills for career advancement and may generate incisive preliminary data for future grant applications. This means that the application will be part of a focused research program that candidates will develop in the next 3-5 years. It is strongly discouraged to conduct several unrelated projects that may dilute research impact during the Award Period.

b. Research Areas for a 2020 Program: Research may be related to basis science research, translational research, clinical outcome research, clinical cohort study, therapeutic trials, comparative research, or health care delivery science with **primary research emphasis on sarcoma** (cancer in the connective tissues). It will be critical to consider universal grant review criteria such as Significance, Innovation, Investigators, Approach, Environment, and Overall Impact. Research topics will be within the mission of MSTS and SarcomaStrong organization led by Dr. Matthew DiCaprio. These topics on oncogenesis, invasion, cancer biology, surgical treatments, surgical reconstructions, and clinical outcome may include cancer biology, bone biology, molecular biology, biomechanics, imaging, novel clinical interventions, tissue regeneration of massive skeletal and joint defects, infection, clinical studies, reconstructive techniques, tissue engineering for sarcoma surgeries, targeted therapy, gene sequencing, and others. In general, mechanism-based research is highly regarded while descriptive research is considered less impactful in basic or translational research. Impactful clinical research usually means sustained influence on the research field or introduction of new conceptual or technical innovations that may change our current clinical practice. Applicants are encouraged to spend enough time to come up with a highly impactful research theme in consultation with mentors.

II. Award Description

1. Candidate
At the time of award, the candidate must have a “full-time” appointment at the academic institution. The award does not provide salary support for the PI. This award is to supplement academic time of each candidate.

2. Mentor(s)
One of the major goals of this Mentored Training Research Award is to interactions between the applicants and senior faculty in orthopaedic surgery and other disciplines. Before submitting the application, the candidate must identify a mentor who will supervise the proposed career development and research experience. The mentor should be an active investigator in the area of the proposed research and be committed both to the career development of the candidate and to the direct supervision of the candidate’s research. The mentor must document the availability of sufficient research support and facilities for high-quality research. Candidates are encouraged to identify **one mentor or more than one mentors in orthopaedic oncology or other fields**, i.e., a mentoring team (or advisory committee) **at home or outside institutions**, if this is deemed advantageous for providing expert advice in all aspects of the research career development program. In such cases, one individual must be identified as the primary mentor who will coordinate the candidate’s research.

The candidate must work with the mentor(s) in preparing the application. The mentor, or a member of the mentoring team, should have a successful track record of mentoring individuals at the candidate's career stage.

Tips for finding mentors:

There are several ways of identifying mentors who have necessary expertise and resources to guide candidates. It is advantageous to have more than one mentors with complementary expertise.

a. Established members of MSTS: Contact information may be obtained from MSTS (info@msts.org).

b. MSTS or AAOS members who have received major federal funding. These information were collected from the NIH Public Website (https://projectreporter.nih.gov/reporter.cfm) and list of grant recipients from the OREF website.

i. NCI/NIH K08 Award: Kurt Weiss, M.D.(Osteosarcoma); Nocholas Bernthal, M.D.(Osteosarcoma); Bang Hoang, M.D.(Osteosarcoma); Hue Luu, M.D.(Osteosarcoma);
ii. NCI/NIH R01 Award: Kevin Jones, M.D. (Sarcoma); Michael Yaszemski, M.D.(Spine Metastasis & Biomechanics); Regis O'Keefe, M.D.(Osteoclastogenesis); Denis Clohisy, M.D.(Cancer-Induced Pain); Francis Y. Lee, M.D.(Metastatic Cancer-Induced Bone Loss); Timothy Damron, M.D.(Radiation and Bone Biomechanics); Richard Terek, M.D.(Chondrosarcoma); Benjamin Alman, M.D.(Soft Tissue Sarcoma); Brian Snyder, M.D.(Cancer and Bone Biomechanics); Constance Chu, M.D.(Arthritis); Ted Miclau, M.D.(Fracture Healing)
iii. OREF Research Grants: Cynthia Emory, M.D.(Radiation and Soft Tissue Sarcoma)
iv. Clinical Trial Grants: Michelle Ghert, M.D.; Kurt Spindler, M.D; Jay Keener, M.D.
v. Department Chairs and MSTS Leadership
v. Professional Organizations: Ruth Jackson Society, AAOS, AOA, ABJS, ORS, and others

c. NIH RePorter Website (https://projectreporter.nih.gov/reporter_searchresults.cfm) : This website lists all investigators who are conducting or have conducted NIH-funded studies over the past 30 years.

d. Corresponding authors of published papers; Speakers of institutional, regional, national, or international workshops or meetings.

e. MSTS Mentor List: MSTS Research Committee is in the process of preparing a MSTS Mentor List that will include MSTS members who may share expertise in research, education, administration, community service, and others. This list will be provided upon submission of a Letter of Interest to apply for the award.

3. Budget Description

a) Total Award Amount: up to $25,000 for one year (No Cost Extension for another year as needed)
b) Award Components
i) **Mentorship and Collaboration**: Approximately <$2,000.00 (+ additional cost by individual travel allowance)
ii) **Research Training**: Approximately <$3,000.00 (+ additional cost by individual travel allowance). The awardee should attend Grant Writing Workshop and at least one high-quality educational courses on research methodology, precision medicine, targeted therapy, immune-oncology, and other new emerging technologies .
iii) **Mentor-Guided Research**: Direct Research Cost Only in the amount of $20,000; No-Indirect Cost; No Salary Support for the PI; A small portion of salary for Research Assistant, Technician, or Students up to $10,000 is allowed. The remaining budget should be spent for Direct Research Cost.
Note) Mentorship/Collaboration Traveling + Research Training cannot exceed $5,000.

Description	Traveling Mentorship	Research Training Workshops/Meetings	Direct Research Cost	Total Amount
	<$2,000	<$3,000	Around $20,000	Up to $25,000

4. Proposal Preparation Instruction and Form
- Use the NIH K08/23 Template and Forms
- 11 Arial Font; 0.5 inch page margin (left, right, upper and lower)
- The application package is almost identical to NIH K08 (Translational Research)/ K23 (Patient-Oriented Research)
- Instruction
https://grants.nih.gov/grants/how-to-apply-application-guide/forms-e/career-forms-e.pdf

5. Page Limits

Individual Career Development Award (K, excluding K12) Applications

Section of Application	Page Limits * (if different from FOA, FOA supersedes)
Project Summary/Abstract	**<=30 lines of text (Summary of Specific Aims Page)**
Project Narrative	**Not Required**
Introduction to Resubmission or Revision Application (when applicable)	**Not Required**
Candidate Information and Goals for Career Development and Research Strategy	**Up to 12 Pages (for both attachments combined: Career Development Plan 3-6 pages + Research Plan 6-9 pages <= 12 pages); Please Refer to Samples**
Specific Aims	**1 Page (Please refer to Samples)**
Training in the Responsible Conduct of Research	**1 (On-line or Lecture Courses at Home Institution)**
Plans and Statements of Mentor and Co-mentor(s)	**<=6 (Descriptions on guidance or support for the application by a Mentor/Mentors**
Letters of Support from Collaborators, Contributors, and Consultants	**Up to 6 Letters**
Description of Institutional Environment	**1**

Section of Application	Page Limits * (if different from FOA, FOA supersedes)
Institutional Commitment to Candidate's Research Career Development	**1 (Department Chair's Support Letter_**
Biographical Sketch (Candidate, Mentors, Collaborators)	**<=5/person for each personnel (Use NIH Biosketches Template)**

6. Progress Report

It is mandatory to submit progress reports with the following elements by December 31, 2019. If no cost extension is planned, a request for 'No Cost Extension' and Interim Progress Report should be submitted by November 30, 2019. A final report for No Cost Extension may be submitted by December 31, 2020.

- Financial Report: Expense Report
- Scientific Research Data, Description, Figures
- Training Courses or Workshops Attended
- Interactions with Internal and External Mentors
- List of grants that were applied or are in preparation
- Presentations or Publications
- Scholarly Career Advancement
- Others

III. Review Criteria

Review Committee: Review committee members have served on NIH/VA Grant Study Sections. Constructive critiques will be provided.

General Review Criteria

- Are the proposed research question, design, and methodology of significant scientific and technical merit?
- Is there a strong scientific premise for the project?
- Has the candidate presented strategies to ensure a robust and unbiased approach, as appropriate for the work proposed?
- Has the candidate presented adequate plans to address relevant biological variables, such as sex, for studies in vertebrate animals or human subjects?
- Is the research plan relevant to the candidate's research career objectives?
- Is the research plan appropriate to the candidate's stage of research development and as a vehicle for developing the research skills described in the career development plan?

In addition, for applications where an independent clinical trial is required:

- Are the scientific rationale and need for a clinical trial, feasibility or ancillary study well supported by preliminary data, clinical and/or preclinical studies, or information in the literature or knowledge of biological mechanisms?
- If proposing a small feasibility study, is the study warranted and will it contribute to planning and preliminary data needed for design of future larger scale clinical trials?
- Is the clinical trial or ancillary study necessary for testing the safety, efficacy or effectiveness of an intervention, or in the case of a feasibility study necessary to establish feasibility of future clinical trial?
- Is the study design justified and relevant to the clinical, biological, and statistical hypothesis(es) being tested?

- Are the plans to standardize, assure quality of, and monitor adherence to, the protocol and data collection or distribution guidelines appropriate?
- Are planned analyses and statistical approach appropriate for the proposed study design and methods used to assign participants and deliver interventions, if interventions are delivered?
- For trials focusing on mechanistic, behavioral, physiological, biochemical, or other biomedical endpoints, is this trial needed to advance scientific understanding?

In addition, for applications where an independent clinical trial is not allowed:

- If proposed, will the clinical trial experience contribute to the proposed research project?

IV. Appendices

1. Funding Agencies for Additional Grant Applications: It is mandatory to apply for external grant applications within 1-2 years of the award initiation date.

- OREF (Success Rate <15-30%)
- Ruth Jackson Society (Unpublished Success Rate)
- **NIH K08 or K23 Awards** (**Success Rate 30-45%: The success rate is highest among all grants!!!!)**
- Musculoskeletal Transplantation Foundation (Success Rate<10-15%)
- Department of Defense
- Institutional Pilot Grant (K12, Cancer Center Pilot Award)
- Sarcoma Foundation
- American Cancer Society
- Susan Komen Breast Cancer Research Foundation
- ORS Traveling Award
- Others

2. Grantsmanship Tutorial (Excerpt from 2018 AAOS ICL on Research Grants and 2018 ORS/OREF Grant Writing Workshop: Moderator: Francis Y. Lee, M.D.; Translational Research by Dr. Brian Snyder, M,D,; Clinical Cohort Study by Dr. Jay Keener, M.D.; and Clinical Trials by Dr. Kurt Spindler, M.D.; Getting the First Grant by Francis Y. Lee, M.D.)
Please send an email request for Grantsmanship Tutiorials to francis.lee@yale.edu

3. Sample Grants: Serious Applicants may inquire grant formats and ask sample grants by contacting our MSTS members; Face-to-face mentoring may done at the National Meetings or Visiting established mentors. These are confidential and privileged information but mentors are willing to share successful grantsmanship.

a. NCI/NIH K08 Award: Kurt Weiss, M.D.; Nocholas Bernthal, M.D.; Bang Hoang, M.D.; Hue Luu, M.D.;
b. NCI/NIH R01 Award: Kevin Jones, M.D.; Michael Yaszemski, M.D.; Regis O'Keefe, M.D.; Denis Clohisy, M.D.; Francis Y. Lee, M.D.; Timothy Damron, M.D.; Richard Terek, M.D.; Benjamin Alman, M.D.; Brian Snyder, M.D.;
c. OREF Research Grant: Cynthia Emory, M.D.
d. Clinical Trial Grants: Michelle Ghert, M.D.; Kurt Spindler, M.D; Jay Keener, M.D.

e. Public Sample Applications (https://www.niaid.nih.gov/GRANTS-CONTRACTS/SAMPLE-APPLICATIONS#k08):

K08 Sample Applications and Summary Statements
The Mentored Clinical Scientist Research Career Development Award (K08) supports those with current work in biomedical or behavioral research, including translational research, a clinical doctoral degree such as M.D., D.V.M., or O.D., and a professional license to practice in the United States. Read more about NIAID Career Development Awards (K).

PI and Grantee Institution	Application Resources
Lenette Lu, M.D., Ph.D., of the Massachusetts General Hospital “Antibody Mediated Mechanisms of Immune Modulation in Tuberculosis” (Forms-D)	Summary Statement**pdf** Full Application**pdf**

4. Grant Review Score Sheet

MSTS Mentored Research and Scholar Development Award Program Review Score Sheet

(Adopted from NIH K Awards Review Criteria)

If you cannot access the hyperlinks below,

visit http://grants.nih.gov/grants/peer/critiques/k.htm.

Application #:

Principal Investigator(s):

OVERALL IMPACT

Reviewers will provide an overall impact score to reflect their assessment of the likelihood for the candidate to maintain a strong research program, in consideration of the following five scored review criteria, and additional review criteria. An application does not need to be strong in all categories to have a major impact.

Overall Impact *Write a paragraph summarizing the factors that informed your Overall Impact score.*

SCORED REVIEW CRITERIA

Reviewers will consider each of the five review criteria below in the determination of scientific and technical merit, and give a separate score for each.

1. Candidate
Strengths • **Weaknesses** •

2. Career Development Plan/Career Goals & Objectives/Plan to Provide Mentoring
Strengths • **Weaknesses** •

3. Research Plan
Strengths • **Weaknesses** •

4. Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s)
Strengths • **Weaknesses** •

5. Environment and Institutional Commitment to the Candidate
Strengths • **Weaknesses** •

5. Biosketches Sample: Downloaded from the NIH Website

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

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

NAME: Hunt, Morgan Casey

eRA COMMONS USER NAME (credential, e.g., agency login): huntmc

POSITION TITLE: Associate Professor of Psychology

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of California, Berkeley	B.S	05/1990	Psychology
University of Vermont	Ph.D.	05/1996	Experimental Psychology
University of California, Berkeley	Postdoctoral	08/1998	Public Health and Epidemiology

A. PERSONAL STATEMENT

I have the expertise, leadership, training, expertise and motivation necessary to successfully carry out the proposed research project. I have a broad background in psychology, with specific training and expertise in ethnographic and survey research and secondary data analysis on psychological aspects of drug addiction. My research includes neuropsychological changes associated with addiction. As PI or co-Investigator on several university- and NIH-funded grants, I laid the groundwork for the proposed research by developing effective measures of disability, depression, and other psychosocial factors relevant to the aging substance abuser, and by establishing strong ties with community providers that will make it possible to recruit and track participants over time as documented in the following publications. In addition, I successfully administered the projects (e.g. staffing, research protections, budget), collaborated with other researchers, and produced several peer-reviewed publications from each project. As a result of these previous experiences, I am aware of the importance of frequent communication among project members and of constructing a realistic research plan, timeline, and budget. The current application builds logically on my prior work. During 2005-2006 my career was disrupted due to family obligations. However, upon returning to the field I immediately resumed my research projects and collaborations and successfully competed for NIH support.

1. Merryle, R.J. & Hunt, M.C. (2004). Independent living, physical disability and substance abuse among the elderly. Psychology and Aging, 23(4), 10-22.
2. Hunt, M.C., Jensen, J.L. & Crenshaw, W. (2007). Substance abuse and mental health among community-dwelling elderly. International Journal of Geriatric Psychiatry, 24(9), 1124-1135.
3. Hunt, M.C., Wiechelt, S.A. & Merryle, R. (2008). Predicting the substance-abuse treatment needs of an aging population. American Journal of Public Health, 45(2), 236-245. PMCID: PMC9162292 Hunt, M.C.,

Newlin, D.B. & Fishbein, D. (2009). Brain imaging in methamphetamine abusers across the life-span. Gerontology, 46(3), 122-145.

B. POSITIONS AND HONORS

Positions and Employment

1998-2000	Fellow, Division of Intramural Research, National Institute of Drug Abuse, Bethesda, MD
2000-2002	Lecturer, Department of Psychology, Middlebury College, Middlebury, VT
2001-	Consultant, Coastal Psychological Services, San Francisco, CA
2002-2005	Assistant Professor, Department of Psychology, Washington University, St. Louis, MO
2007-	Associate Professor, Department of Psychology, Washington University, St. Louis, MO

Other Experience and Professional Memberships

1995-	Member, American Psychological Association
1998-	Member, Gerontological Society of America
1998-	Member, American Geriatrics Society
2000-	Associate Editor, Psychology and Aging
2003-	Board of Advisors, Senior Services of Eastern Missouri
2003-05	NIH Peer Review Committee: Psychobiology of Aging, ad hoc reviewer
2007-11	NIH Risk, Adult Addictions Study Section, members

Honors

2003	Outstanding Young Faculty Award, Washington University, St. Louis, MO
2004	Excellence in Teaching, Washington University, St. Louis, MO
2009	Award for Best in Interdisciplinary Ethnography, International Ethnographic Society

C. CONTRIBUTION TO SCIENCE

1. My early publications directly addressed the fact that substance abuse is often overlooked in older adults. However, because many older adults were raised during an era of increased drug and alcohol use, there are reasons to believe that this will become an increasing issue as the population ages. These publications found that older adults appear in a variety of primary care settings or seek mental health providers to deal with emerging addiction problems. These publications document this emerging problem but guide primary care providers and geriatric mental health providers to recognize symptoms, assess the nature of the problem and apply the necessary interventions. By providing evidence and simple clinical approaches, this body of work has changed the standards of care for addicted older adults and will continue to provide assistance in relevant medical settings well into the future. I served as the primary investigator or co-investigator in all of these studies.
    a. Gryczynski, J., Shaft, B.M., Merryle, R., & Hunt, M.C. (2002). Community based participatory research with late-life addicts. American Journal of Alcohol and Drug Abuse, 15(3), 222-238.
    b. Shaft, B.M., Hunt, M.C., Merryle, R., & Venturi, R. (2003). Policy implications of genetic transmission of alcohol and drug abuse in female nonusers. International Journal of Drug Policy, 30(5), 46-58.
    c. Hunt, M.C., Marks, A.E., Shaft, B.M., Merryle, R., & Jensen, J.L. (2004). Early-life family and community characteristics and late-life substance abuse. Journal of Applied Gerontology, 28(2),26-37.
    d. Hunt, M.C., Marks, A.E., Venturi, R., Crenshaw, W. & Ratonian, A. (2007). Community-based intervention strategies for reducing alcohol and drug abuse in the elderly. Addiction, 104(9), 1436-1606. PMCID: PMC9000292

2. In addition to the contributions described above, with a team of collaborators, I directly documented the effectiveness of various intervention models for older substance abusers and demonstrated the importance of social support networks. These studies emphasized contextual factors in the etiology and maintenance of addictive disorders and the disruptive potential of networks in substance abuse treatment. This body of work also discusses the prevalence of alcohol, amphetamine, and opioid abuse in older adults and how networking approaches can be used to mitigate the effects of these disorders.
    a. Hunt, M.C., Merryle, R. & Jensen, J.L. (2005). The effect of social support networks on morbidity among elderly substance abusers. Journal of the American Geriatrics Society, 57(4), 15-23.
    b. Hunt, M.C., Pour, B., Marks, A.E., Merryle, R. & Jensen, J.L. (2005). Aging out of methadone treatment. American Journal of Alcohol and Drug Abuse, 15(6), 134-149.
    c. Merryle, R. & Hunt, M.C. (2007). Randomized clinical trial of cotinine in older nicotine addicts. Age and Ageing, 38(2), 9-23. PMCID: PMC9002364

3. Methadone maintenance has been used to treat narcotics addicts for many years but I led research that has shown that over the long-term, those in methadone treatment view themselves negatively and they gradually begin to view treatment as an intrusion into normal life. Elderly narcotics users were shown in carefully constructed ethnographic studies to be especially responsive to tailored social support networks that allow them to eventually reduce their maintenance doses and move into other forms of therapy. These studies also demonstrate the policy and commercial implications associated with these findings.

    a. Hunt, M.C. & Jensen, J.L. (2003). Morbidity among elderly substance abusers. Journal of the Geriatrics, 60(4), 45-61.
    b. Hunt, M.C. & Pour, B. (2004). Methadone treatment and personal assessment. Journal Drug Abuse, 45(5), 15-26.
    c. Merryle, R. & Hunt, M.C. (2005). The use of various nicotine delivery systems by older nicotine addicts. Journal of Ageing, 54(1), 24-41. PMCID: PMC9112304
    d. Hunt, M.C., Jensen, J.L. & Merryle, R. (2008). The aging addict: ethnographic profiles of the elderly drug user. NY, NY: W. W. Norton & Company.

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/collections/public/1PgT7IEFIAJBtGMRDdWFmjWAO/?sort=date&direction=ascending

D. ADDITIONAL INFORMATION: RESEARCH SUPPORT AND/OR SCHOLASTIC PERFORMANCE

Ongoing Research Support

R01 DA942367 Hunt (PI) 09/01/08-08/31/16
Health trajectories and behavioral interventions among older substance abusers
The goal of this study is to compare the effects of two substance abuse interventions on health outcomes in an urban population of older opiate addicts.
Role: PI

R01 MH922731 Merryle (PI) 12/15/07-11/30/15
Physical disability, depression and substance abuse in the elderly
The goal of this study is to identify disability and depression trajectories and demographic factors associated with substance abuse in an independently-living elderly population.
Role: Co-Investigator

Faculty Resources Grant, Washington University 08/15/09-08/14/15
Opiate Addiction Database

The goal of this project is to create an integrated database of demographic, social and biomedical information for homeless opiate abusers in two urban Missouri locations, using a number of state and local data sources.
Role: PI

Completed Research Support

R21 AA998075 Hunt (PI) 01/01/11-12/31/13
Community-based intervention for alcohol abuse
The goal of this project was to assess a community-based strategy for reducing alcohol abuse among older individuals.
Role: PI

Funded NIH Grant Samples (Grantsmanship Tutorials)

SPECIFIC AIMS (Kindly Provided by Kurt Weiss, M.D. Sample for K08 Award; Funded by NCI)

Osteosarcoma (OS) is the most common primary malignancy of bone. Despite aggressive treatment, overall survival is only 60-70%. Patients with metastatic disease have especially poor prognoses with survival rates of 15-30%. These statistics have not improved in over a generation, and the same challenges and failures of OS exist for other sarcoma subtypes as well. **The greatest obstacle to progress in the treatment of sarcoma is inadequate understanding of the basic biology that governs sarcoma metastases. Novel, biologically-driven treatment paradigms are required to improve the prognoses of sarcoma patients.**

K7M2 and K12 are related cell populations derived from a spontaneously occurring murine OS. **K7M2 metastasizes vigorously to the lung** in the mouse model of OS, whereas **K12 is much less metastatic**. As these populations are related but vary in their metastatic rates, they are powerful tools through which the qualities that confer **metastatic potential** may be elucidated. We have published that K7M2 and K12 produce different quantities of cytokines, and that cytokine inhibition alters OS cell behavior *in vitro*. We have recently demonstrated differences between K7M2 and K12 in terms of cancer stem cell factors.

Aldehyde dehydrogenase (ALDH) enables cells to resist oxidative stress. ALDH has been implicated as a **cancer stem cell marker**. Cells with high ALDH activity have demonstrated enhanced tumorigenicity in multiple cancers, and high ALDH activity predicted poor survival in breast and ovarian cancer. We observed that K7M2 cells display greater resistance to oxidative stress than K12 cells when challenged with H_2O_2 and hypothesized that differences in ALDH expression and activity might explain this difference. Indeed, we found greater ALDH expression and activity in highly metastatic K7M2 cells than in less metastatic K12 cells. These data suggest that ALDH is a cancer stem cell factor in OS.

Notch1 has been called **"the stem cell master switch"** and influences morphogenesis, lineage specification, and proliferation. Notch1 contributes to cancer progression by promoting survival, neo-angiogenesis, drug resistance, and metastasis. We demonstrated that K7M2 cells display greater baseline expression of Notch1 and its downstream targets than less metastatic K12 cells. Treatment with the Notch1 inhibitor DAPT decreased K7M2 cells' invasiveness and resistance to oxidative stress with H_2O_2. We observed that Notch1 inhibition correlated with substantial decreases in ALDH expression and activity in OS cells. Taken together, these data suggest a relationship between ALDH, Notch1, and OS metastatic potential.

In order to develop anti-metastatic therapies for OS and other sarcomas, we suggest the following:

Aim #1, *In Vitro* (years 1-2): *Evaluate the importance of ALDH in the metastatic potential of OS, and investigate the* in vitro *effects of ALDH and Notch1 inhibition on OS metastatic potential.* Using flow cytometry, we will sort K7M2 and K12 populations into high ALDH activity (ALDH-high) and low ALDH activity (ALDH-low) fractions. We will evaluate their metastatic phenotypes by assessing and comparing their abilities to migrate, proliferate, invade, and resist chemotherapeutic stress. We will determine the effects of ALDH and Notch1 inhibition on the metastatic phenotypes of OS cells *in vitro*. This will be accomplished by treating OS cells with the ALDH inhibitor disulfiram and the novel Notch1 inhibitor MK-0752. The aforementioned phenotypes of proliferation, migration, invasion, and resistance to chemotherapy will be evaluated. **We hypothesize that ALDH-high cells will display enhanced metastatic potential compared with ALDH-low cells. We also hypothesize that both disulfiram and MK-0752 will decrease the metastatic potentials of OS cells *in vitro*.**

Aim #2, *In Vivo* (years 2-3): *Determine the effects of disulfiram and MK-0752 alone and with cytotoxic chemotherapy in the mouse model of OS.* The clinical efficacy of ALDH and Notch1 inhibition will be rigorously tested with the animal model of metastatic OS. We will compare disulfiram treatment, MK-0752 treatment, and combination therapy in terms of the *in vivo* metastatic biology of OS. In order to enhance the clinical relevance of our model, we will combine the most promising biologic therapy with doxorubicin, a standard OS chemotherapeutic agent. **We anticipate that biologic treatment with disulfiram and/or MK-0752 will have an additive effect when combined with doxorubicin.**

Aim #3, Human Cells (years 1-4): *Use the Musculoskeletal Oncology Tumor Registry and Tissue Bank (MOTOR) to correlate the clinical histories of sarcoma patients with* in vitro *metastatic phenotypes.* The MOTOR is an IRB-approved mechanism by which patients' clinical data can be correlated with the biological activity of their tumor cells. We will evaluate gene expression, metastatic potential, and therapeutic sensitivity in novel human sarcoma cell lines. These data will be compared with the above findings to search for trends that might be exploited for therapeutic benefit in other sarcomas besides OS.

Through the completion of the experiments described above we will refine our understanding of the factors and molecular pathways that drive the growth and metastasis of OS specifically and sarcoma in

general. The ultimate goal of these experiments is to uncover novel strategies and treatment options that will alter the metastatic behaviors of pediatric and adult sarcomas and improve the prognoses of these diseases.

6. Specific Aims Sample I (K08 Award Sample; Funded in 2017): Many successful grant writers spend lots of time in preparing a single-page Specific Aim section. Specific Aims page contain significance, overall goal, long-range goal, purpose, innovation, preliminary data, hypothesis, description of Aims (usually 2-3, rarely over 4), and overall impact. There are many different styles and styles may vary among investigators.

Specific Aims (This sample is for a NIH K08 Award; Funded in 2017; R03/21/01 Specific Aims are more science focused)

Overview (Candidate and Institutional Commitment): This XXX K08 Proposal seeks to provide the most ideal scholarly infrastructure for Dr. XXX, a promising orthopaedic XXX surgeon scientist with clinical expertise in shoulder reconstructive surgeries. The candidate was fortunate to work on rotator cuff injuries under Dr. XXX, an exemplary orthopaedic surgeon scientist at the XXX, during his medical school in 2007. The candidate became proficient in animal experiments on clinically relevant rotator cuff injury and repair models and has been one of authors of series of papers. The candidate was naturally inducted into clinical shoulder surgery and science. Most ideally, the candidate is working under Dr. XXX, M, D, another pioneer of rotator cuff inflammation and biology, at the XXX School of Medicine, which is one of top research-intensive universities and health care systems. The Chair provides XX% of Protected Research Time. A team of the primary mentor, co-mentors, and collaborators consisting of Drs. XXX, XXX, XXX, XXX, , and Steven XXX are ready to provide rigorous guidance for infusing multi-disciplinary mechanistic research knowledge and skills that will be most critical for the candidate to develop into an independent orthopaedic surgeon scholar. The pool of orthopaedic surgeon scientist with NIH R01 funding has been very scarce and the total number of such investigators are just around dozen. Rotator cuff disorders are one of most common musculoskeletal disorders and yet treatments need further improvements. Scientific discoveries from the rotator cuff disorders can be applied to other inflammatory, degenerative, or traumatic disorders in different anatomic locations. Our research project was deliberately chosen to provide training opportunities that will lead to clinically impactful discoveries, thereby fulfilling all necessary elements of the most successful K08 Award requirements.

Mentored Training Plan: The primary mentor, Dr. XXX, is a leading orthopaedic surgeon scientist with several NIH R01 grants and high-impact publications, and has interacted with the Candidate during his fellowship and AAOS/ORS/OREF New Investigator as a mentor. Dr. XX and Dr. XX, the most enthusiastic chair for the candidate, share a long-term history of productive collaboration, leading to early establishment of rotator cuff inflammatology. The mentoring committee will have weekly face-to-face meetings to provide the necessary guidance. Most mentors are faculty members of Department of Orthopaedic Surgery. Through intensive training courses offered by the XXX University Graduate School of Medicine and CTSI, the candidate will acquire necessary knowledge and skills in research methodology, experimental designs, biostatistics, bioethics, and the Responsible Conduct of Research (RCR). There are ample K01/08/23/99 recipients who will share critical information for successful transition from K08 to R01 in the next 5 years, a signature of successful clinician scientists.

Significance, Premise, and Research Strategy: Shoulder joint is the most mobile joint in the human body, steered by a rotator cuff consisting of tendons arising from the scapular inserting in a tight region on the top end of the humerus. As a result, a rotator cuff is vulnerable to wear-and-tear of the tendons and inflammation of the bursa, resulting in shoulder pain and dysfunction. Our **long-range scientific goal** is to establish a molecular therapeutic approach for rotator cuff disorders. We can apply this therapeutic approach to treat other inflammatory disorders in the musculoskeletal system. Our **Preliminary Data** showed that inflamed human rotator cuff tissues and cells express higher levels of cytokines and chemokines compared to normal rotator cuff tissues [XXX & XXX 2005, 2006, 2011, 2014]. One of the most striking inflammatory mediator is stromal cell derived factor-1 (SDF-1) that is a chemokine responsible for mobilizing inflammatory cells bearing CXCR4, a specific receptor for SDF-1. We have established rat rotator cuff tear models that simulate human rotator cuff inflammation. Our **central hypothesis** is that SDF1-CXCR4 targeting mitigates rotator cuff inflammation and promotes healing of the tendon repair around the shoulder joint. We will conduct rigorous scientific experiments to accomplish the following **Specific Aims**.

Specific Aim 1. To determine whether SDF1-CXCR4 targeting reduces infiltration of inflammatory cells and cytokine expression at the site of tendon degeneration *in vivo* and *in vitro*.
3-5 line summary of Approach (Hypothesis, Key methodology, Readouts, Expected Outcome, etc.)
Specific Aim 2. To determine whether SDF1-CXCR4 targeting enhances repair of torn rotator cuff tendons *in vivo*.
3-5 line summary of Approach
Innovation and Impact: In the era of Precision Medicine, we are introducing a new therapeutic paradigm for musculoskeletal inflammatory disorders targeting key mediators of inflammation with locally delivered inhibitors. The proposed study should provide rigorous training for mechanistic impactful research.

Specific Aims Sample II (R01 Specific Aims; Funded by NIH in 2009; Kindly Provided by Francis Y. Lee, M.D.)

Our **long-range goal** is to enhance the clinical success of bone implants by optimizing bone formation and suppressing the inflammatory response at the bone-implant interface. The long-term clinical success of bone implants depends on osseous integration, which is often impeded by the host innate immune response and subsequent bone resorption at the bone-implant interface. To this end, in our original ongoing proposal (2007-2011), we verified the role of Ca^{++} /calcineurin/ Nuclear Factor of Activated T cells (NFAT) as a common signal transducer for COX-2, osterix and TNF-alpha gene induction in the context of wear particle-induced inflammatory bone loss and mechanotransduction. While the identification of NFATc in bone mechanotransduction and innate immunity highlights the intimate interactions among immune cells, osteoprogenitor cells, physical force and biomaterials, there is a ***knowledge gap*** concerning the mechanism by which physical and inflammatory signals are transduced into specific cellular responses such as cytokine gene expression or osteogenesis. The **objective** of this proposal is to elucidate the *mechanobiological mechanisms* governing osteogenic differentiation and inflammation at the host-bone implant interface using clinically relevant *in vitro* simulation and *in vivo* animal experiments. We will employ the techniques of molecular optical imaging, molecular biology, and genetically engineered mice. **Our central hypothesis is that combinatorial mechanical perturbation and biomaterial particles amplify the innate immune response at the bone-implant interface by activating osteo-immuno-mechano-regulatory pathways.** We propose the following 3 specific aims:

Specific Aim 1: To examine the molecular mechanism by which NFATc regulates osteogenic or inflammatory cellular processes in response to combinatorial mechano-inflammatory signals consisting of particulate biomaterials and pathologic mechanical strain in osteoprogenitor cells.
Hypothesis: NFATc and ERK co-regulate the dual roles of osteogenic differentiation and inflammatory cytokine production by osteoprogenitor cells.

We identified that osteoprogenitor cells produce RANKL and MCSF in response to stimulation by wear particles and/or superphysiologic mechanical strain, and that this response is mediated by extracellular receptor kinase 1/2 (ERK1/2) signalling. We also observed that NFATc activation by mechanical loading is linked to induction of master osteogenic genes such as Osx. We will examine NFATc regulation of Osx gene induction and osteogenic differentiation in osteoprogenitor cells and examine the interaction between NFATc and ERK1/2 signalling using pharmacologic inhibition, siRNA and osteoblast-specific ERK1/2 dysfunctional murine osteoblasts.

Specific Aim 2. To examine the osteoblast-macrophage interaction and macrophage activation after wear particle stimulation and mechanical perturbation.
Hypothesis: Inflammatory osteoclastogenesis requires concurrent activation of ERK and NFATc during osteoblast-macrophage interaction.

We have optimized NFATc1 gene silencing and have established osteoblast-specific ERK 1/2 dysfunctional mice. We will examine osteoclastogenesis and bone resorption after co-culturing macrophages and osteoblasts with defective NFATc1 or ERK1/2 in a simulated effective joint space subject to wear particle stimulation and mechanical perturbation. We will measure osteoclast formation and related cytokine expression after genetic or pharmacologic manipulation of NFATc and ERK1/2 in a co-culture system.

Specific Aim 3. To examine the combinatorial effect of wear particles and mechanical perturbation on inflammatory osteolysis in a mouse tibia implant model and in a mouse calvarial osteolysis model
Hypothesis: NFATc and ERK1/2 co-activation are required for superphysiologic mechanical perturbation-induced inflammatory bone loss *in vivo*.

We will apply axial and three-point loading to the tibiae with intramedullary implants in wild-type and ERK1/2 defective mice and will examine osteoclastic activity by molecular imaging of cathepsin K activity. Inflammatory bone resorption will be measured with microradiographs and microCT. We will also measure the bone loss after NFATc targeting by administering cyclosporine A in mice with tibial implants.

Summary and Overall Impact: Our proposal is a pre-clinical extension of the role of NFATc in mechanotransduction and inflammation *in vitro.* Here, we introduce new experiments using genetically engineered mice and a clinically relevant tibial implant animal model. We plan to address the fundamental issues on molecular mechanisms of osteogenesis and inflammation at the host bone-implant interface.

Career Goals Sample for K08 (Funded in 2017; Kindly Provided by Francis Y. Lee, M.D.)

Career Development Plan/Career Goals & Objectives/Plan to Provide Mentoring

A.1. Background: The candidate's prior training, research experience, and commitment

The desire to supplement my clinical education with extensive research training began in medical school, when I spent a year between medical school years three and four conducting research as a student research fellow under Dr. [redacted] at the Hospital for Special Surgery. I was introduced to numerous methods pertinent to translational orthopaedic research while contributing to a highly-impactful study investigating the bone-tendon junction of the shoulder rotator cuff and anterior cruciate ligament in rodents. As a resident at the Cleveland Clinic Foundation, I continued to demonstrate my passion for orthopaedic research by devoting another full academic year to a research fellowship under Drs. [redacted]. I later applied the knowledge and experience gained from these prior fellowships as I subspecialized in shoulder and elbow surgery as a clinical fellow at Columbia University. There I worked under the mentorship of the renowned scientist and shoulder surgeon Dr. Francis Y. Lee. I continued interactions with Dr. Francis Y. Lee when I attended the American Academy of Orthopaedic Surgeons/Orthopaedic Research Society/Orthopaedic Research and Education Foundation Workshops for New Investigators and Junior Faculty. I joined the Yale University School of Medicine in 2015. Dr. [redacted] is currently Department Chair and supports translational research beyond my expectations. The Dean and Institutional Leadership at the Yale University School of Medicine emphasize NIH-funded research programs in the surgical departments, which is very uncommon at other universities in this day and age. As a result, I continued working with Dr. Francis Y. Lee, who joined the Yale University School of Medicine, under the full support of research faculty at my current department. Drs. [redacted] and Lee have worked on shoulder inflammation for more than 10 years, assuring institutional and scientific commitment to my K08 Award Application (**Figure 1**). I am fully committed to devote 50% of my time and effort to solve clinical dilemmas that I encounter at the shoulder clinic and in the operating room. In other words, my current work environment allows me to commit 100% of my effort to shoulder-related research and clinical care. Analysis of imaging and pathologic specimens from my shoulder clinic will further enhance my performance and the likelihood of success in my K08-related research activities.

Currently, I am conducting a small foundation grant-funded research project investigating a means of increasing the mechanical and structural strength of the supraspinatus tendon-to-bone enthesis and decreasing fatty degeneration of muscles following rotator cuff injury. This funding naturally allowed me to establish translational research animal models at the Yale University School of Medicine. This has involved the development of a surgical model for rotator cuff injury and repair, which builds upon the utility of the rat models I helped develop as a resident. While most previous rodent models pertinent to rotator cuff surgery have involved either an injury only or an acute repair model involving injury and immediate repair, a secondary goal of my current project is the optimization of a chronic repair model. The rotator cuff repair occurs four weeks after the initial tendon transection, which gives sufficient time for severe atrophy and fatty degeneration of the muscles. This more accurately models the human chronic injury condition, where it is common for patients to avoid seeking medical intervention for months or even years after the initial injury. The development and perfection of a rodent model for this condition has provided me valuable experience working through the unforeseen issues that inevitably arise while conducting innovative research. Furthermore, it has provided me

comprehensive knowledge about numerous surgical techniques and clinical methods that I will utilize in this proposed K08 award project. Through didactic interactions and SWOT (Strength-Weakness-Opportunities-Treats) analysis with Drs. [redacted], I will be able to contribute more if I acquire further knowledge and skills in cell signaling, molecular biology, basic biomechanical testing, mechanistic experiments, rigor, biological viable, gene editing, and responsible conduct of research. I have read many orthopaedic translational research papers that can be further strengthened by the application of these methods.

A.2. My career goals and specific timeline toward scientific independence

I ultimately aspire to become a leading clinician-scientist in the field of upper extremity orthopaedic surgery. Definition of a clinician-scientist according to the AAOS workshop consensus requires meeting the following 3 criteria;

1) Active clinical practice in focused area: In my case, it is the field of shoulder reconstructive surgery. I have learned about and seen firsthand critical barriers in shoulder reconstructive surgeries. I am in a very strong position of conducing clinically impactful translational research once I receive NIAMS K08 and subsequent awards.

2) High-impact research to advance the field: Regarding this, I need to move from descriptive research toward more mechanism-based research.

3) Continued peer-reviewed funding from external agencies: Most private organizations have one-time or limited opportunities for junior faculty members to receive funding. In order to sustain my research programs, I ultimately plan to conduct a series of NIH R01 grants to maintain high-impact research programs. In order to become a successful NIH-funded orthopaedic surgeon scientist, I need a structured mentorship plan for several years. I keep hearing a phrase that '**orthopaedic surgeon scientists are an *endangered species* or *disappearing*'**. I have had a very rare opportunity of working with NIH-funded orthopaedic surgeon mentors, such as Drs. [redacted], who showed persistent passion for science. I am committed to maintaining the presence of a rare pool of orthopaedic surgeon scientists.

As I continue developing animal models, I seek to continue conducting experiments that evaluate biologic augmentation of rotator cuff healing. By utilizing the mentorship of the numerous renowned faculty researchers at the Yale Department of Orthopaedics and Rehabilitation, I hope to first secure a NIAMS K08 Award to reinvent my descriptive research *projects* toward a mechanism-based research *program* in treating diseases of the rotator cuff. This NIMAS K08 Award will commence in 2017 and proceed for up to five years. I will apply for NIAMS Limited Time R03 Grants for K08/23 Awardees (PAR-16-268) in 2018 and ultimately R01 awards beginning in 2020 and thereon to fund the development and operation of a comprehensive shoulder research program at Yale. An R21 mechanism is not necessarily desirable for a New Investigator, but it will be considered if I come up with a high-risk, high-reward exploratory research topic. My central research program leading to R01 funding will focus on screening candidate endogenous and exogenous targetable inflammatory mediators to prevent tendon degeneration and to augment rotator cuff healing. Beginning around 2022, upon successful completion of this rigorous preclinical screening and evaluation process, I plan to clinically evaluate newly-identified therapeutic agents through clinical trials and the use of comprehensive standardized patient reported outcome surveys to assess the agents' ability to improve the musculoskeletal healing and overall quality of life of my and other clinicians' patients. My plans are in line with personalized medicine in the field of orthopaedic surgery. Concerning research community service, I will participate in early career Center for Scientific Review Study Section opportunities and will volunteer for journal reviews.

The ultimate purpose of the development of this comprehensive system is to ensure the highest possible standard of care is provided to patients suffering from shoulder and elbow diseases. Although these conditions are incredibly common, the paucity of orthopaedic surgeons who conduct impactful translational research has hampered the progress toward higher surgical and nonsurgical success rates. I aspire to join Drs. Francis Lee, [redacted], and a limited set of other orthopaedic surgeon-scientists whose research is aimed at ensuring that the subpar success rate of shoulder treatments is improved upon. In doing so I hope to become a

leading surgeon and researcher in the orthopaedic community while providing the same attentive mentorship that I am currently provided to the next generation of young investigators.

A.3. Plans for monitoring and evaluating the candidate's research and career development progress

I will consistently interact with the Mentoring and Research Support Committee consisting of Drs. [redacted] by hosting weekly Translational Research Meetings. Dr [redacted] will join the meeting via Skype. I will also present a detailed overview of my current projects and findings to all members of the Orthopaedics department at least twice per year at grand rounds. This will ensure that I am constantly in the process of producing presentable results while allowing my colleagues and mentors to evaluate the progress of my research. I also hope to share my experience with orthopaedic residents and medical students who will be next generation scholars. Additionally, I will publish my findings in the most impactful orthopaedic journals and present my findings at national and international conferences to ensure that the orthopaedic surgeons from other institutions as well as the greater scientific community as a whole can evaluate the quality of my research and track my career development.
Furthermore, since the ultimate goal of my career as a clinician-scientist is to secure R01 funding for my translational research, the time at which I do so is the most effective means of tracking my career development. I aim to apply for R01 grants beginning in 2020 after successfully securing K08 and possibly R03 funded projects. My K08 mentors, most of which are Yale faculty members, will remain close colleagues in the near and distant future and will undoubtedly ensure I am adequately prepared to apply for and conduct R01 research at that time.

A4. Training, Courses, Mentoring, Responsible Conduct of Research, and Institutional Commitment

The major efforts of this NIAMS K08 Award are training toward scientific independence and institutional commitment. We have described all necessary elements in the ***Plans and Statements of Mentor and Co-mentors***, ***Institutional Commitment***, and ***Responsible Conduct of Research*** sections. Most importantly, the Research Plan is integrated with specific training components to fulfill the mission of the K08 Mechanism. The timeline of research and career plans are briefly summarized in **Table 1**.

Table 1. Milestones toward Independent Investigators

	Year 1	Year 2	Year 3	Year 4	Year 5
Aim 1	Acquisition of Research Skills; In vitro Mechanistic Experiments				
Aim 2		In vivo Animal Surgeries, Data Collection, Analysis			
Career Plans	RCR	Limited Time R03 Application for K08 Awardees			
	Yale R01 Writing Course/Traveling Fellowship			R01 Submission & Revision	
	Weekly Meetings with Mentoring and Research Support Committee; Courses; Seminars				

Concluding Remarks: My prior training, current clinical expertise, training plan, career goals, institutional support, institutional environment, and Mentoring & Research Support Committee are well aligned for me to become an Independent Investigator. Proposed experiments not only are innovative but also provide a fundamental set of data for my NIAMS L

Mentor Statement Sample for K08 (Funded in 2017)

Plans and Statements of Mentor and Co-mentors (Sample; Funded in 2017)

1. Overview: A Compelling Case

This NIAMS K08 Award stems from an ideal combination of scientific passion, qualifications, well-defined career goals, mentoring infrastructure, and research environment shared by [redacted] David Kovacevic (K08 Candidate), Francis Y. Lee, M.D., Ph.D. (Mentor), the Department of Orthopaedic Surgery ([redacted], Professor & Chair), Yale University, and PA16-191/Special Notice NOT-AR-16-012 promoting K08 Awards for Orthopaedic Surgeons (**Figure 1**). Dr. David [redacted] is an exceptional and promising young orthopaedic surgeon who has shown persistent motivation to develop into a scholarly, independent investigator since his pre-doctoral period. Dr. [redacted] is an ideal candidate for a NIAMS K08 award because providing him with the means to develop new research skills and knowledge will complement his rapidly evolving clinical expertise in shoulder reconstructive surgery and add high-impact mechanistic research armamentarium to

enhance his research portfolio. Our department has a dedicated mentoring committee consisting of Francis Y. Lee, M.D. (Primary Mentor, NIAMS R01-funded orthopaedic surgeon), [redacted], M.D. (Co-Mentor, Professor & Department Chair, orthopaedic surgeon scientist), [redacted], Ph.D. (Co-Mentor, Vice Chair of Research, immunologist), and [redacted], M.D. (Co-Mentor; Past NIAMS P30 Musculoskeletal Center Director, bone and cartilage biologist, endocrinologist). Our mentors are pioneers in basic and translational research with extensive research funding and programs that will provide comprehensive training opportunities for Dr. [redacted]. Yale University School of Medicine is a highly ranked research-intensive health science institution that has generated numerous independent investigators. Yale School of Medicine supports the high-impact research activities of 14 Howard Hughes Medical Investigators, 41 Institute of Medicine members, and 62 members of National Academy of Science. Furthermore, Yale School of Medicine currently supports the career development and training of 106 K01/K08/K23/K99 new junior investigators, reflecting a serious commitment to the development of the next generation of scholarly leaders. Unfortunately, there has been no Orthopaedic Surgeon K08 awardee in the past at the Yale School of Medicine. Concerning NIH-funded Principal Investigators, there are only about 10-15 orthopaedic surgeons (an endangered species) with NIH R01 grants. The number has been decreasing over the past 10 years. There is an urgent need to identify and support promising junior orthopaedic surgeon scientists. This need is in line with public health care issues because musculoskeletal disorders are among the most common health conditions nationwide, with costs totaling more than 5% of GDP (Bone and Joint Burden Report in 2014). It is of the utmost importance to enhance musculoskeletal health through rigorous scientific research focused on the development of more effective novel therapeutic approaches. To this end, we are presenting a very compelling proposal of a NIAMS K08 Award that will meet all necessary criteria and will generate a very impactful outcome. Our team has specific plans to train Dr. [redacted] and add him as one more Orthopaedic Surgeon Independent Investigator to a shrinking pool of NIH-funded orthopaedic surgeon scientists (an endangered species).

2. Qualifications of the mentors in the area of the proposed research and expected roles. Evidence of the mentor's, consultant's and/or collaborator's previous experience in fostering the development of independent investigators? Evidence of the mentor's current research productivity and peer-reviewed support. Is active/pending support for the proposed research project appropriate and adequate?

Mentors, collaborators, and a consultant will provide research training through face-to-face didactic sessions, formulation of hypotheses, experimental design, hands-on experiments, data acquisition, data interpretation, formulation of new or alternative hypotheses, planning independent research projects, grantsmanship training, and independent investigator status. This training paradigm is embedded in the culture of the Yale School of Medicine as evidenced by the school's more than 100 K01/08/23/99 awardees in 2016. Most importantly, the senior leadership faculty members of the Department are well-funded scientists or clinician scientists who provide dedicated mentorship to younger investigators (**Table 1**). All listed internal mentors and collaborators meet face-to-face at the weekly Translational Research Science Conference, which is unique among clinical orthopaedic departments. More convincingly, the Department Chair is a clinician scientist with a long-term history of collaboration with the primary mentor. As the Department Chair, protected time, space, internal funding and administrative support is ideally aligned without bureaucratic barriers. Brief descriptions on the qualifications and expected roles of each mentor and collaborator are as follows.

Table 1. Peer Reviewed Support and Mentees (Recent & Current Research Base for Training)

	Agency	Grants	Period	Role	Previous Trainees
[redacted], M.D.	ASES	Shoulder Research	2016-2017	PI	K08 Candidate
Francis Y. Lee, M.D. Primary Mentor	NIAMS	R01 AR0683531	2015-2020	PI	12 Posdoctoral Fellows; 8 premedical students (Current MDs); 6 Faculty
	NIAMS	R01 **AR056246**	2013-2018	PI	
	NCI	R01 CA203011	2015-2020	PI	
	NIBIB	R01 **EB006834**	2012-2017	PI	
[redacted], M.D. Co-Mentor	NIAMS	P50 NIAMS (*Pending*)	2017-2022	PI	20 Fellows & Junior Faculty Members
	Industry	Ultragenyx, Inc.	2014-2016	PI	
	NIH	1UL1 RR024139; CTSI	2016-2021	Co-I	CTSI Medical Director
[redacted]	OREF	OREF Grant	Past	PI	Department Chair; Fellowship Director
	ASES	ASES Grant	Past	PI	
[redacted], Ph.D. Collaborator	NIDCR	R01DE021088	2010-2016	PI	5 Postdoctoral Fellows
	NIDCR	R01DE020823	2010-2016	PI	
[redacted], Ph.D.	NIAMS	1R01AR069088-A1	2016-2020	Co-I	

Collaborator	NIAMS	R01AR067185	2016-2021	Co-I	3 Postdoctoral Fellows; 6 BME Students
[redacted], Ph.D.	NIAMS	R01 AR063649	2012-2018	PI	5 Postdoctoral Fellows
Collaborator	Mark Cuban	Foundation	2015-2017	PI	
[redacted], Ph.D.	NIAMS	1R01AR069088-A1	2016-2021	MPI	20 Postdoctoral Fellows; 5 Faculty; 1 K99/R00
	NIDDK	2R24DK092759	2011-2020	MPI	
	NIAMS	1R13AR069961	2016-2017	PI	

Francis Y. Lee, M.D., Ph.D. (Primary Mentor)

Qualifications: Dr. Lee was a Professor with Tenure and the Robert Carroll Professor at Columbia University until 2016. He became the first tenured professor as a clinician in the Department of Orthopaedic Surgery in recognition of his exceptional success in translational research at the Columbia University. He was recently recruited by the Yale University School of Medicine as a Professor with Tenure and a Southwick Endowed Professor, with protected time for promoting translational research and junior faculty career development. In the past, Dr. Lee has devoted himself to mentoring young basic science investigators and junior clinical faculty throughout the AAOS Clinician Scholar Development Program and ORS/AAOS/OREF New Investigator Workshop, where he has served as a Program Chair or Faculty Member since 2009. He was recently recruited to Yale University School of Medicine to promote high-impact mechanistic orthopaedic translational research and career development of junior faculty members, and is currently running four active NIH R01 grant-supported research programs, two of which are supported by NIAMS. Dr. Lee has also committed himself to promoting K08 grant participation among orthopaedic surgeons. He has been consulting or supporting 3 recent NIAMS K08 Awardees at Wake Forest, NYU, and UCLA. He will provide space and directly cover research-related costs in order to create an ideal research environment for the Candidate. Additionally, Dr. Lee's office is located in the same building as the Candidate's and he will interact with the Candidate on a daily basis. Most importantly, this K08 Award Proposal is a natural extension of more than 10 years of collaboration with Dr. [redacted] and more than 2 years of external mentor-mentee interactions with Dr. [redacted] (the K08 Candidate).

Expected Role: Dr. Lee will serve as the Primary Mentor and Chair of the Mentoring & Research Support Committee that will consist of [redacted]. Dr. Lee will provide laboratory space and costs that exceed the $30,000/year provided by the NIAMS. These committee members hold Departmental Research and Educational meetings in the department's conference room every Friday morning at 7:00 am. At these meetings, Dr. Lee will facilitate discussions regarding research progress, grant applications, and publications. Dr. [redacted] will call in either via phone or Skype. We will have additional face-to-face meetings at the Orthopaedic Society Meeting or other annual conferences. If there are any logistical issues, Dr. Lee will intervene immediately in order to maintain the most ideal research environment. Dr. Lee's research programs are diversified under the central theme of inflammation in the context of wear particles, infection, and metastatic cancers. An additional area of his research focuses on tissue regeneration. Dr. Lee's research team will provide full support so that Dr. [redacted]'s didactic and independent research programs progress in the most efficient and effective way. Preliminary data in this proposal were prepared by the collaborative work of Dr. [redacted].

[redacted], M.D. (Co-Mentor; Department Chair)

Qualifications: Dr. [redacted] is a Professor and Chairman of the Department of Orthopaedics and Rehabilitation at the Yale University School of Medicine. He is a renowned shoulder surgeon with expertise in shoulder inflammation research. Dr. Lee and Dr. [redacted] have extensively collaborated on shoulder inflammation research projects and have published several papers together. Due to well-aligned departmental and institutional commitment, well-established long-term scientific collaborative expertise, as well as additional resources, Dr. [redacted]s a very effective mentor and supportive Department Chair. Furthermore, because Dr. [redacted] and the Candidate share the same clinical specialty in Shoulder Disorders, Dr. [redacted] and the Candidate meet on a daily basis. Dr. Blaine will teach the Candidate about mechanisms of shoulder inflammation, cytokines and chemokines associated with shoulder research, as well as mentor him in career development. Most importantly, it is very fortunate for Dr. Kovacevic to have a clinician mentor, Dr. [redacted], to provide immediate access to well-aligned academic and clinical infrastructure.

Expected Role: Dr. [redacted] is the Chair of the Department of Orthopaedics. He coordinates the activities of research faculty members in order to provide comprehensive multi-disciplinary expertise within the close

proximity of the Department. He will secure 50% protected time for the Candidate as per NOT-AR-16-012. As Departmental Chair, Co-Mentor, and Clinician Scientist, Dr. [redacted] is an ideal senior clinical partner who can fully promote Dr. [redacted]'s career in the fields of both clinical and basic science research. Dr. Blaine will serve as an established mentor in the field of translational shoulder research while providing instruction and expertise regarding the role of CXCR4 inhibition on tendon injury and rotator cuff healing. Additionally, [redacted], the Primary Mentor, who has been successful as an NIH-funded orthopaedic surgeon scientist and a long-term collaborator, in order to foster the career development of talented junior faculty members such as Dr. Kovacevic.

[redacted], Ph.D. (Co-Mentor)
Qualifications: Dr. [redacted] is a Tenured Professor and the Vice Chair of Research at the Department of Orthopaedics and Rehabilitation at the Yale University School of Medicine. His research in immunology and bone biology has been funded by NIAMS for more than 2 decades. Additionally, he strongly supports the translational research conducted by collaborating orthopaedic surgeons. He will teach the Candidate about mechanistic signal transduction, fundamental laboratory techniques such as PCR, immunoblotting, genetic models of human diseases, and immunologic assays, as well as the responsible conduct of research. He has been the leading organizer of the International Osteoimmunology Conference for the past 10 years.
Expected Role: Dr. [redacted] is the Vice Chair for Research in the Department of Orthopaedic Surgery. He is a bone immunologist, stem cell biologist, educator, and mentor. Dr. [redacted] successfully transformed his prior postdoctoral fellow into an independent investigator through the NIDDK K99/R00 Award Mechanism. He will provide his expertise in inflammation, chemokines, cytokines, immunology, and stem cells.

[redacted], M.D. (Co-Mentor)
Qualifications: Dr. [redacted] is a Professor of Pediatrics and Orthopaedic Surgery at the Yale University School of Medicine. His laboratory is located in the same section as those of Drs. [redacted] is a world-renowned bone biologist and pediatric endocrinologist. He was previously the Director of the NIAMS P50 Center of Orthopaedic Translation (CORT) at the Yale School of Medicine. He is an expert in FGF-23 and his mouse models exhibit abnormal enthesis (Liang G et al. Survey of the enthesopathy of X-linked hypophosphatemia and its characterization in Hyp mice. Calcif Tissue Int. 2009 Sep;85(3):235-46.). Dr. [redacted] will teach the candidate about clinical bone biology, growth factors, bone & cartilage metabolism, academic promotion, and the responsible conduct of research.
Expected Role: Dr. [redacted] is an exceptional clinician scientist as evidenced by his pivotal clinical trial using FGF23 to treat hypophosphotamic rickets. FGF23 is secreted by osteocytes and promotes secretion of phosphate in the kidney. Nowadays, this is basic science knowledge. He translated this finding into clinical trials through NIAMS P50 CORT and other funding sources. He has trained several pediatric endocrinologists into clinician scientists. He will provide his expertise in clinical bone and cartilage biology and Phase I/II/III Clinical Trials, which are the emblem of the ultimate triumph of successful translational research.

Steven Tommasini, Ph.D. (Collaborator)
Qualifications: Dr. [redacted] is an Assistant Professor of Orthopaedic Surgery and Biomedical Engineering at Yale University. He is an expert in biomechanics. Dr. [redacted] will collaborate with Dr. [redacted] in biomechanical testing of tendon substance and enthesis. He will share his knowledge and skills in biomechanics and biomedical engineering with Dr. [redacted]. Dr. [redacted]'s laboratory is located in the same section as the aforementioned Orthopaedic investigators.
Expected Role: Dr. [redacted] is a biomedical engineer with expertise in biomechanics and tissue engineering. He has recently been collaborating with Dr. [redacted] on tensile properties of tendons. Dr. [redacted] will provide his expertise in material testing, jig design, stiffness/strength/energy absorption density, mathematical modeling, tendon fiber structural analysis, computer interfaces, and biostatistics.

[redacted], Ph.D. (Collaborator):
Qualifications: Dr. [redacted] is an Assistant Professor of Orthopaedic Surgery at the Yale University School of Medicine. He is a developmental bone & cartilage biologist and molecular biologist. He conducts highly mechanistic experiments using genetically altered mice that show abnormal bone and cartilage phenotypes. Dr. [redacted] will assist in sample preparation and interpretation of data as it relates to the

fibrocartilage-bone junction. Dr. [redacted] will share his expertise, knowledge, and skills with the Candidate.
Expected Role: Dr. [redacted] will provide essential knowledge of signal transduction and mechanistic experiments using gain/loss-of function genetic manipulation of mice so that Dr. [redacted] will implement more mechanistic research elements in his future independent projects. Dr. [redacted] will provide scientific perspectives on tendon insertion sites that have a complex transition from tendon to fibrocartilage to bone.

[redacted]**, Ph.D. (Consultant/ Long-Term Collaborator since 2012/External Mentor;)**:
Qualifications: Dr. [redacted] is an Assistant Professor of Orthopaedic Surgery and Molecular & Integrative Physiology at the University of Michigan. He has been collaborating with the Candidate on enhancement of enthesis healing for a number of years. Due to successful ongoing collaboration, Dr. [redacted] will continue collaborating with the candidate as a consultant in order to ensure successful tendon research and training. The candidate will communicate with Dr. [redacted] via e-mail, Skype, or on-site visits. Additionally, Dr. [redacted] attends annual meetings of the Orthopaedic Research Society.
Expected Role: Dr. [redacted] has been a collaborator and external consultant for Dr. [redacted] during past and ongoing research projects. Dr. [redacted] has been assisting Dr. [redacted] in conducting more comprehensive analysis of muscle-tendon-bone units by adding knowledge on muscle atrophy and fat infiltration, which are very common symptoms following chronic rotator cuff tears. Dr. [redacted] will continue providing expertise in tendon biomechanics in addition to our in-house expertise provided by Dr. [redacted]i.
3. Assessment of the candidate's potential, strengths and areas needing improvement
Our committee members identified Dr. [redacted] as the most qualified candidate based on his passion and commitment to scholarly research (**Please refer to Biosketches**). The department's infrastructure, resources, and institutional commitment are ready to support Dr. [redacted]'s career development. Dr. [redacted] has expertise in animal surgery models, translation of scientific findings into clinical applications, scientific analysis of diseased human specimens, design of clinically relevant experiments, and credentials, with early publication records in a focused area of tendon and shoulder research among more than 20 publications. Dr. [redacted] received numerous awards. Most importantly, Dr. [redacted] is surrounded by the most ideal institutional environment and mentoring committee members. Our committee felt that Dr. [redacted] would benefit from additional multidisciplinary training in the following areas;

1) **Responsible Conduct of Research (RCR)**: Dr. [redacted] will take in-person and web-based courses provided by the Yale School of Medicine (IMED 630: Ethical Issues in Biomedical Research).
2) **Rigor and Transparency**: Our Mentoring and Research Support Committee will provide guidance and feedback for each experiment.
3) **Biostatistics**: Our Research Core Curriculum and Committee (Dr. [redacted]) will provide basic knowledge and skills in selecting and conducting relevant biostatistical testing. Commonly used statistics programs such as SAS and Multitab will be available for candidates to assess sample size, study power, and suitability of analyses.
4) **Mechanistic Experimental Design and Tools**: Common patterns of orthopaedic literature are descriptive in nature. We will emphasize specific cause-and-effect relationships in each experiment so that Dr. Kovacevic can develop insight into the design of mechanistic experiments. The candidate will present his experimental designs and our committee will enhance the impact of Dr. [redacted]'s science by rigorously applying time-course, dose-dependency, loss-of-function, and gain-of-function experiments. Our committee will provide creative suggestions on the use of pharmacologic inhibitors, agonists, peptides, viral vectors, gene silencing, CRISPR/Cas-9, and genetically altered mice.
5) **Big Data Science**: Our committee and the Yale School of Medicine core service will provide information regarding the advantages and disadvantages of Next Generation Sequencing (NGS) and Proteomics. We will provide training for heat map clustering analysis, pathway analysis, R-program, and functional verification steps.
6) **Sensitivity, Specificity, False Positive, and False Negative Issues of Basic Laboratory Techniques**: Our committee will extensively share issues related to common laboratory techniques such as immunohistochemistry, immunoblotting, PCR, ELISA, biomechanical testing, bone histomorphometry, molecular optical imaging, optical strain analysis, microCT and functional outcome analysis.
7) **Transition into Scientific Independency & Grantsmanship**: Our committee members are experienced grant writers and CSR reviewers. We will provide necessary constructive criticism to enhance the Rigor and

Transparency of each proposal. The Yale University School of Medicine offers a regular course on the R01 grant application process as a part of the Postgraduate School of Medical Science. The candidate will attend Orthopaedic Research Society or U.S. Bone and Joint Decade Grant Writing Workshops. Dr. [redacted] will apply for NIAMS Limited Time R03 Grants for K08/23 Awardees (PAR-16-268) in 2018 and thereon. He will apply for an R01 grant in or around 2020. Given his drive, persistence, and qualifications, our Mentoring and Research Committee strongly feels that Dr. [redacted] will meet all requirements needed to reach scientific independence.

4. Mentor's description of the elements of the research career development activities and formal course work. Plans for monitoring and evaluating the career development awardee's progress toward independence

1) **Mentoring and Research Support Committee**: Our Department has core research faculties covering clinical, immunology, stem cell, biomechanics, and orthopaedic translational research. Our committee members and collaborators who will provide research training, support, and career development are described in Section 2. We will ensure that the candidate gets sufficient support and guidance in order to become an independent investigator. Our Mentoring Committee Members are seasoned investigators with sustained research funding. We will guide Dr. [redacted] to apply for R03 (Limited R03 for K Award Recipients) and R01 grants. Revision (Preparation of A1 Proposals) is a very critical element because the success rate for A0 is 3 times lower than for A1. However, the success rate of R01 funding for K Award recipients is in the range of 40%. We will specifically monitor Dr. [redacted]'s progress by reviewing quality of data, grant submissions, and publications of high-quality manuscripts. 5-year time-line is summarized in **Table 1** of the **Candidate's Career Goal** section.

2. **Institutional Courses, Seminars, and Lectures**: The Yale University School of Medicine offers several programs that augment the transition of K Awardees into R01 Investigators. The major goals of the curriculum are to provide the rigorous training needed to ensure success in a career in biomedical investigation. The curriculum is designed to provide core training, but also sufficient flexibility, to meet the individualized needs of each early-state investigator. Pertinent courses for our Candidate are as follows;

i) IMED 630: Ethical Issues in Biomedical Research: This semester-length course addresses topics that are central to the conduct of biomedical research, including the ethics of clinical investigation, conflicts of interest, misconduct in research, data acquisition, and protection of research subjects. Practical sessions cover topics such as collaborations with industry, publication and peer review, responsible authorship, and mentoring relationships. Satisfactory completion of this course *fulfills the NIH requirement for training in* ***Responsible Conduct of Research.*** Format consists of lecture presentation followed by discussion.
ii) IMED 635: Directed Reading in Investigative Medicine: An independent study course in the Investigative Medicine program. Topics are chosen by the student for weekly meetings. Six sessions are required; dates/times by arrangement.
iii) IMED 645: Introduction to Biostatistics in Clinical Investigation: This course introduces statistical concepts and techniques commonly encountered in medical research. Previous coursework in statistics or experience with statistical packages are not a requirement. Topics to be discussed include study design, probability, comparing sample means and proportions, survival analysis, and sample size/power calculations. The computer lab will incorporate lecture content into practical application by introducing the statistical software package SPSS to describe; analyze data.
iv) IMED 670: Writing Your First Independent Investigator-initiated (R-type) Grant: Students will gain intensive, practical experience in evaluating and preparing grant proposals, including discussion of NIH study section format. The course is particularly designed to help investigators in the "K to R" transition period. The course is limited to students who plan to submit an R-type (eg, R01 or R-21), but also VA and Foundation grant proposals. Attendance and active participation are required. Course Director: Eugene Shapiro, M.D. Co-Director: David Fiellin, M.D.
v) IMED 680: Topics in Human Investigation: The human investigation course will teach Dr. Kovacevic about the process through which novel therapeutics are designed, clinically tested, and approved for human use. It is divided into two main components, with the first devoted to moving a chemical agent from the bench to the clinic, and the second to outlining the objectives and methods of conducting clinical trials during the FDA

approval process. The format will include background lectures with discussions, labs, and computer tutorials. Practical experience will also be part of these latter sessions, with opportunities for students to observe the Yale Human Investigations Committee and the enrollment of patients in clinical protocols at the Hospital Research Unit. In the final lectures, clinical trials and data analysis will be discussed in the context of the FDA new drug approval process.

3) External Meetings, Conferences, Workshops, Traveling Fellowships, External Organizations: The Mentoring and Research Committee Faculty Members are organizers and leaders of many scholarly research groups. The Candidate will attend the American Academy of Orthopaedic Surgeons Clinician Scholar Development Program of which faculty members are NIH-funded orthopaedic surgeons and basic scientists. Dr. Lee has been a course faculty since 2010 (Chair: 2011-2013). The Candidate will attend Gordon Research Workshops on musculoskeletal tissues or inflammation during the K08 award period. He will visit distinguished tendon biologists (Dr. [redacted], Ph.D.; Dr. [redacted], Mount Sinai School of Medicine; Dr. [redacted], Hospital for Special Surgery; Dr. [redacted], Cleveland Clinic) during the Award Period for face-to-face didactic sessions. Traveling expenses will be supplemented by the K08 Award and Departmental Academic Fund. In order to understand the intensity of research rigor of contemporary research peers, the Candidate will apply for the NIH CSR Early Career Reviewer (ECR) program. Our Mentoring and Research Support Members have been serving on SBSR, SBDD, and MTE Study Sections where fundamental musculoskeletal science proposals are reviewed. Our Committee Members will promote the Candidate by nominating him for various positions to serve as a committee member, moderator, reviewer, and presenter.

Research Plan Sample for K08 (Funded in 2017: Kindly Provided by Francis Lee, M.D./David Kovacevic, M.D.)

a) Significance

1. Clinical Premise: Inflammation and Rotator Cuff Disorders

More than 6 million out-patient visits are made to orthopaedic surgeons for shoulder pain each year, with rotator cuff tendonitis being the most common etiology. Rotator cuff tendinitis represents a spectrum of disorders of tendons, surrounding soft tissue structures, and bones, all of which cooperate to create inflammatory milieu. Inflammation (Latin: *inflammare*, to set on *fire*) is a complex biological response of tissues to harmful physical, biological, or chemical stimuli. As early as 2000 years ago, Celcius and Galen described cardinal signs of inflammation such as rubor (redness), calor (increased heat), tumor (swelling), dolor (pain), and function laesa (loss of function), all of which are present in rotator cuff disorders. Modern molecular and biological interpretations of these signs are related to cytokines, recruitment of inflammatory cells by chemokines, tissue destroying enzymes, angiogenesis, pain mediators, and impaired tissue regeneration by the inflammatory milieu [27,50]. Charles Neer, a pioneer of modern shoulder surgery, defined subacromial impingement syndrome (also known as rotator cuff tendonitis) in a classic paper in 1972, where he noted three stages of impingement: 1) edema and hemorrhage, 2) fibrosis and tendonitis, and 3) spur formation and tendon rupture. Others later implicated acromial morphology and the presence of subacromial spur formation in the development of bursitis, rotator cuff tendonitis and rotator cuff tears [46,71]. This theory postulates that decreased space under the acromion leads to external or internal compression of the soft tissues (bursa, rotator cuff tendons) in the subacromial space, leading to *inflammation*. Recent investigations have helped to define the cellular and biochemical events that occur in the process of subacromial inflammation and rotator cuff syndrome. [7,50] Inflammatory mediators have been shown to cause cytokine-induced tendonitis [90]. Santavirta and coauthors found a preponderance of CD-2 and CD11b mononuclear cells in the subacromial bursa of patients with bursitis [81]. This means that somehow mononuclear inflammatory cells are recruited from local or systemic sources to the rotator cuff region. Similarly, Yanagisawa demonstrated increased expression of Vascular Endothelial Growth Factor in patients with impingement, implying chronic inflammation and increased vascularity [109] Soifer and colleagues demonstrated the presence of neurological elements in the subacromial space [87] and Gotoh related increased expression of substance P (a pain mediator) in the subacromial bursa with shoulder pain in patients with impingement [28]. This series of historical and recent findings forms clinical and scientific standards of targeting inflammatory pathways as a logical therapeutic avenue. Currently, nonsteroidal anti-inflammatory drugs (NSIADs), the most commonly used pharmacologic agents to treat inflammation, do not adequately relieve clinical symptoms and have significant side effects,

including peptic ulcer disease, cardiac toxicity, and inhibition of tendon healing. Local steroid injection is often referred to as 'a magic shot' by patients due to its dramatic short-term pain-relieving effects. However, it is associated with tendon degeneration, rupture, predisposition to infection, and impaired tendon repair. Furthermore, in the inflammatory milieu, surgical repair of rotator cuff tendons do not heal well. As a result, many technical modifications of suturing techniques have been tried without dramatic improvement in surgical outcomes. Therefore, new pharmacologic agents that specifically target the aforementioned inflammatory pathways would significantly advance the treatment of this common clinical problem.

2. Further Scientific Premise: Screening of therapeutic targets and clinically relevant animal models

i) Inflammatory milieu: Screening experiments to identify novel mediators of shoulder inflammation

In 2015, President Obama and Vice President Biden declared a moon-shot program to conquer cancer. The fundamental premise of the plan involves targeted therapies and personalized medicine. The United States public has since become familiar with cytokines and kinases as every-day non-professional words. Over the past 10 years, Dr. [redacted] (Our NIAMS K08 Candidate) and mentors of our team have been working on identifying specific cytokine and kinase targets in the context of shoulder rotator cuff disorders, thereby providing an ideal institutional environment to undertake more mechanism-based experiments on shoulder inflammation. Studies in our laboratory showed that surgical specimens from patients with painful rotator cuff disorders contain common suspect cytokines or mediators such as IL-1, IL-6, TNF, and PGE2 (COX-2), which activate downstream pathways that are modified by NSAIDs or steroids (**Figure 2**) [8,10,50]. We then undertook more broad screening experiments by comparing 300 inflammatory genes in normal and symptomatic rotator cuff tissues. Normal tissues were obtained at the time of surgical resection of cancers in the humerus, while pathologic tissues were removed for debridement of inflamed tissues, both of which are common standards of care. The most striking difference we found that distinguishes inflammatory rotator cuff disorders from normal was the expression of stromal cell derived factor-1 alpha (SDF-1; also known as CXCL12) [50,56]. Chemokines derives from the fusion of Chemo-attractive + Cytokines play critical roles in inflammation, recruitment, and regeneration (**Figure 3**). Furthermore, ligand-receptor interactions are rigorously detailed, providing an ideal training research topic for orthopaedic surgeons who are most familiar with surgical techniques and descriptive studies. In the context of tendon healing, the existing literature gives controversial opinions of whether SDF-1/CXCR4/7 targeting is beneficial or harmful in terms of alleviating local inflammation or tendon healing [7,12,27,57,58,69,84].

ii) Clinically relevant animal models of chronic rotator cuff tendon rupture, inflammation, and repair:

Orthopaedic research frequently involves clinically relevant animal models. Despite excellent animal models using surgical techniques and outcome analysis, most results of published studies end up as descriptive in nature. Dr. [redacted] has a determined passion for translational shoulder research and has re-established such animal models after he completed pre-doctoral traini ng under Dr. Scott Rodeo, an exemplary clinician scientist, as well as Drs. Ianotti/Derwin/Mendias during residency. This animal model entails two surgeries (**Figures 1** and **4**). The first involves releasing the rotator cuff tendons to simulate tendon rupture and the resulting inflammatory environment. The second involves repair of the ruptured tendons after 4 weeks to simulate degeneration of tendon substance, muscle atrophy, fatty infiltration of rotator cuff muscles, and degeneration of the proximal humerus tendon insertion sites (foot prints) [4,30,31,33,34,53]. Concerning choice of animal species, age, sex and other biological variable, we described aforementioned issues in the Experiment sections.

3. Central hypothesis and training paradigms

Our proposal presents an ideal training environment for Dr. [redacted] because i) our team identified a new therapeutic target, an SDF-1/CXCR4/7 axis, in humans; ii) we re-established clinically relevant animal models that also validate the importance of SDF-1; iii) we comply with rigorous criteria regarding the age and sex of animals; and iv) we provide ample training opportunities for gain-/loss-of-functional experimental methods in animal models and *in vitro* (**Figures 3-6**). Based on our preliminary data, we posit a **central hypothesis** that **the proposed SDF1-CXCR4/7 targeting mitigates rotator cuff inflammation and promotes healing of the tendon repair around the shoulder joint**. It should be noted that we are using the SDF-1/CXCR4/7 axis as a training paradigm for thorough mechanistic experiments. We are aware that optimal inflammation is beneficial for rotator cuff healing while excessive prolonged inflammation is detrimental for healing. Once rigorous training is underway, we will start to formulate alternative hypotheses and test new Specific Aims accordingly in the format of NIAMS limited R03 and hypothesis-driven R01 grants.

b) Innovation

i) Conceptual and Therapeutic Innovation: Until recently, there has been little scientific advancement regarding the use of novel targeted or personalized therapies other than NSAIDs and corticosteroids in the nonoperative treatment of painful rotator cuff disorders. Corticosteroids, in addition to their systemic risks, may inhibit or impair rotator cuff tendon healing and cause infection, and limited use (less than three injections) is recommended. There is significant concern, however, about the use of oral NSAID agents because of high rates of gastrointestinal adverse events (conventional NSAIDs) and potential for cardiovascular risk (both conventional NSAIDs and COX-2-selective agents). Due to the important role of SDF-1 in other diseases such as HIV and cancer, clinical agents blocking the SDF-1 pathway are a realistic alternative to NSAIDs and corticosteroids for the treatment of shoulder inflammation. Several of these have already been developed and are currently in clinical trials for other indications (AMD3100, T140 and TN14003, CTCE-9908 (Chemokine Therapeutics Corp.,Vancouver, BC, Canada)). The proposed experiments will investigate the role of these new pharmacologic agents in the treatment of rotator cuff syndrome.

ii) A multi-disciplinary Mentoring and Research Support Committee: Our Mentoring and Research Support Committee consists of a diverse set of mentors including Dr. Francis Lee [7,8,14,50,55,56,68] (An Orthopaedic Clinician Scientist), [redacted]. Dr. [redacted] [2-4,11,30-35,52-54,89] the K08 Candidate, is positioned for outstanding research training by well-funded scholars at one of the best research-intensive institutions in the world.

c) Approach

Specific Aim 1. To determine whether SDF1-CXCR4 mediates infiltration of inflammatory cells, cytokine expression, and macrophage polarization at the site of tendon degeneration *in vitro*.

Overview: We have confirmed the expression of SDF-1 by the soft tissues at the site of tendon degeneration and painful impingement syndrome in human patients (**Figures 1** and **3**). We have verified expression of IL-1, IL-6, COX-2, and TNF (**Figure 2**). Therefore, IL-1 will be used as a surrogate simulation of rotator cuff inflammation in vitro, similar to in vitro simulation of inflammatory arthritis. We will examine diverse roles of the SDF-1/CXCR4/7 axis on inflammation of peritendon soft tissues, tendon substance, and tendon-peritendon soft tissue (bursal cell) interactions. In addition, we will conduct loss-of-function experiments using clinical-grade pharmacologic agents and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR-associated (Cas) genes. We will examine signaling pathways downstream of the SDF-1/CXCR4/7 interactions in the context of macrophage migration and M1/M2 polarization. Aim 1 will provide detailed training for cell signaling and functional experiments that have not been a part of prior research training for the candidate.

Rationale: Under the classical paradigm of impingement syndrome, supraspinatus tendon and adjacent soft tissues between the proximal humerus and acromion are squeezed together. This has recently lead to a "chicken-or-egg" discussion about whether inflammation starts first in the peri-tendon soft tissues or in the tendon itself. *First*, the "chicken" side of the story suggests that peri-tendon soft tissues including the so-called subacromion bursa get inflamed before tendons get inflamed. Inflamed peri-tendon tissues produce chemokines, cytokines, and recruit inflammatory cells that cause pain and weaken the tendon. *Second*, the "egg" side of the story is that damaged tendon expresses chemokines and cytokines that cause secondary inflammation in the peri-tendon soft tissues. A *third* theory postulates that both tendon and peri-tendon soft tissues are simultaneously affected by the impingement between the acromion and proximal humerus. These three possibilities are not mutually exclusive and it is difficult to prove which occurs in vivo. Therefore, *in vitro* simulation of inflammatory milieu between tendon and peri-tendon tissues is important. Concerning SDF-1, it has diverse functions such as chemotaxis, inflammation, mobilization of stem cells, mobilization of inflammatory cells, proliferation, and tissue repair among others (**Figure 6**). This means that our proposed experiments will add more than just an incremental gain of knowledge by shedding light on the roles of SDF-1 on tendons and peri-tendon soft tissues. We intend to focus on SDF-1 as a first phase of *in vitro* experimentation because unlike other chemokines, SDF-1 has a limited number of receptors, thereby providing

a straightforward training platform for Dr. [redacted] to learn about upstream and downstream signaling pathways. *We will investigate other chemokines or cytokines in future projects as needed*.
Preliminary Data: Our team has identified a dramatic increase of SDF-1 in subacromial bursal tissues obtained from patients with peri-tendon inflammation (so-called bursitis) and tendon rupture (**Figures 2,3,4,6**). CXCR4 positive inflammatory cells are present. The inflammatory soft tissue cells produced IL1, IL6 and TNF, of which expression was decreased by AMD3000, a well-known SDF-1/CXCR4 blocker ([redacted], 2005; 2006). However, there is a possibility of nonspecific targeting. Therefore, we adopted a gene editing strategy so that we can overcome the limitations of off-target effects of the pharmacologic inhibitors.

Experimental Design (Effects of the SDF-1/CXCR4/7 axis on rotator cuff inflammatory milieu)
1) Isolated culture and co-culture experiments:
Biological Variable (Clinically relevant age, sex, and species): As a part of Dr. [redacted]'s rigorous research training, we will use *both human and rat cells* prepared from supraspinatus tendon and peri-tendon soft tissues. Both male and female subjects will be used. Age range of the animals will correspond to the human age of 45-55 years, when painful shoulder disorders are most frequently seen. For rats, we will use both male and female rats of 18-24 months representing similar human ages. We chose rats because we used them to gain our preliminary data, they have similar anatomic structures to humans, and dimension of their tissues allows clinically relevant surgical techniques. Each sex will have *5 different donors* because our previous in vitro experiments showed statistical significance with sample sizes varying from 4-5 ($p<0.05$; post hoc study power≥0.8). We will use Sprague-Dawley rats because our preliminary data were generated with Sprague-Dawley rats and relevant literature extensively supports the use of this strain.
Controls and Experimental Groups (Inflammation & Inhibition of SDF-1/CXCR4/7): We will isolate cells from peri-tendon soft tissues and supraspinatus tendons that were debrided or resected as part of standard surgical care (shoulder reconstruction or cancer resection with normal rotator cuff and loose areolar tissues). Dr. [redacted] has extensive experience in tendon soft tissue cultures. Dr. Lee has extensive experience in primary cultures of human and animal musculoskeletal tissues as a part of his previous cancer and inflammation research. Dr. [redacted] has experience in culturing peri-tendon soft tissues and human rotator cuff tissue. Drs. [redacted] have extensive experience in culture of cells derived from endodermal, mesodermal, and ectodermal origin. All listed mentors and collaborators will train Dr. [redacted] with respect to aseptic harvesting of tissues, digestion, selection of culture media, primary culture, and subcultures. We will also conduct co-cultures of tenocytes and peri-tendon soft tissue cells by using double tray culture dishes in order to simulate the human disease condition. With respect to experiments using human cells, control (untreated) groups will consist of isolated normal tendon cultures, isolated normal peri-tendon soft tissues, and co-culture of normal tendon/peri-tendon soft tissue cells. Experimental groups will be isolated from inflamed debrided tendon cells and inflamed peri-tendon soft tissue cells. In order to have uniform inflammatory stimuli, additional normal control groups will be treated with IL-1 or SDF1 to represent controlled inflammatory events in response to a single most commonly used cytokine for inflammation research. In these groups, we will pre-stimulate with IL-1 to induce inflammation and then we will co-culture inflamed tendon cells with bursal cells or vice versa. We included SDF-1 because it may trigger an autocrine or paracrine loop of inflammation or anti-inflammation. We will treat aforementioned cells with AMD3000 (plerixafor, FDA Approved, distributed by Genzyme; a CXCR4 antagonist). We will conduct parallel experiments using 18-24-month-old Sprague-Dawley male and female rats respectively.
Time Course & Readouts: Time course experiments will be done to represent early, mid-term, and late time points. Cells will be prepared for mRNA extraction and protein lysates at 3 hours, 6 hours, 12 hours, 24, 48, and 72 hours. Expression of mRNAs for IL1, IL6, TNF, SDF-1, COX-2, and MMP1 will be our primary readouts because we verified aforementioned genes in human samples. We will examine expression of other mRNAs representing various cytokines and chemokines using a pre-assembled array system. These arrays contain 384 control and target genes that are pertinent to musculoskeletal inflammation and repair (RT^2 qPCR Arrays and Assays, www.Qiagen.com). Dr. Tommasini will assist Dr. Kovacevic regarding statistical validation for hierarchical clustering and heat map analysis (**Figure 7**). A phospho-protein kinase array will be used to screen for key kinases and transcription factors such as pERK1/2, STAT, Akt, NFkb, and NFATc1 that are reciprocally regulated by SDF-1 or AMD3000. We included these screening experiments so that Dr. [redacted] will learn candidate target vs. open-ended approaches for additional discovery opportunities. We will make sure that he is aware of the pros and cons of big data experiments. While the primary advantage of these methods lies in the ability to screen a large number of candidate molecules, delineating the specific function of

each candidate is prohibitively time consuming. Additional complexity arises from the potential for functional associations between candidate molecules and pathways.

Data Interpretation: We expect that SDF-1 itself stimulates cells in addition to its well-known chemotactic effects. Normal tendon or peri-tendon cells may show increased expression of various kinds of cytokines on candidate RT-PCR experiments as well as on gene arrays. We confirmed expression of SDF-1 in the peri-tendon soft tissues and recruited inflammatory cells in human pathologic specimens as well as in the rat animal experimental model, thereby making our proposal a strong translational research. Our co-culture experiments will determine whether activation of one side of the rotator cuff complex (i.e. tendon itself vs peri-tendon soft tissues) will trigger inflammation in the other counterpart.

Potential Problems, Alternative Approaches, and Future Direction: We are aware of the inherent limitation that in vitro experiments do not completely portray *in vivo* human pathology. However, co-culture experiments using tendon cells and peri-tendon soft tissues provide a unique opportunity to explore inflammatory interactions between tendons and bursal cells that cannot be readily examined in human patients. Regarding animal species, when we identify any downstream targets or other promising candidate molecules, we will consider using mouse tendons and soft tissues so that we can utilize systemic or scleraxis-promoter driven conditional knock out mice. We will use larger animals such as rabbits or sheep for preclinical experiments. Our mentors have used Next Generation Gene RNA Sequencing and Proteomics in the past. Although these techniques are fancy, functional validation takes very long time. These are likely not ideal first screening tools for a K08 candidate. However, we will use these techniques when there is a need. Regarding the choice of therapeutic-targeting agents, we will expand the experiments as needed. Throughout this experiment, we will examine the pathophysiologic significance of SDF-1/CXCR4-Kinase X?-Transcription Factor Y?-Cytokine / MMPs Z that will provide additional targets for personalized or precision shoulder therapeutics (**Figure 6**).

2) Inflamed Tendon or Peri-tendon cells recruit macrophages by secreting SDF-1

Rationale & Hypothesis: Our previous experiments showed that inflamed peri-tendon tissues express TNF, IL1, and IL-6, all of which are known to be Type I macrophage polarizers. Type I macrophages are polarized by classical pathways and are involved in adding more fire (pain, edema, pro-inflammatory cytokines, tissue destroying enzymes) while Type II macrophages provide soothing acute inflammatory responses toward a resolution stage. Macrophages in the tissue and monocytes in the peripheral blood have CXCR4, a specific receptor for SDF-1. We posit a **hypothesis** is that inflamed tendon cells or peri-tendon cells express SDF-1, recruit inflammatory macrophages, and triggers M1 polarization of macrophages.

Biological Variables and Rigor: We will use both human and rat tendon and peri-tendon cells as described in the previous experiments. For human monocytes, we will use two different sources. First, we will use well-established THP-1 cells (ATCC, MD) for reproducibility because many other investigators have used THP-1 cells extensively in the past. Second, we will isolate monocytes from the fresh whole blood packs that will be purchased from the American Red Cross blood bank. We will have five male and five female donors. Regarding rat monocytes, we will obtain monocytes from the bone marrow. Drs. Lee, Blaine and Horowitz have used both rat bone marrow and human peripheral blood monocytes in the past [50,79,82].

Experiments: We will conduct cell migration assays by co-culturing monocytes in the presence of normal or inflamed tendon cells or peri-tendon cells. We will quantify the migration of monocytes toward normal vs inflamed human tendon or peri-tendon cells (**Figure 8**). For rat tendon or peri-tendon cells, we will pre-stimulate them with IL-1, which has been identified in our human samples and was commonly used for inflammation research by other investigators. We will conduct parallel experiments using neutralization antibodies or AMD3000 in order to define SDF-1 as a major contributing chemotactic factor. We will quantify monocyte recruitment at 6, 12 and 24 hours. In addition, we will examine whether the SDF-1/CXCR4 signaling contributes to M1/M2 polarization of macrophages. In this regard, we will stimulate naïve human and rat macrophages with interferon gamma (for M1 Polarization) or IL-4 (for M2 polarization) in the presence or absence of supplemental SDF-1 (**Figures 4** and **9**). We will conduct RT-PCR and flowcytometry probing for M1 phenotypic markers (IL-1 beta, IL-6, IL-12, TNF, Interferon gamma, and MCP-1) and M2 phenotypic markers (IL-1 alpha, IL-10, TGF beta 1/3; VEGF, Dectin 1, CD206, CCL18). Samples will be collected at 1, 3, 6, 12, and 24 hours. Dr. Eswarakumar, a developmental bone biologist and immunologist by training, will teach Dr. Kovacevic with respect to PCR primer designing. Dr. Horowitz will teach Dr. Kovacevic techniques of flowcytometry.

Data Interpretation: Research by orthopaedic surgeons is often very descriptive. Many published papers list expression of cytokines or growth factors by various musculoskeletal tissues. Our Mentoring and Research

Support Team will participate in the analysis of whether SDF-1 is indeed important by aggravating inflammation at the site of tendon or peri-tendon soft tissue damage. Evidence on a role of the SDF-1/CXCR4 macrophage polarization in the musculoskeletal tissues has not been well elucidated. Based on our findings, Dr. Kovacevic will formulate new hypotheses.

Potential Problems, Alternative Approach, and Future Direction: If SDF-1 turns out to be a major chemotactic factor, SDF-1/CXCR4 blockers can be used for therapeutic intervention in human patients. For example, orthopaedic surgeons routinely inject steroids in the subacromial space of the shoulder joint. Instead of injecting steroid, future orthopaedic surgeons will inject specific SDF-1 blockers so that patients do not suffer from steroid-associated complications such as infection or tendon degeneration. If SDF-1 turns out to be less important than expected, we will conduct similar experiments by blocking other chemokines such as MCP-1, MIP-1 and other proteins that will be identified on RT-PCR Arrays. Concerning M!/M2 polarization, we are aware that M1/M2 polarization is a dynamic process. In vitro and in vivo M1/M2 polarization may be different. Dr. [redacted] will mentor Dr. Kovacevic regarding experimental limitations and alternative experiments. We are aware that IL-1 is not the sole pro-inflammatory stimulant in shoulder inflammation. We will use alternative ways of stimulating the tendon or peri-tendon tissues by mechanical abrasion, LPS, or TNF as deemed necessary.

3) Loss-of-Function using CRISPR/Cas-9 Gene Editing or Pharmacologic Inhibitors

Rationale and Hypothesis: For any clinical translation, rigorous verification of the specificity of targeting drugs is needed. For example, many small molecules have side off-target effects leading to the blockage of unwanted targets. To this end, we will verify the role of SDF-1 as a new contributor of shoulder inflammation by genetically deleting SDF-1 in human tendon or peri-tendon soft tissue cells. Genetic manipulation gene therapy is unrealistic in orthopaedic clinical practice. Therefore, the Mentoring team will teach Dr. [redacted] Gene Editing that has been introduced into biological research fields in the early 2010s. This tool will be complementary to loss-of-function experiments using short interfering RNAs or pharmacologic drugs. As the main goal of the NIAMS K08 Award is rigorous training, we will use a gene editing technique and a shoulder inflammation research protocol as the training platform. Second, small molecules or peptides have different bioavailability and potency. To this end, we will test different types of inhibitors such as AMD3100, T140, TN14003, and CTCE-9908 among more than 25 available SDF-1/CXCR4 targeting drugs. The primary learning objective is that bioavailability, migration inhibition efficiency, and side effects are all different. In this regard, Dr. Thomas Carpenter is a pioneer in translating FGF23 science into a novel therapeutic agent for hypophosphatemic rickets. As Medical Director of Yale CTSI/ Yale Center for Clinical Investigation, Dr. [redacted] regarding drug design and development. Lastly, we envision that we will use SDF-targeting drugs as a local agent. Therefore, we will use commonly used hydrogels that were extensively used by Drs. Lee and [redacted]. We will train Dr. [redacted] regarding release kinetics and functional assays for the successful development of locally delivered personalized shoulder anti-inflammatory agents.

CRISPR/Cas-9 Gene Editing: We will delete the SDF-1 gene in human tendon or peri-tendon cells. As a proof-of-concept and didactic demonstration, Dr. Lee deleted the MEK1 gene in human MDA231 breast cancer cells (**Figure 10**). Briefly, Le ntivirus CRISPR constructs targeting hMEK1 and hMEK2 were purchased from Genscript (Piscataway, NJ, USA). To generate knock out cell lines for each lentiCRISPRv2 construct, MDA-MB-231 cells were transfected with 10 μg of the respective lentiCRISPRv2 constructs along with lipofectamine 2000. Single cell clones were selected using 5μg/ml puromycin. MEK1 gene deletion was confirmed by immunoblotting. MEK1 is one of known SDF-1/CXCR4 downstream kinases. Dr. [redacted] will teach additional details of molecular biology techniques such as the use of vectors, target gene selection, and gRNAs. After deleting SDF-1 in tenocytes or peri-tendon soft tissue cells, we will conduct human monocyte migration assays. This experiment will overcome the limitations of off-target effects of small molecules.

Sustained Release and Functional Cell Migration Assay: Dr. Lee has used a gel-based delivery of growth factors in the past. Briefly, target drugs are suspended in 2.0 mL of Glycosil + Gelin-S. The hydrogel was formed by the addition of Extralink to the Glycosil + Gelin-S mix in a 1:4 volume ratio (0.5 mL Extralink to 2.0 mL Glycosil + Gelin-S) [15,40,51,75,88]. In order to examine the release mechanism, we took scanning electron microscopic pictures at various time points ranging from 30 minutes to 7 days. We observed the gel surface become more porous, which facilitates fluid transport between the inner gel and local environment (**Figure 11**). We will use SDF-1 as a testing protein because specific antibodies and ELISA techniques are well established. Once we load AMD3100, T140, TN14003, and CTCE-9908 in the gels, we will then conduct monocyte migration assay in the presence of SDF-1 or SDF-1 expressing inflamed cells in the counter part

wells in order to determine the efficacy and potency of AMD3100, T140, TN14003, and CTCE-9908-loaded gels. We will test 3 samples for each drug. We will conduct an ANOVA test to compare the potency of small molecules for statistical validation ($p<0.05$; study power≥ 0.8).

Data Interpretation, Potential Problems, Alternative Approach, and Future Direction: We expect to provide fundamental genetics training for Dr. Kovacevic by deleting SDF-1 completely with CRISPR/Cas-9. Regardless of the positive or negative results, we can apply time-course, dose-response, or loss-of-function experiments using CRISPR/Cas-9, shRNA, antibodies, or small molecules to different mechanistic research.

Specific Aim 2. To determine whether SDF-1/CXCR4/7 targeting enhances healing of torn rotator cuff tendons *in vivo*.

Rationale and Hypothesis: Therapeutic translational research involves pre-clinical animal trials that meet the standards of human clinical trials. Among many drugs, we have used AMD3000 for 8 years with reproducible success in vitro. AMD3000 is currently used in Phase II Clinical Trials (nih.clinicaltrials.gov). The goal of Specific Aim 2 is to train Dr. [redacted] with respect to clinically relevant translational research. We will modify drugs as needed. We will design randomized blinded rodent trials. Dr. [redacted] has established a clinically relevant chronic rotator cuff rat animal model at our institution. We will deliberately choose biological variables, age, sex, dose escalation, and readouts for future additional experiments. Concerning SDF-1, we are open to accept multiple effects on inflammation vs. tissue healing.

Biological Variable (Clinically relevant age, sex, and species): We will begin with rats because we can use the same surgical technique Dr. [redacted] uses in his current animal studies, consisting of repairing the rotator cuff with a drill-hole suture pass-through. We will use mice for genetic approaches or larger animals for preclinical studies. Regarding age and sex, we will consider using retired rat breeders of age 18-24 months, which corresponds to 45-55 years of human age and the time when inflammatory rotator cuff arthropathy occurs most commonly in male and female patients.

Control & Experimental Groups: We are going to conduct animal experiments to establish comprehensive control groups. These groups include various types of age-matched normals, sham surgery, SDF-1, tendon division without repair, tendon division with immediate repair, and tendon division with delayed repair. The goals are to understand the natural history and time course changes of cytokines, chemokines, tendon degeneration, repair failure, muscle atrophy, muscle fatty infiltration, foot print fibrocartilage degeneration, and subchondral bone osteopenia in the proximal humerus. Our current model involves an open exposure of the supraspinatus tendon and a physical perturbation of the peri-tendon soft tissues, including subacromial bursal tissues. Therefore, we will be able to determine whether inflammatory changes in the peri-tendon soft tissues cause subsequent tendon degeneration. Inflammatory changes invariably result in the recruitment and infiltration of macrophages. We will examine whether inhibition of SDF-1/CXCR4 interactions between rotator cuff cells and monocytes is beneficial or detrimental for the rotator cuff complex (muscle-tendon-foot print-subchondral metaphyseal bone). We will examine how surgical disruption and SDF-1 affect M1/M2 polarization and expression of cytokines by immunohistochemistry of the tissues and RT-PCR of mRNA extracts from the tissues. Concerning tendon repair (immediate or delayed), SDF-1 may play a dual role by either promoting or inhibiting the tendon repair process because stem cells express CXCR4 and locally delivered SDF-1 may recruit more reparative stem cells. We will examine muscle, foot print, and subchondral bone changes using special staining, micromechanical architectural analysis, microCT, and biomechanical testing. Our proposed experiments are poised to generate more than the anticipated cookbook-like results.

Inflammatory changes in rotator cuff disorders: Animal Models

Control Groups: The purpose of these groups is to provide time-course data on inflammation following the disruption of peri-tendon soft tissues or tear of the supraspinatus tendon in rodent animal models. In order to represent chronic rotator cuff tears and delayed surgical repair in human patients, we established a two-stage animal model in rats as follows. The procedure will be performed in the right shoulder. Male and female rats of 18-24 months of age will be randomized for different treatment groups. Randomization will be performed using a web-based software. Each group at each time point will consist of 6 rats for immunohistochemistry probing for inflammatory cytokines, macrophage makers, and M1/M2 markers. Time points will be 1, 2, 4, 6 and 8 weeks. We will include the primary repair group for comparison with the delayed repair group. At 4 weeks, delayed surgical repairs will be performed after re-opening the tendon rupture sites. The time-course changes following surgical repair will be examined at 1 week, 2 weeks, and 4 weeks following the delayed repairs. 8 rats in both the immediate repair and delayed repair groups will be designated for biomechanical testing.

SDF-1 or AMD3000 Intervention Groups: These groups represent either augmented or suppressed SDF-1 signaling in the supraspinatus regions after peri-tendon inflammation, tear of the supraspinatus tendon, or delayed surgical repair. Depending on the timing of SDF-1 signaling augmentation, there may be either increased inflammation or increased stem cell mobilization leading to a better quality repair because both macrophages and stem cells have CXCR4 receptors. Our proposed experiments will generate baseline data in defining a role of SDF-1/CXCR4 signaling in various settings of inflammation and rotator cuff tears. **Table 1** summarizes Control and Intervention Groups. In order to minimize repeated anesthesia and injections, we will use hydro-gel based delivery. Our previous experiments and published literature showed that the release of loaded drugs continues up to 5-7 days (**Figure 11**) [15,40,51,75,88].

Table 1. Control and Intervention Groups

Control & Intervention Groups		Time Points	Outcomes
Surgical Procedures	**Drug Interventions**	**Weeks**	**Comprehensive Analysis**
Control (Contralateral)	None	1*2* 4* 6* 8*	• Function (Gait, Active Motion, Appearance) • Immunohistochemistry (Inflammation & Stem Cells): CD14, CD45, CD68, SDF-1, CXCR4, CD105, RANKL, MCSF, TRAP (Osteoclasts) • Immunohistochemistry (M1/M2 Macrophages) -M1: IL-1 beta, IL-6, IL-12, TNF, Interferon gamma, & MCP-1) -M2: IL-1 alpha, IL-10, TGF beta 1/3; VEGF; Dectin 1, CD206, CCL18 • Muscle Examination: Fat, Atrophy • MicroCT: Resorption of Enthesis & Bone • Biomechanical Tensile Testing* • Enthesis: Safranin O; Type I/II/III/X Collagens
Bursitis Peri-Tendon Disruption Intact Tendon	Ia. Gel	1*2* 4* 6* 8*	
	Ib. SDF-1 Gel	1*2* 4* 6* 8*	
	Ic. AMD3000 Gel	1*2* 4* 6* 8*	
Tendon Laceration No Repair (Chronic Tear)	IIa. Gel	1*2* 4* 6* 8*	
	IIb. SDF-1 Gel	1*2* 4* 6* 8*	
	IIc. AMD3000 Gel	1*2* 4* 6* 8*	
Tendon Laceration Immediate Repair (Primary Repair)	IIIa. Gel	1*2* 4* 6* 8*	
	IIIb. SDF-1 Gel	1*2* 4* 6* 8*	
	IIIc. AMD3000 Gel	1*2* 4* 6* 8*	
Tendon Laceration Delayed Repair @4 weeks (Repair of Chronic Tear)	IVa. Gel	6* 8*	
	IVb. SDF-1 Gel	6* 8*	
	IVc. AMD3000 Gel	6* 8*	

Tendon Biomechanical Testing: Drs. Tommasini and [redacted]s will train Dr. [redacted] in planning and performing biomechanical testing. Cross sectional areas of the supraspinatus and infaspinatus tendons are measured near the attachment sites using microCT. Humeri are then potted in poly-methyl-methacrylate-filled brass tubes. Tests are performed on a uniaxial mechanical testing frame (Instron 5542, Norwood, MA) with an in-line 2kN load cell and custom fixtures and grips (**Figure 12**). Tests are performed for tensile loading first with 10 cycles of preconditioning to 5% strain at 0.1%/sec, followed by stress relaxation (100%/sec ramp, 5% strain hold for 300 sec), a period of rest (300 sec), and extension to 3N at a rate of 0.2%/sec. Strain is measured globally as grip-to-grip displacement relative to the initial gauge length for each tendon. The initial gauge length is measured using digital calipers. Stiffness and Young's modulus are calculated by measuring the slope of the linear region of force-length and stress-strain curves. Failure mechanisms are assessed. Maximum load, maximum stress, and toughness are calculated for the tendon.

Muscle & tendon histomorphologic analyses: Dr. [redacted] has extensive experience in muscle testing and will train Dr. [redacted] with respect to muscle testing [66-68]. Rotator cuff tears are associated with muscle atrophy and degeneration. Therefore, the analysis of shoulder muscles provides an important set of data. For muscle biomechanical testing, passive single-fiber and fiber-bundle muscle mechanics are tested as described in Dr. [redacted]' previous publications. With respect to tendon architecture remodeling, we will conduct optical reflection analysis (**Figure 13**).

Immunohistochemistry, RT-PCR, and Histology: Immunohistochemistry techniques were extensively used by [redacted] in their previous publications. In addition, RNA preparation and subsequent RT-PCR or in situ hybridization will be instructed by Drs. [redacted] as needed. Histologic preparation for enthesis was successfully done by Mentors (**Figures 6, 9,13**).

MicroCT: [redacted] regarding acquisition of data, analysis and interpretation.

Sample Size, Power Analysis, and Statistical Validation: Dr. [redacted]'s publications with Dr. [redacted] and existing literature suggest a sample size of 6 for immunohistologic examination for staining intensity changes and 7-8 specimens for biomechanical testing/microCT at $p \leq 0.05$, study power ≥ 0.8 and medium effect size 0.4-0.6 in order to detect 30-40% improvement in tensile strengths. Dr. [redacted] will conduct several *post hoc* power analyses as the pilot experiments progress. Sample size will be adjusted accordingly.

Data Interpretation, Potential Problems, Alternative Approach, and Future Direction: First, Groups Ia, IIa, and IIIa will provide time course changes of inflammation, tendon stiffness/strength, and repair efficacy following subacromial inflammation, tendon rupture, and primary repair. Group IVa will represent time-course

healing of delayed repair (repair of chronic rotator cuff tear). This will provide a set of fundamental data that Dr. Kovacevic will use for any type of therapeutic preclinical experiment. Concerning augmentation or inhibition of SDF-1 signaling, we expect beneficial effects of AMD3000 during the acute phase of inflammation. However, SDF-1 may or may not interfere with tendon repair depending on systemic recruitment of circulating stem cells or regional stem cells when it is applied 4 weeks after the injury. The interpretation of data will be exciting because a series of new hypotheses will be generated. Similar experimental approaches can be conducted to target other cytokines or chemokines. Likewise, other growth factors such as PDGF or Platelet Rich Plasma may be tested following a similar experimental paradigm. For genetic loss of function experiments, we will conduct experiments using knockout mice. For preclinical experiments, we will conduct large animal experiments using rabbits or sheep. Yale University School of Medicine

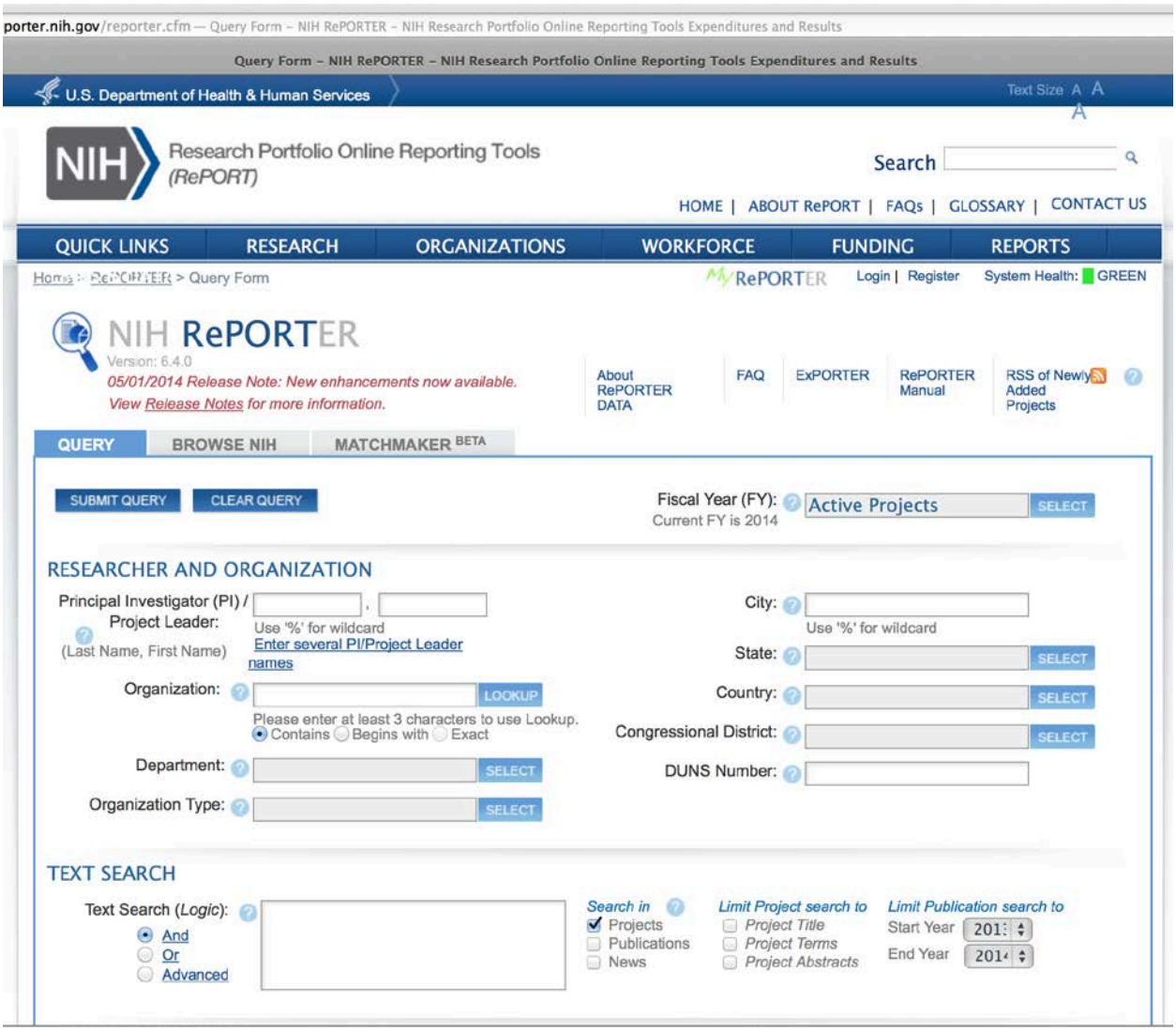
Other Resources

Funding Resources (Grant Types, Funding Agencies, Review Process, Grant Samples, Funded Projects)

NIH R01 Sample Grants:	http://www.niaid.nih.gov/researchfunding/grant/Documents/Ratnerfull.pdf
	(There are best samples of NIH-funded grants with perfect scores!)
NIH Grant Strategy:	http://www.niaid.nih.gov/researchfunding/grant/strategy/pages/default.aspx
Department of Defense:	http://cdmrp.army.mil/funding/prorp.shtml
OREF Grants:	http://www.oref.org/site/PageServer?pagename=grants_homepage
MTF Grant Page:	http://www.mtf.org/research_grant_programs.html
Clinical Trials Tutorial:	http://clinicaltrials.gov/ct2/show/NCT01631669
NIH RePORTER:	https://projectreporter.nih.gov/reporter.cfm

NIH RePORTER: *You can search abstracts of NIH-funded research projects and PI profiles. If you click topics or PI names, the website provides abstracts, PI names, and amount of grants. You may use this link to find internal and external collaborators and mentors. All NIH-funded project abstracts are public information.*

NIH Statistics:	http://report.nih.gov/success_rates/index.aspx
NIH Grant Reviewers:	http://public.csr.nih.gov/StudySections/Standing/Pages/default.aspx
	(You can find out members of the NIH Grant Review Panels. Example: SBSR, SBDD, MTE, and Other Orthopaedic Surgery-Related Reviewers)



NIAMS Paylines: http://www.niams.nih.gov/about_us/budget/funding_plan_fy2013.asp
NIH Grant Types: http://grants.nih.gov/grants/funding/funding_program.htm
NIH Grant Forms: http://grants.nih.gov/grants/forms.htm
NIH Success Rate: http://report.nih.gov/success_rates/index.aspx
NIH Grant Gossips: http://writedit.wordpress.com
VA System Grants: http://www.research.va.gov/funding/
Academic Promotion: http://sites.jhu.edu/council/transit
http://www.columbia.edu/cu/vpaa/docs/guideline.html
http://www.college.ucla.edu/personnel/handbook/asst-prof_e.htm
http://www.cumc.columbia.edu/dept/facultyaffairs/app_prom/officers_instruction.html
(List of Academic Titles & Promotion Tracks)
Others Grant Societies: http://www.rjos.org/web/awards/index.htm
http://ota.org/research/research-studies/
http://www.posna.org/research/awards/researchAward.asp
http://www.aofas.org/medical-community/Pages/Research-Grants.aspx
http://www.acsm.org/find-continuing-education/awards-grants/research-grants

Informal Bogs http://writedit.wordpress.com
(Gossips or information from many different investigators)