| | | |
|---|---|---|
| PI: | Title: Molecular basis of MED12 in the pathogenesis of uterine fibroids | |
| Received: 03/04/2016 | FOA: | Council: 10/2016 |
| Competition ID: FORMS-C | FOA Title: RESEARCH PROJECT GRANT (PARENT R01) | |
| | Dual: | Accession Number: |
| IPF: | Organization: | |
| Former Number: | Department: | |
| IRG/SRG: ICER | AIDS: N | Expedited: N |
| Subtotal Direct Costs<br>(excludes consortium F&A)<br>Year 1: 250,000<br>Year 2: 250,000<br>Year 3: 250,000<br>Year 4: 250,000<br>Year 5: 250,000 | Animals: Y<br>Humans: Y<br>Clinical Trial: N<br>Current HS Code: 30<br>HESC: N | New Investigator: N<br>Early Stage Investigator: N |
| | | |
| *Senior/Key Personnel:* | *Organization:* | *Role Category:* |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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

| 3. DATE RECEIVED BY STATE | State Application Identifier |
|---|---|
| | |

**1. TYPE OF SUBMISSION***

❍ Pre-application ● Application ❍ Changed/Corrected Application

**4.a. Federal Identifier**

**b. Agency Routing Number**

**c. Previous Grants.gov Tracking Number**

**2. DATE SUBMITTED**
2016-03-04

**Application Identifier**

**5. APPLICANT INFORMATION**

**Organizational DUNS*:**

Legal Name*:
Department:
Division:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
ZIP / Postal Code*:

Person to be contacted on matters involving this application

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

Position/Title:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
ZIP / Postal Code*:
Phone Number*: Fax Number: Email:

**6. EMPLOYER IDENTIFICATION NUMBER** *(EIN) or (TIN)**

**7. TYPE OF APPLICANT*** H: Public/State Controlled Institution of Higher Education

Other (Specify):

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

**8. TYPE OF APPLICATION***

❍ New ● Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).

❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

**Is this application being submitted to other agencies?*** ❍Yes ●No What other Agencies?

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

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

**11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT***
Molecular basis of MED12 in the pathogenesis of uterine fibroids

**12. PROPOSED PROJECT**
Start Date* Ending Date*
12/01/2016 11/30/2021

**13. CONGRESSIONAL DISTRICTS OF APPLICANT**

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



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

Prefix: First Name*: Middle Name: Last Name*: Suffix:
Position/Title:
Organization Name*:
Department:
Division:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
ZIP / Postal Code*:
Phone Number*: Fax Number: Email*:

**15. ESTIMATED PROJECT FUNDING**

| | |
|---|---|
| a. Total Federal Funds Requested* | $1,882,315.00 |
| b. Total Non-Federal Funds* | $0.00 |
| c. Total Federal & Non-Federal Funds* | $1,882,315.00 |
| d. Estimated Program Income* | $0.00 |

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

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

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

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

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

● I agree*

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

**18. SFLLL or OTHER EXPLANATORY DOCUMENTATION** File Name:

**19. AUTHORIZED REPRESENTATIVE**

Prefix: First Name*: Middle Name: Last Name*: Suffix:
Position/Title*:
Organization Name*:
Department:
Division:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
ZIP / Postal Code*:
Phone Number*: Fax Number: Email*:

**Signature of Authorized Representative***

**Date Signed***
03/04/2016

**20. PRE-APPLICATION** File Name:

**21. COVER LETTER ATTACHMENT** File Name:R01_HD087417_01A1_Cover_Letter1017715510.pdf

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

OMB Number: 4040-0010
Expiration Date: 06/30/2016

# Project/Performance Site Location(s)

**Project/Performance Site Primary Location**

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:

Duns Number:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:
Project/Performance Site Congressional District*:

---

**Project/Performance Site Location 1**

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:
DUNS Number:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:
Project/Performance Site Congressional District*:

---

File Name

**Additional Location(s)**





Funding Opportunity Number: PA-13-302. Received Date: 2016-03-04T09:02:52.000-05:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

# RESEARCH & RELATED Other Project Information

**1. Are Human Subjects Involved?*** ● **Yes** ○ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ● No

If YES, check appropriate exemption number: 1 2 3 4 5 6 If

NO, is the IRB review Pending? ● **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number 00005928

**2. Are Vertebrate Animals Used?*** ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number A3345-01

**3. Is proprietary/privileged information included in the application?*** ○ **Yes** ● **No**

**4.a. Does this project have an actual or potential impact - positive or negative - on the environment?*** ○ **Yes** ● **No**

4.b. If yes, please explain:

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

4.d. If yes, please explain:

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

5.a. If yes, please explain:

**6. Does this project involve activities outside the United States or partnership with international collaborators?*** ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

| | Filename |
|---|---|
| **7. Project Summary/Abstract*** | R01_HD087417_01A1_Summary_Abstract1017816810.pdf |
| **8. Project Narrative*** | R01_HD087417_01A1_Narrative1017715501.pdf |
| **9. Bibliography & References Cited** | BIBLIOGRAPHY_R01_HD087417_01A11017715594.pdf |
| **10.Facilities & Other Resources** | R01_HD087417_01A1_Facilities1017715502.pdf |
| **11.Equipment** | R01_HD087417_01A1_Equipment1017715503.pdf |
| **12. Other Attachments** | Authentication_of_Key_Resources_Plan1017715591.pdf |





Funding Opportunity Number: PA-13-302. Received Date: 2016-03-04T09:02:52.000-05:00

## PROJECT SUMMARY/ABSTRACT

Uterine leiomyomas (LM; fibroids) are monoclonal neoplasms of the myometrium (MM) and represent the most frequent tumors in women worldwide. Although benign, they nonetheless account for significant gynecologic and reproductive dysfunction. As no long-term non-invasive treatment option exists for LM, deeper insight regarding tumor etiology is key to the development of newer targeted therapies. Accordingly, this proposal is impactful as it suggests an etiologic basis for the predominant LM subtype and further offers proof of concept for therapeutic intervention involving new druggable targets in this specific genetic setting. LM arise from the genetic transformation of a single MM stem cell (SC) into a tumor initiating cell (LM SC) that seeds and sustains fibroid growth through asymmetric cell divisions. Heretofore, the dominant drivers of cell transformation have been largely identified. The most prevalent among these, accounting for ~70% of LM, are recurrent somatic mutations in the gene encoding the MED12 subunit of Mediator, a multiprotein signal processor through which regulatory information conveyed by gene-specific transcription factors is transduced to RNA polymerase II (Pol II). However, the impact of these mutations on MED12 function and the molecular basis for their tumorigenic potential remain unknown. Herein, we show that LM-linked mutations in MED12 disrupt its ability to activate Cyclin C (CycC)-dependent kinase 8 (CDK8) in Mediator, leading to reduced site-specific RNA Pol II phosphorylation and global gene dysregulation. We also identify genetic programs uniquely dysregulated in MED12-mutant fibroids, leading us to hypothesize that Mediator kinase disruption as a consequence of MED12 mutations elicits transcriptional reprogramming and altered signaling sufficient to drive MM SC transformation. We further hypothesize that MED12-mutant LM are therapeutically susceptible to reactivation of CDK8 or pharmacologic modulation of uniquely dysregulated signaling pathways. To test these hypotheses we will: (1) Establish the pathogenic role of Mediator kinase disruption in MED12-mutant LM. We will ask if genetic or chemical disruption of CDK8 (or its paralog CDK19) in Mediator can induce fibrotic transformation of MM SCs and, conversely, if WT MED12 can restore CDK8/19 kinase activity and suppress the fibrotic phenotype of MED12-mutant LM SCs; (2) Elucidate the pathogenic mechanism of Mediator kinase disruption in MED12-mutant LM. We will define the biochemical basis by which MED12 mutations disrupt CycC-CDK8/19 kinase activity and employ an integrated genome-scale approach to acquire the unique transcriptomic and epigenomic profiles of MED12 WT and mutant LM SCs; (3) Examine the therapeutic implications of Mediator kinase disruption in MED12-mutant LM. We will ask if reactivation of CDK8/19 or pharmacologic manipulation of signaling pathways uniquely dysregulated in MED12-mutant LM SCs can reverse their fibrotic phenotype. We expect these studies to significantly impact personalized treatment of women with LM.

## PROJECT NARRATIVE

Uterine leiomyomas (LM; fibroids) are the most frequent tumors in women worldwide and, although benign, are nonetheless associated with significant gynecologic and reproductive dysfunction. Current treatment options are limited beyond surgery, and the development of alternative effective medical therapies will require a better understanding of the underlying molecular etiology of LM. The studies proposed herein, focused on the etiological role of MED12, mutated in ~70% of uterine fibroids, address these looming issues and are therefore expected to significantly impact the treatment of women with this prevalent and clinically significant disease.

## FACILITIES AND OTHER RESOURCES (INCLUDING CLINICAL COLLABORATIVE RESEARCH PLAN)

### OFFICE

occupies a 140 square foot office located in the Research Facility on the campus of . The office is equipped with a Macintosh Pro desktop computer that is fully networked, along with a high-resolution 30-inch LCD flat panel monitor, an HP laserjet color printer and an HP Scanjet 5590 scanner. The services of an administrative assistant (shared by two other faculty members) and complete printing, FAXing, Xeroxing, and computer services are provided by the / . The administrative services of three additional assistants are available as needed.

### LABORATORY

The laboratory of occupies 1,270 square feet in the Research Facility. The laboratory is outfitted with state-of-the-art equipment sufficient to permit biochemical as well as cell and molecular biological analyses pertinent to the proposed studies. Equipment comprises: two 4°C cold boxes, an upright 4°C refrigerator, three -20°C freezers, two fume hoods, a standing New Brunswick Scientific Innova 4335 refrigerated incubator shaker, two IEC Centra CL3R table-top refrigerated centrifuge, two Eppendorf 5424R refrigerated microcentrifuge, six eppendorf 5418 microcentrifuges, two Diagenode Bioruptor 200-UCD sonication devices, six water baths, three shaking platform shakers, two Savant gel dryer and speed vacuum/concentrator systems, two pH meters, three heat blocks, power supplies, a top loading balance, an analytical balance, gel electrophoresis and immunoblotting equipment, one 1 Applied Biosystems 7500HT Real-time PCR machine, two MJ Research, Inc. PTC 200 programmable thermal controllers with gradient cycling capabilities, and one Molecular Dynamics Storm phosphorimager, one Luminoskan Ascent Luminometer, and one Thermoscientific Fluoroskan Ascent microplate fluorometer. Protein purification equipment includes Pharmacia AKTA Prime FPLC and AKTA Explorer protein purification systems and access to a Biorad programmable HPLC system and a Pharmacia SMART system. Computing facilities include one Mac Pro desktop, one iMac desktop, and one HP PC desktop, all of which are ethernet-connected to all other computers and printers in the building and to the main frame computer at the main campus providing internet access in addition to an HP Laserjet 4200dn printer. Both Apple computers are connected to digital scanners.

### SHARED CORE FACILITIES

A 4,000 square-foot common use core facility houses centralized cell culture facilities with laminar flow hoods and incubators, -80°C freezers, refrigerated incubators, high-speed and super-speed centrifuges, two darkrooms with automated X-ray film developers, UV spectrophotometers, a Kodak Gel Logic 2000 imaging work station, Biacore Model 2000, scintillations counters, two Molecular Dynamics Storm phosphorimaging systems, ABI real time PCR machines, Nanopure water systems, two cold rooms, and a warm room located immediately adjacent to the laboratory. In addition, the following core facilities are readily available and fully operational: (1) Equipment Core, including environmentally controlled rooms, centrifuges, freezers, incubators, and cell culture facilities; (2) Optical Imaging Core, including a Nikon N-Storm super resolution microscope system, a Nikon LiveScan Swept Field Confocal system, a Prairie Technologies Ultima Multiphoton Microscopy systems, a Nikon Eclipse TE2000 confocal microscope, a Zeiss Axioplan 2 compound immunofluorescence microscope, and a Nanoscope III atomic force microscope;; (3) Cell Biology Core, including FACS and FISH services; (4) Molecular Biology Core, including DNA sequence and oligonucleotide synthesis capabilities; (5) Transgenic Mouse Core; (6) Monoclonal Antibody Production Core; and (7) Proteomics Core that includes an Applied Biosystems Voyager DE-PRO Biospectrometry workstation consisting of a high performance integrated MALDI-TOF mass spectrometer and a SymBiot I Sample Workstation and a Thermoquest integrated LC/MS system consisting of a Surveyor HPLC coupled in line with a Finnigan LCQ DUO ion trap mass spectrometer with MS/MS capability.

### GENOME SEQUENCING FACILITY

The is housed within a 2000 square foot research space in the located adjacent to the on the campus. The includes several professional staff supervised by a , our collaborator on this application. This core facility contains six bench stations, standard equipment for a fully operational molecular biology lab, and provides equipment and expertise to promote a variety of functional genomics studies by using advanced technologies, notably next generation sequencing.

**Relevant equipment, includes, but it not limited to:**
- Illumina HiSeq 2000 Sequencing Systems
- Illumina MiSeq sequencing systems
- Illumina cBot Cluster Generation Station
- Covaris S220 Ultra Sonicator
- Thermo Scientific NanoDrop 2000
- Agilent 2100 Bioanalyzer
- Invitrogen Qubit 2.0 Fluorometer
- Beckman Coulter SPRIWorks Fragment Library System for Illumina Genome Analyzer
- Eppendorf Realplex Quantitative PCR and Eppendorf thermocyclers
- Caliper LabChip XT Fractionation System

Specifically, with the Illumina HiSeq 2000 systems and other necessary peripherals as listed above, the is capable of carrying out genome sequencing (including de novo and re-sequencing), transcriptome analysis (including RNA-seq and small RNA seq), WGBS, MeDIP-seq, MBD-seq, and ChIP-seq for genomic studies aiming to understand genetic variation, expression regulation and epigenetic modification. The Genome Sequencing Facility is also a research and development laboratory with experience in customizing experiments and developing new applications.

**LABORATORY ANIMAL RESOURCES FACILITIES**

A housing unit for mice with full AAALAC credentials is located on the campus. Animals will be housed in the facilities for laboratory animals provided by the under the direction of . The complies with the Animal Welfare Act (AWA), the Public Health Service Policy on Humane Care and Use of Laboratory Animals, the Guide for the Care and Use of Laboratory Animals, and the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training. The Health Science Center has an Animal Welfare Assurance on file with the NIH Office of Protection from Research Risks; the assurance number is . The is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) since 1974. In assuring that both the letter and the spirit of these regulatory requirements are met, the DLAR provides a contemporary program of Laboratory Animal Medicine and Care which includes:

- State-of-the-art husbandry, veterinary care, and management support of colony animals,
- Routine surveillance and quality assurance of vendor- and colony-produced animals,
- Clinical, gross, and histopathologic support of both clinical and research subjects,
- Extensive research support (assistance with experimental surgery, anesthesia, and recovery)
- Training in the handling, humane care, and manipulation of research animals,
- Consultation, collaboration, and professional assistance in animal model development,
- Monitoring of *in vivo* maintained tumor and hybridoma tissues, and
- Assisting in the review process of the Application for the Use of Laboratory Animals.

**COMPUTATIONAL BIOLOGY AND BIOINFORMATICS**

***Computational Biology Initiative***

The Computational Biology Initiative (CBI) is a joint computational facility located at the , which is 7.5 miles distance from the campus. CBI provides support and on-site training with workstations for students to access licensed bioinformatics tools such as MATLAB and GeneSpring. CBI is also serves as a portal for computation resources from the . Both and are members of the with access to this facility, one of the top 10 systems in the world with about 63 thousand processing cores and an outstanding computational biology educational program that we will try to host at (One of the Advisory Committee members, ).

***Advanced Computing Center***

A number of computational resources available to system investigators will be utilized to support this study including the . provides access to high performance

computing resources and large storage backup space. In addition, they offer regular training opportunities intended to provide life scientists with all the basic information and Linux/Unix skills necessary to effectively use array of Computational Biology resources for their research. Hands-on examples are heavily incorporated into the material, giving attendees experience working with systems directly. We plan to host such workshops locally in order to promoter the effective use of this resource. In addition, the System project is underway and progress is being made toward providing researchers at System institutions access to enhanced computer, storage, and networking infrastructure. Lonestar, a new resource began production on February 1, 2011 and is available to System researchers through .

is a . In addition to facility, , the , also has sufficient staff and faculty support to the educational program conducted at . Other than large memory Linux servers and two small Beowolf cluster, the have a collection of bioinformatics tools such as Ingenuity Pathway Analysis, Pathway Studio, Cytoscape, TRANSFAC database and other functional data analysis tools.

*Equipment*

*Computers dedicated to NGS pipeline*

The has acquired an IlluminaCompute dedicated to NGS pipeline. It contains 1 40URack with a High-performance cluster of 3 Dell R610 servers, Isilon IQ12000x storage modules with 30TB disk space, and dedicated Cisco 3750 network Switches. Illumina NGS data process pipeline is installed for data processing, genome alignment, and variation detection.

*Server Computers for Bioinformatics Tasks*

1x Linux server with 2x Quad-core Xeon 3GHz, 32GB RAM as dedicated MySQL database server
1x Linux large memory Server with 4x Quad-core Xeon 3GHz, 128GB RAM, for complex computation.
1x Linux Large Memory Server with 4x 10-core Xeon 2.2GH, 512GB RAM, on order.
1x Linux Cluster with 6 nodes (2x Dual-core Xeon per node) for software development
1x 30TB storage system dedicated to efforts in Next-generation sequencing support
1x 6TB disk storages for general research activity shared within .
1x 10Gig internet connection to University's central network and storage support, as well as to the system-wide support, including the facility.

*University-wide Service and Support to efforts*

*Network connectivity*: University provides enterprise network with Gig connection from to the entire campus.

*Storage Backup and Service*: Data Center is managing and servicing computer servers and data storage for the , including backup (weekly incremental and monthly full backup), additional data storage requests and handling. Also providing new equipment acquisition.

*Web hosting and virtualization*: websites are hosted at Data Center with virtual machines hosted on multiple Linux servers.

**CLINICAL COLLABORATIVE RESEARCH PLAN**

***Rationale:*** Studies proposed in Aims 1-3 of this application, including those designed to elucidate the requirement, mechanism, and therapeutic implications of Mediator kinase disruption in transcriptional reprogramming and fibrotic transformation of MED12-mutant LM, will rely on patient-derived myometrial and uterine leiomyoma primary cells and stem cells for both in vitro and in vivo (fibroid xenograft) analyses. For this purpose, collaborative interactions with local clinicians of pertinent expertise and established affiliations with area hospitals will be essential to the success of this project. In this regard, we are fortunate to have recruited as study collaborator (Collaboration letter included). is a noted clinician-scientist and leading expert in the fields of clinical infertility, assisted reproduction, reproductive endocrinology, and endometriosis. is a past , a former

, as well as a past
. As part of ongoing clinical practice in the , he maintains affiliations with local hospitals, including
. and his faculty perform hysterectomies/myomectomies (approximately 10:1 ratio) on more than 300 patients annually. As documented in his letter of support, will continue to provide us with myometrial and uterine leiomyoma tissues from informed consent patients undergoing such procedures, as well as histopathological confirmation of LM status.

***Design:*** In total, 120 patients are expected to participate in this study, which has already commenced following Institutional Review Board approval on November 24, 2014 ( ). Due to the characteristic biology of uterine fibroids, which are hormone responsive and thus relevant to reproductive age women, participating subjects will meet the following criteria for inclusion: premenopausal cancer-free women of unrestricted race/ethnicity who have received no hormonal treatments (including contraceptives or GnRH analogues) for 3 months prior to surgery. In addition, subjects undergoing immunomodulatory therapies or those with established gynecologic comorbidities, such as endometriosis, will be excluded. Beyond written informed consent, subjects will provide information (via questionnaire) concerning their reproductive history, parity, fibroid symptoms, hormonal use, last menses, and age. Additional clinical information collected by attending physicians related to patient biometrics, including BMI and menstrual phase (proliferative *vs* secretory), and clinicopathological features including fibroid type, location, size, and characteristics including tumor cellularity, matrix deposition, and vascularity will also be made available to the study PI. Subjects will be assigned a unique study number to permit de-identifiable coding. This code, and clinical data linked to each subject will be stored on private computer, which is University encrypted and password protected. Individually identifiable private information will be accessible only by the physician/surgeon ( ), while de-identifiable private information will be provided to as study PI.

We will devote considerable effort to ensure that our study cohort includes a precisely phenotyped patient population. In this regard, the use of autologous patient-matched tissues (i.e., myometrium and corresponding MED12 WT and MED12 mutant fibroids from the same patient uterus) would obviously reduce biological heterogeneity between sample tissues. Therefore, to minimize heterogeneity between samples, we will restrict our study analysis to patient-matched sample sets comprising myometrium, as well as MED12 WT and mutant fibroids from single patients. To minimize biological heterogeneity between patients, we will work closely with to ensure that study patients are matched as closely as possible for biometric and clinicopathological features, including age, race/ethnicity, BMI, menstrual phase (proliferative *vs* secretory), and endometrial histology. Finally, to reduce biological heterogeneity among tumors, we will strive to ensure uniformity with respect to fibroid location (intramural fundal) and size (4-8 cm).

Paired myometrial and leiomyoma tissues from patients will be harvested at the time of surgery for study purposes. Study team members from my laboratory, including and have been processed and approved for hospital privileges at and for purposes of collecting tissue at surgeries. Surgically recovered tissue will be transferred to maintenance media for subsequent dissociation and stem cell isolation, or aliquots immediately snap frozen in liquid nitrogen for subsequent RNA, DNA, and protein analyses as described in our research plan. Thus far, from a total of 38 race-diverse patients at, we have banked a total of 38 myometrium samples, 36 MED12 WT fibroids, and 67 MED12 mutant fibroids; some of these tissues have been used for isolation and characterization of stem cell populations as described in our accompanying research plan. We continue to accrue patient samples once or twice weekly from surgeries. Notably, although our patient cohort from is predominantly Hispanic (59% of the local population compared to 17% nationally), our preliminary studies indicate no significant difference in the frequency or spectrum of MED12 mutations found in fibroid tumors of Hispanic women compared to other racial or ethnic populations. In this regard, among 88 fibroid tumors thus far recovered and sequenced from 31 different Hispanic patients on site, 56 (or 64%) harbor MED12 mutations, the vast majority (81%) of which correspond to missense mutations in codon 44 (127). These data from Hispanic patients at corroborate those of two prior studies (128, 129) and support the conclusion based on numerous additional reports that MED12 mutation frequencies in uterine fibroids are similar in patients of diverse racial and ethnic origins (25, 27, 28, 32, 128, 129).

In addition to surgically recovered tissue, we will also have access, should we require it, to the Gynecology Biologic Specimen Repository and Associated Data Base. This biorepository, under the direction of , maintains stored patient-derived reproductive tract tissues including leiomyomas, endometriosis, eutropic endometrium, and malignancies as well as an associated database containing relevant clinical and follow-up information. As documented in his accompanying support letter, is committed to working closely with us, and this collaborative clinical research plan will be an integral component of this interaction. Our overlapping research interests, complementary expertise, and close proximity on the campus will significantly enhance the prospect of productive collaborative interactions between my research team and that of in pursuit of our study aims.

**EQUIPMENT**

laboratory is equipped with all of the necessary equipment for the proposed studies, including the following equipment, located in the , and can be accessed for the purposes of this study:

4 96-well thermocyclers
1 Eppendorf Mastercycler Gradient
1 Diagenode Model no.202162 Bioruptor
1 Agilent Technology G2545A Hybridization Oven
1 Applied Biosystems 7500HT Real-time PCR machines
2 MJ Research, Inc. PTC 200 programmable thermal controllers with gradient cycling capabilities
2 Diagenode Bioruptor 200-UCD sonication devices
1 Hydra 96-well microdispensor
1 TJ-25 high-speed centrifuge for 96-well microplates (Beckman-Coulter)
1 Savant speedvac for 96-well microplates
1 Regular speedvac
2 Spectrophotometer (NanoDrop) for nucleic acid and Cy dyes quantification
2 RAGE system for 96-well format gel electrophoresis
1 Hybridization ovens
4 Vertical tube Rotator
2 3-D Bidirectional Rotator
3 Horizontal platform shakers
6 Adjustable temperatue water baths
3 Matrix Impact$_2$ 12- and 16-channel pipetters
2 Savant gel dryer systems
2 UV light boxes
1 Zeiss Immunofluorescent Inverted Microscope
1 New Brunswick Scientific Innova 4335 refrigerated incubator shaker
2 Eppendorf 5424R refrigerated micrcentrifuge
6 eppendorf 5418 microcentrifuges
2 pH meters
2 IEC Centra CL3R table-top refrigerated centrifuge
1 Thermoscientific Luminoskan Ascent Luminometer
1 Thermoscientific Fluoroskan Ascent microplate fluorometer
2 Pharmacia AKTA Prime FPLCs
1 AKTA Explorer protein purification system
1 Biorad programmable HPLC system
1 Pharmacia SMART system
6 Regular freezers/refrigerators and -70$^{o}$C freezers
6 Regular horizontal and vertical gel electrophoresis apparatus for DNA, RNA, and protein separation
5 Cell culture incubators, Therma Scientific Forma 3110 CO2 adjustable; water jacketed, 6.5 cu. ft
1 Cell culture incubator, Therma Scientific Forma 3110 CO2/O2 adjustable; water jacketed, 6.5 cu. ft

is a . In addition to facility, , the and , also has sufficient staff and faculty support to the educational program conducted at . Other than large memory Linux servers and two small Beowolf cluster, the have a collection of bioinformatics tools such as Ingenuity Pathway Analysis, Pathway Studio, Cytoscape, TRANSFAC database and other functional data analysis tools.

***CBBI Equipment***

*Computers dedicated to NGS pipeline*

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dedicated Cisco 3750 network Switches. Illumina NGS data process pipeline is installed for data processing, genome alignment, and variation detection.

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1x Linux Large Memory Server with 4x 10-core Xeon 2.2GH, 512GB RAM, on order.
1x Linux Cluster with 6 nodes (2x Dual-core Xeon per node) for software development
1x 30TB storage system dedicated to CBBI's efforts in Next-generation sequencing support
1x 6TB disk storages for general research activity shared within .
1x 10Gig internet connection to University's central network and storage support, as well as to the system-wide support, including the facility.

*University-wide Service and Support to efforts*

*Network connectivity*: University provides enterprise network with Gig connection from to the entire

*and Service*: Data Center is managing and servicing computer servers and data storage for the , including backup (weekly incremental and monthly full backup), additional data storage requests and handling. Also providing new equipment acquisition.

*Web hosting and virtualization*: websites are hosted at Data Center with virtual machines hosted on multiple Linux servers.

***State-wide Service and Computing Systems***

is a part of Systems and provides access to high performance computing resources and large storage backup space. It has three powerful computing systems: Stampede (Dell Power Edge C8220 Cluster with Intel Xeon Phi coprocessors), Ranger (Sun Constellation Linux Cluster), and Lonestar (Dell Linux Cluster); as well as other storage and visualization servers.

Stampede system is a 10 PFLOPS (PF) Dell Linux Cluster based on 6,400+ Dell PowerEdge server nodes, each outfitted with 2 Intel Xeon E5 (Sandy Bridge) processors and an Intel Xeon Phi Coprocessor (MIC Architecture). The aggregate peak performance of the Xeon E5 processors is 2+PF, while the Xeon Phi processors deliver an additional aggregate peak performance of 7+PF. The system also includes a set of login nodes, large-memory nodes, graphics nodes (for both remote visualization and computation), and dual-coprocessor nodes. Additional nodes (not directly accessible to users) provide management and file system services.

The Lonestar Linux Cluster consists of 1,888 compute nodes, with two 6-Core processors per node, for a total of 22,656 cores. It is configured with 44 TB of total memory and 276TB of local disk space. The theoretical peak compute performance is 302 TFLOPS. In addition, for users interested in large memory, high throughput computing, Lonestar provides five nodes, six cores each, each with 1TB of memory. For visualization and GPU programming, Lonestar also provides eight GPU nodes each with 12 CPU cores. The system supports a 1 PB global, parallel file storage, managed by the Lustre file system. Nodes are interconnected with InfiniBand technology in a fat-tree topology with a 40Gbit/sec point-to-point bandwidth. A 10 PB capacity archival system is available for long-term storage and backups.

## AUTHENTICATION OF KEY RESOURCES PLAN

### BIOLOGICAL RESOURCES

1. **Patient-derived myometrial and fibroid tissues:** Uterine tissue samples used in this study derive from informed consent female patients undergoing routine hysterectomy/myomectomy for symptomatic fibroids at under the clinical care of our study collaborator, . and his clinical staff will authenticate surgically recovered myometrial and uterine fibroid tissues based on histopathological analysis. Due to the characteristic biology of uterine fibroids, which are hormone responsive and thus relevant to reproductive age women, participating subjects will meet the following criteria for inclusion: premenopausal cancer-free women of unrestricted race/ethnicity who have received no hormonal treatments (including contraceptives or GnRH analogues) for 3 months prior to surgery. In addition, subjects undergoing immunomodulatory therapies or those with established gynecologic comorbidities, such as endometriosis, will be excluded. Beyond written informed consent, subjects will provide information (via questionnaire) concerning their reproductive history, parity, fibroid symptoms, hormonal use, last menses, and age. Additional de-identified clinical information collected by attending physicians related to patient biometrics, including BMI and menstrual phase (proliferative *vs* secretory), and clinicopathological features including fibroid type, location, size, and characteristics including tumor cellularity, matrix deposition, and vascularity will also be collected and made available to the study PI. To minimize biological heterogeneity between patients, we will work closely with to ensure that study patients are matched as closely as possible for biometric and clinicopathological features, including age, race/ethnicity, BMI, menstrual phase (proliferative *vs* secretory), and endometrial histology. To reduce biological heterogeneity among tumors, we will strive to ensure uniformity with respect to fibroid location (intramural fundal) and size (4-8 cm).
2. **MM/LM SC lines:** Tissue-derived clonal SC lines at low-passage (between 2-4) will be used for study purposes. However, parental clones will be validated for mesechymal SC status at 6-month intervals upon retrieval from cryo-storage by the following methods: (1) chromosomal analysis; (2) FACs-based surface marker expression; (3) global gene expression profile by RNA-seq; (4) directed differentiation assays in culture to ensure multipotent potential; and (5) xenograft assays to ensure tumorigenic potential of LM SC clones. MM SC lines engineered by CRSPR/Cas9-mediated genome editing to express kinase-dead CDK8/19 or mutant MED12 (Aim 1A) as well as LM SC lines engineered by lentiviral-based transduction to ectopically express WT or mutant MED12 (Aim 1B) will be authenticated by: (1) genomic and cDNA sequencing to validate allele-specific mutation and expression; and (2) IP/Kinase assay to validate CDK8/19 activity status.
3. **Antibodies:** Commercial antibodies used for IP, immunoblot, IHC, and ChIP-seq will be vendor-authenticated and certified by product specification materials as well as investigator-validated by prior published studies. Nonetheless, we will endeavor to authenticate key antibodies in our own laboratory prior to use as follows: (1) Mediator subunit-specific antibodies will be authenticated by immunoblot and IP using recombinant Mediator subunits as well as Mediator subunit knockdown cell lines; (2) RNA Pol II total and phospho-CTD-specific antibodies will be authenticated by immunoblot and IP using recombinant site-specifically phosphorylated RNA Pol II large subunit and/or CTD peptides; (3) histone modification-specific antibodies (including point-source histone marks H3K27ac, H3K4me1) will be authenticated by immunoblot and IP using recombinant chemically modified histone tail peptides as well as cell lines treated with histone-specific siRNAs or chemical inhibitors of histone writers/erasers (i.e., HMTases, HDACs, KDMs).

### CHEMICAL RESOURCES

1. **CCT251545** (*Mediator kinase inhibitor*): Commercially available (ApexBio; Cat. No. B5979). This highly specific, potent, and orally bioavailable CDK8/19 inhibitor is vendor authenticated by certificate of analysis (COA). However, along with the Macromolecular Structure Core (MSC), we will independently authenticate chemical purity (>98%) by HPLC and compound structure by mass spectrometry.
2. **ICG-100** (*Wnt/β-catenin pathway inhibitor*): Commercially available (Selleck Chemicals; Cat. No. S22662) This small molecule inhibitor of the β-catenin/CBP coactivator interaction (required for β-catenin transactivation activity) is vendor authenticated by COA. However, along with the MSC, we will independently authenticate chemical purity (>99.75%) by HPLC and compound structure by NMR.
3. **Dexamethasone** (*glucocorticoid receptor agonist*): Commercially available (Selleck Chemicals; Cat. No. S1322) This synthetic glucocorticoid is vendor authenticated by COA. However, along witht the UTHSCA MSC we will independently authenticate chemical purity (>99.50%) by HPLC and compound structure by NMR.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

# RESEARCH & RELATED Senior/Key Person Profile (Expanded)

| PROFILE - Project Director/Principal Investigator | | | | |
|---|---|---|---|---|
| Prefix: | First Name*: | Middle Name | Last Name*: | Suffix: |
| Position/Title*: | | | | |
| Organization Name*: | | | | |
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| Country*: | | | | |
| Zip / Postal Code*: | | | | |
| Phone Number*: | Fax Number: | E-Mail*: | | |
| Credential, e.g., agency login: | | | | |
| Project Role*: PD/PI | | Other Project Role Category: | | |
| Degree Type: PhD | | Degree Year: 1990 | | |
| | | File Name | | |
| Attach Biographical Sketch*: | | | | |
| Attach Current & Pending Support: | | | | |





Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-04T09:02:52.000-05:00

| PROFILE - Senior/Key Person | | | | |
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| Prefix: | First Name*: | Middle Name | Last Name*: | Suffix: |
| Position/Title*: | | | | |
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| Country*: | | | | |
| Zip / Postal Code*: | | | | |
| Phone Number*: | Fax Number: | E-Mail*: | | |
| Credential, e.g., agency login: | | | | |
| Project Role*: Co-Investigator | | Other Project Role Category: | | |
| Degree Type: | | Degree Year: | | |
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| Attach Biographical Sketch*: | | | | |
| Attach Current & Pending Support: | | | | |

| PROFILE - Senior/Key Person | | | | |
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| Phone Number*: | Fax Number: | E-Mail*: | | |
| Credential, e.g., agency login: | | | | |
| Project Role*: Other (Specify) | | Other Project Role Category: | | |
| Degree Type: | | Degree Year: | | |
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| Attach Biographical Sketch*: | | | | |
| Attach Current & Pending Support: | | | | |

PROFILE - Senior/Key Person

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

Position/Title*:
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State*:
Province:
Country*:
Zip / Postal Code*:

Phone Number*: 3 Fax Number: E-Mail*:

Credential, e.g., agency login:

Project Role*: Other (Specify) Other Project Role Category: Collaborator

Degree Type: Degree Year:

File Name

Attach Biographical Sketch*:

Attach Current & Pending Support:

PROFILE - Senior/Key Person

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

Position/Title*:
Organization Name*:
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State*:
Province:
Country*:
Zip / Postal Code*:

Phone Number*: Fax Number: E-Mail*:

Credential, e.g., agency login:

Project Role*: Other (Specify) Other Project Role Category: Collaborator

Degree Type: Degree Year:

File Name

Attach Biographical Sketch*:

Attach Current & Pending Support:

PROFILE - Senior/Key Person

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

Position/Title*:
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State*:
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Country*:
Zip / Postal Code*:

Phone Number*: Fax Number: E-Mail*:

Credential, e.g., agency login:

Project Role*: Other (Specify) Other Project Role Category: Collaborator

Degree Type: PhD Degree Year: 2001

File Name

Attach Biographical Sketch*:

Attach Current & Pending Support:

PROFILE - Senior/Key Person

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

Position/Title*:
Organization Name*:
Department:
Division:
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Street2:
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Country*:
Zip / Postal Code*:

Phone Number*: Fax Number: E-Mail*:

Credential, e.g., agency login:

Project Role*: Other (Specify) Other Project Role Category: Collaborator

Degree Type: PhD Degree Year: 1995

File Name

Attach Biographical Sketch*:

Attach Current & Pending Support:





Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-04T09:02:52.000-05:00

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

## 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:

eRA COMMONS USER NAME:

POSITION TITLE:

EDUCATION/TRAINING

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | Completion Date MM/YYYY | FIELD OF STUDY |
|---|---|---|---|
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### A. PERSONAL STATEMENT

The long-term goal of my laboratory is to understand how fundamental mechanisms of eukaryotic transcription control are subverted during developmental diseases and cancer, with an emphasis on women's reproductive cancers. This objective conforms well to my training and expertise in the field of RNA polymerase II (Pol II) transcription. This training began in the laboratory of my mentor, , where I studied the genetic and biochemical requirements for expression of glycolytic enzymes linked to metabolic disease. As a postdoctoral fellow in the laboratory of , I refined my expertise in the biochemistry and genetics of the core Pol II apparatus. This work led to my seminal discovery of human Mediator, a conserved multisubunit signal-processor through which regulatory information conveyed by gene-specific transcription factors is transduced to Pol II. As an independent investigator, I have leveraged my basic knowledge in the biochemistry of Mediator to investigate how its dysfunction elicits developmental disease and tumorigenesis. In this regard, we discovered that somatic mutations in Mediator subunit 12 (MED12) responsible for ~70% of uterine fibroids disrupt Mediator-associated CDK8 activity leading to aberrant RNA polymerase II function genome-wide. The studies proposed herein aim to extend these novel findings and elucidate the molecular basis and therapeutic implications of Mediator kinase disruption in the pathogenesis of MED12-mutant fibroids. In addition to the intellectual and technological resources that my own research team brings to bear on this problem, I have recruited a world-class team of skilled collaborators who offer leading expertise in genetics, clinical pathology, and translational models of fibroids. In sum, the combined weight of my training, expertise, and effective leadership and communication skills render me ideally suited to successfully complete the proposed studies.

**B. POSITIONS AND HONORS**

**Professional Experience**:

**Professional Service:**

**Honors and Awards**

**C. CONTRIBUTION TO SCIENCE**

1. **BRCA1: Molecular basis for its breast tumor suppressor function**

   Our early studies aimed to understand how germline inactivation of the BReast CAncer susceptibility gene, *BRCA1*, confers a cumulative lifetime risk of breast (and ovarian) cancer. Our work contributed significantly to the current concept of BRCA1 as a cellular caretaker that ensures global genome stability by coupling DNA damage-induced signals to downstream responses, including DNA damage repair and cell cycle checkpoint activation. In this regard, we identified a novel role for BRCA1 as a sequence-specific transcriptional co-repressor of DNA damage-response genes and further linked disruption of this important function to its inherent tumor suppressor properties. We also identified a novel function for BRCA1 in direct repair of damaged DNA through a homologous recombination-independent pathway (non-homologous end-joining). Notably, our work further clarified the molecular basis for the tissue-specific tumor suppressor function of BRCA1, which cannot be adequately explained by its universal role as a genomic caretaker. Thus, we showed that BRCA1 suppresses estrogen signaling, and further linked this activity with its biological function as a breast and ovarian-specific tumor suppressor. Taken together, our early studies helped shape current paradigmatic views of BRCA1 as a ubiquitous genomic caretaker and tissue-restricted breast- and ovarian-specific tumor suppressor.

**2. Human Mediator: An integrative hub for signal-dependent gene regulation**

A central outstanding question in metazoan biology concerns the means by which developmental, environmental, or homeostatic signals are effectively coupled with precise gene expression output sufficient to specify cell fate and function. While the underlying mechanisms have not yet been completely elucidated, this process nonetheless depends to a large extent on intermediary activities that link signal-regulated and chromatin-bound transcription factors with the core RNA Polymerase II (Pol II) transcription machinery. Central among these so-called "co-regulators" is the multi-protein Mediator, originally discovered in the budding yeast *Saccharomyces cerevisiae*, and since found to be broadly conserved among eukaryotes. We were among the first to biochemically isolate and functionally characterize human Mediator, the first to implicate Mediator in metazoan development, and the first to ascribe a function for Mediator as an endpoint in signal transduction pathways. Our work has fundamentally advanced the concept of Mediator as an integrative hub through which regulatory information conveyed by signal-regulated transcription factors is transduced to Pol II. Furthermore, we have contributed significantly to the notion of Mediator as a sensor, integrator, and processor of developmental and oncogenic signals that converge on protein-coding gene promoters, with profound implications for development and disease.

**3. Mediator and human disease**

My laboratory has been at the forefront of efforts to understand how altered signaling as a consequence of genetic variation in Mediator triggers developmental disorders, neurodegenerative disease, and cancer. For example, landmark studies from my laboratory revealed that mutations in Mediator responsible for syndromic forms of X-linked intellectual disability disrupt proper epigenetic controls over neuronal gene expression and normal constraints on Sonic hedgehog signaling. More recently, we elucidated the functional impact and continue to investigate the molecular bases by which Mediator mutations drive the formation of multiple cancer types, including colorectal, prostate, and blood cancers. Together, these studies highlight the component nature and pathogenic vulnerability of Mediator and suggest new therapeutic strategies with possible applications across a range of human pathologies.

**Complete List of published work in My Bibliography**:

**D. RESEARCH SUPPORT**

**Current Research Support:**

**Completed Research Support:**

**BIOGRAPHICAL SKETCH**

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

| NAME | POSITION TITLE |
|---|---|
| eRA COMMONS USER NAME | |

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing,*

| INSTITUTION AND LOCATION | DEGREE | YEAR(s) | FIELD OF STUDY |
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**A. Personal Statement:**

As a practicing Gynecologist-Scientist, I have seen the national trend in which women are electing to start their families and plan pregnancy at a delayed age. All too often, these women are faced with the dilemma of addressing uterine fibroids (UFs)-related health problems while desiring to preserve potential fertility. For the past 19 years, I have been investigating the pathogenesis of UFs and its associated risk factors with a keen interest in this major health disparity disease that exhibits 3-4 times higher prevalence among African Americans and other women of color. I have been fervently exploring non-surgical fertility-preserving options to improve the quality of life for women who are afflicted or prone to this common disease. My ongoing research utilizes human tissues as well as preclinical models to understand the molecular pathogenesis of UFs and develop novel non-invasive therapeutic and preventative strategies. My extensive experience in translational and clinical research embodies more than 116 publications, 197 abstracts, and 17 clinical trials in the field of female reproductive health, specifically the area of UFs. In this current application, I am happy to collaborate with on his important work as to the role of Med12 mutations in human fibroid pathogenesis. We recently described the prevalence of Med12 mutations in up to 85% of all fibroid lesions in women from the United States ( ). As such, Med12 is currently the most relevant somatic mutation associated with this common women's health threat. I am particularly thrilled to serve as Co-Investigator. is the world authority on mediator biology and I remain confident that his team will lead the way in dissecting the exact role of Med12 mutation in fibroid pathogenesis. As Co-Investigator, I will provide access to our large collection of well characterized human fibroid and myometrial samples as well as matched primary and surface-marker specific stem cells ( ). I will also lend my expertise in *in vivo* fibroid animal experiments especially our humanoid mouse kidney capsule model, currently the most reliable model in the fibroid field ( ).

**B. Positions and Honors.**

**Positions and Employment**

## Selected Other Experience and Professional Memberships

## Selected Honors

**C. Contribution to Science (Selected from "116" Original Peer Reviewed Articles, "19" Books/Book chapters/Reviews/Case Reports, and "197" abstracts presented at Scientific Conferences)**

"My Biography" at NCBI

(1) **Molecular Origin and Stem Cell Biology of Uterine Fibroids** There is a collective agreement in the field currently that fibroid lesions originate in dysregulated myometrial stem cells after the emergence of a Med12 mutation. I led the team that first described the prevalence of Med12 mutation in women from the southern United States. I also led the team first to isolate and characterize surface maker specific human myometrial stem cells. I am pursuing this important line of investigations to evaluate the impact of obesity, inflammation, and early life exposures on developmental epigenetics of myometrial stem cells that eventually lead to the emergence of Med12 mutations and development of fibroid lesions. Understanding these formative steps will inform the development of novel, effective preventative and therapeutic strategies for this common women's health threat.

(2) **Why Uterine Fibroids are More Common in Blacks?** I have always been intrigued by the long-standing observation that uterine fibroids are at least 3-4 times more common in women of color. A few years ago, there was no plausible explanation for this finding. I led the team first to report the specific genetic polymorphisms in estrogen signaling (ER) or metabolizing (COMT) genes to support more estrogenic milieu in African American women. I also led the team that was first to connect low serum 25 hydroxy vitamin D3 levels to an increased risk of UFs. Since women (and men) of color are known to have a higher prevalence of hypovitaminosis D, this observation presents a plausible explanation for the ethnic disparity of this disease. This observation was subsequently confirmed by other groups around the world. I continue to pursue investigations of how low vitamin D levels enhance inflammation and lead to the development of UFs.

**(3) Developing targeted non-invasive Gene Therapy for Conservative Treatment of Uterine Fibroids** UFs continue to be a major health challenge for reproductive age women and their doctors. Due to a limited understanding of the molecular pathogenesis of the disease, there is currently no medical treatment for this disease and surgery (usually hysterectomy) is the mainstay. Clearly, this is not acceptable for women with symptomatic uterine fibroids who want to preserve their fertility potential. I led the team that first described adenovirus-based gene therapy as a localized non-invasive treatment option for UFs. This approach allows for the ablation of lesions while preserving uterine integrity without altering the patient's ability to ovulate and conceive. We further targeted this approach and developed an adenoviral vector that is fibroid-ON/normal tissue-OFF. We have validated this *in vitro*, Fibroid Eker rat model and in our unique humanoid mouse kidney capsule model in preparation for future human pilot studies.

(4) **Developing Novel Oral Agents for Treatment of Women with Symptomatic Uterine Fibroids** Pursuant to the development of a non-surgical treatment options for women with symptomatic uterine fibroids (item No. 3 above), I led the team first to demonstrate that COMT inhibitors are an effective therapy for uterine fibroids *in vitro* and in animal models. I also led the first clinical trial demonstrating the efficacy of EGCG [COMT inhibitor, green tea extract] in shrinking uterine fibroids by an average of 33% with significant improvement in symptoms and quality of life. I also led the team first to demonstrate that vitamin D receptor analogues (such as Vitamin D3, Paracalcitol) are effective and safe treatment options for UFs. These agents are used currently to treat women with UFs who are seeking a non-surgical treatment options.

**(5) Molecular Biology, Gene and Stem cell Therapy of Premature Ovarian Failure (POF)** POF is a devastating condition in which younger women prematurely enter menopause. These women are infertile and only have the option of egg donation from a healthy donor to be able to conceive albeit the child will not be biologically theirs. I have participated in the team that characterized first animal model (FORKO) of this condition. I have also led the team that was first to report on novel approaches, including gene therapy and stem cell therapy, to treat the POF phenotype in female FORKO mice. Our work open the door for innovative methods to reactivate folliculogenesis in POF patients. I am currently leading a multidisciplinary team to translate these findings into a pilot human clinical trial.

## C. Research Support

**ONGOING:**

**COMPLETED:**

**BIOGRAPHICAL SKETCH**

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NAME:

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

POSITION TITLE:

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 |
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**A. Personal Statement**

As a physician-scientist, I have been conducting both basic and clinical research in steroid-related aspects of human disease since 1991. I have collaborated with many groups of scientists over the past 20 years to study the roles of steroid hormone receptors, signaling pathways, and somatic stem cells in the pathogenesis and growth of uterine fibroids (leiomyomas). My combined experience, knowledge and expertise in the clinic and bench research will contribute substantially to this application.

**B. Positions and Honors**

**Positions and Employment:**

**Other Experience and Professional Memberships**

**Awards and Honors**

## C. Contribution to Science

I have made a significant global impact on medicine via pioneering contributions to the genetics and regulation of steroid production and action in human physiology and pathology including understudied areas of gynecology such as uterine fibroids and endometriosis, which impact >15 million people women in the U.S.

1. We isolated somatic stem cells from the uterine fibroids for the first time. These cells make up 1- 6 % of the entire population, but serve an indispensable role for fibroid tumorigenesis and progesterone-dependent growth. Targeting these cells effectively shrink or eradicate a fibroid tumor.

2. My group revolutionized our understanding of the epigenetic basis of endometriosis and single-handedly introduced aromatase inhibitors as an effective and novel class of drugs to treat it. I received a NIH-MERIT award for this exceptional achievement.

3. My team cloned the gain-of-function mutations of the aromatase gene in several families and thus discovered the genetic basis of the aromatase excess syndrome, which is characterized by estrogenization of pre-pubertal boys and girls, gynecomastia, premature thelarche, and short adult stature. I also defined the role of breast adipose tissue aromatase in the pathogenesis of breast cancer.

**D. Research Support**

**Ongoing Research Support**

**Ongoing Research Support**

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

## BIOGRAPHICAL SKETCH

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### A. PERSONAL STATEMENT

The overarching goal of research laboratory to unravel the molecular background of human tumor susceptibility, utilizing tools provided by the Human Genome Project and other recent advances in biosciences. The aim is to create information on human tumorigenesis, to facilitate cancer prevention, diagnosis, and treatment. More recently, has focused on the utilization of registry-based approaches to identify unique cancer family materials, for whole genome/exome sequence analysis and disease gene identification. In this regard and most pertinent to proposed studies, discovered the RNA Polymerase II Mediator Subunit *MED12* as a key mutational target found in ~70 of uterine leiomyomas, and has published extensively on the genetics/genomics of uterine fibroids. He is an expert in the acquisition and analysis of large-scale genomic an proteomic data using next generation sequencing methodologies and mass spectrometry, particularly in the realm of uterine fibroids. Furthermore, and directly related to application, was a key collaborator with on recent studies that identified the molecular basis of uterine leiomyoma-linked mutations in MED12, namely disruption of Mediator-associated CDK activity. is committed to continue this productive collaborative enterprise with , and he is committed to sharing his expertise in cancer genomics, along with his platform of next generation genomics- and proteomics-based technologies, in support of proposed studies.

## B. POSITIONS AND HONORS

### Positions

### Awards and Honors

## C. CONTRIBUTION TO SCIENCE

**1. Hereditary predisposition to colorectal cancer**

An historic focus of research has been hereditary colorectal cancer, where he contributed to several key discoveries, including identification of the loci, through linkage analysis, and later the

genes (MSH2, MLH1) responsible for Hereditary NonPolyposis Colorectal Cancer (HNPCC). has been at the forefront of efforts to identify and functionally characterize cancer susceptibility genes and oncogenes in familial colorectal cancer.

**2. Genetic analyses of tumor predisposition syndromes**

identified and characterized two novel tumor predisposition syndromes and their responsible genes: hereditary leiomyomatosis and renal cell cancer (HLRCC) and pituitary adenoma predisposition arising from germline mutations in the fumarate hydratase (FH) and aryl hydrocarbon receptor interacting protein (AIP), respectively. He also identified the gene responsible for the hereditary tumor predisposition syndrome Peutz-Jeghers syndrome (PJS).

**3. Tumor genetics and genomics**

More recently, has focused on the utilization of registry-based approaches to identify unique cancer family and other materials for whole genome/exome sequence analysis and disease gene identification.

## D. RESEARCH SUPPORT

### Ongoing Research Support

### Completed Research Support

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

**BIOGRAPHICAL SKETCH**

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| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | Completion Date MM/YYYY | FIELD OF STUDY |
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**A. Personal Statement**

is

He is Board certified in Ob/Gyn and in Reproductive Endocrinology and Infertility. He served as a and He has been a member of the . He served as and . He received the . He was elected to the in 1998. He served as in 1994 and in 1996. He served on the 1997-2000. He served on the 2002-2007 and was in 2005. He is for the . His clinical and basic research interests are endometriosis, mature women's health, assisted reproduction, and regenerative medicine. was the . experience as a clinician-scientist with a focus in women's reproductive health will add significant value to proposal. will provide with surgically excised myometrial and fibroid tissues recovered during hysterectomy/myomectomy of informed consent patients as well as pathological analyses to confirm fibroid status. This will effectively continue what has been an ongoing productive collaboration between and . Their overlapping research interests, complementary expertise, and immediate proximity on the campus will significantly enhance productive collaborative interactions important to advance the aims of proposal.

**B. Positions and Honors**

**Professional Positions**

**Honors**

**C. Contribution to Science**

The Women's Health Initiative (WHI) was a randomized controlled trial from September 1993-2002 in which 16,608 postmenopausal women aged 50 to 79 years with an intact uterus at baseline were recruited to 40 US clinical centers and followed up for an average of 5.6 years. I was the Principal Investigator for one of the US clinical centers where we enrolled 2,949 women of which approximately 30% were Hispanic. Clinical findings from this study have changed the way physicians approach the care of post-menopausal women and hormone replacement therapy.

Endometriosis is a common gynecologic disease affecting up to 10% of reproductive-age women. It is the third leading cause of gynecologic hospitalizations in the United States, a leading cause of hysterectomy in reproductive-age women, and responsible for $22 billion in U.S. health care costs annually. Despite the high prevalence and the severe symptoms associated with the disease, the pathogenesis of endometriosis is unclear. One widely accepted theory proposes that fragments of menstrual endometrium pass retrograde through the fallopian tubes, then attach and grow on peritoneal surfaces. Three lines of evidence support this theory: (1) retrograde menstruation is common as evidenced by the recovery of endometrial tissue from the

peritoneal fluid of women undergoing laparoscopy during menstruation (2) menstrual endometrium remains viable as evidenced by *in vivo* and *in vitro* studies and (3) release of menstrual endometrium into the pelvic cavity of non-human primates results in endometriosis. However, the factors involved in the adherence of endometrial cells to peritoneal surface mesothelium have not been identified until recently when we established the importance of endometrial cell CD 44 expression in the pathogenesis of endometriosis. Specifically, we reported that endometrial cells from women with endometriosis have greater ability to adhere to peritoneal mesothelial cells (PMCs) and overexpression CD44 splice variants when compared to women without endometriosis. These findings were confirmed in our recent study showing decreased development of experimental endometriotic lesions in CD44 knockout mice.

We evaluated the effect of daily low-dose follicular-phase cocaine administration on menstrual cyclicity, ovulation rates, corpus luteum function, and hormone levels in rhesus monkey. We determined through this study that Daily low-dose follicular-phase cocaine administration disrupts menstrual cyclicity and folliculogenesis. This effect is independent of weight loss, caloric intake, and basal gonadotropin levels. Cocaine exposure may have a persistent effect on menstrual and ovarian cyclicity in some monkeys.

**D. Research Support**

**Completed Research Support**

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

# 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:

eRA COMMONS USER NAME (agency login):

POSITION TITLE:

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

| INSTITUTION AND LOCATION | DEGREE (if applicable) | Completion Date MM/YYYY | FIELD OF STUDY |
|---|---|---|---|
| | | | |

## A. Personal Statement

Based on my extensive experience in next generation sequencing technologies and applications, I feel that I am highly qualified to supervise the utilization of Illumina HiSeq 3000 sequencers proposed in this grant application led by . I am in the at . I devote 100% time to sequencing operation of the . I hold a with more than 15 years of extensive research experience, and have authored or coauthored more than 30 peer-reviewed research articles. I was a research scientist as Functional Genomics Unit Leader in , where I started to use next generation sequencer from Roche 454 to Illumina Genome Analyzer and HiSeq platforms. Whiling leading the Functional Genomics team and managing large-scale next generation sequencing (NGS) activities of an NSF grant, I have acquired hands-on knowledge and expertise in high-throughput sequencing sample preparation, instrument operation, technology development, biological application, and associated basic bioinformatics studies. I was fascinated on the NGS technology development and applications, and decided to make a move to lead the new established in 2011. At the , I have been leveraging my experience toward establishing and managing internal and external collaborations in the strategic planning and execution of big and small sequencing projects and performed high-throughput sample preparation and large-scale sequencing (genome, transcriptome, exome, methylome, ChIP, small RNA, targeted genome). Under my leadership, With the data generated from the GSF, supported by the analytic and bioinformatics division at , The NGS has quickly become essential for support of many predominately NIH funded projects at , and the has contributed significantly to biomedical genomic research with numerous high impact publications. Overall, I have the knowledge, expertise, leadership, training, management skill and motivation necessary to successfully collaborate with on this innovative and fast-moving grant project.

## B. Positions and Honors

## Positions and Employment

**Other Experience and Professional Memberships**

**Honors**

## C. Contribution to Science

1. My early scientific investigations focused on the molecular mechanism of self-incompatibility, with Antirrhinum as study materials. Plant self-incompatibility has been known for at least two centuries and was viewed by the great biologist Darwin as “one of the most surprising facts which I have ever observed”. Understanding this unique cell-cell recognition system will enrich our understanding of the immune systems of plants, animals and humans, and also have huge potential usage for agricultural hybrid production. My graduate work demonstrated the first cloning of a pollen S gene in an S-RNase based self incompatibility system, and the first evidences that the ubiquitin pathway may involved in the response of self incompatibility. A patent related to the gene was filed and has been granted ( ). This work is regarded as a milestone in the field of self-incompatibility and has been widely cited by papers in Nature, Plant Cell, Trends in Immunology, Trends in Genetics, Trends in Plant Science, Annual Review of Plant Biology and others.

2. After my graduation, I joined laboratory for my postdoc training, where I focused on understanding the molecular genetic changes underlying plant speciation, adaptation and weediness in the Compositae, the largest and most economically important families of flowering plants, comprising one-tenth of all known flowering plant species. My research work has made substantial contribution to understand the role of chromosome rearrangement, the gene expression regulation and DNA polymorphism underlying the phenotypic variation we observed in the Compositae family.

3. When I took a position of working in the , I actively led and participated many genomic studies to generate permanent genomic resources for Compositae research community. I started to get more genomic experience from microarrays to high throughput sequencing, and was really excited to the molecular application with the cutting edge technology development.

4. With my current position as , I actively communicate with the principal investigators to set up the experimental design, protocol development and associated data analysis. I contributed in the experimental setup, data collection and analysis in some projects regarding to RNA-seq and rare cancer mutation identification.

**Complete List of Published Work in MyBibliography**:

## D. Research Support

## Ongoing Research Support

## Completed Research Support

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

# 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:

eRA COMMONS USER NAME (agency login):

POSITION TITLE:

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

| INSTITUTION AND LOCATION | DEGREE (if applicable) | Completion Date MM/YYYY | FIELD OF STUDY |
|---|---|---|---|
| | | | |

## A. Personal Statement

For this project, we will continue our active collaboration with lab in the , involving RNA-seq and ChIP-seq using the . We will also perform analytical, functional and statistical analysis for studying the molecular basis of MED12 in the pathogenesis of uterine fibroids. Currently I am the director of the computational biology and bioinformatics program at , and . Prior to joining the , I was a staff scientist at specializing in Bioinformatics, and (1996-2006), leading the bioinformatics efforts for microarray technology. I authored and co-authored about 140 peer-reviewed papers in journals such as , in the area of prostate tumor, melanoma, breast cancer and participated and lead the bioinformatics effort in hepatoblastoma (HB) research (funded by )), which has a higher incidence rate in . In addition, I am also the of the , and and providing campus-wise deep sequencing services and research collaboration.

## B. Positions and Honors

## Positions and Employment

## Other Experience and Professional Memberships

## Honors

## C. Contribution to Science

1. My earlier research work mainly focused on microarray data analysis, including the pioneering ratio statistics for gene expression measurement, multidimensional scaling for tumor sample clustering and classification since 1997. Since then, we have applied these methods in many cancer studies, such as melanoma, breast cancer, and hepatocellular carcinoma.

2. Recently, we have implemented and developed a set of analysis tools for next generation sequencing tasks to support our Genome Sequencing Facility. Particularly, tools for RNA-seq, mutation analysis, MBDCap-seq and ChIP-seq has been employed in many cancer studies and result in many peer-reviewed publications

3. We have applied our novel methodologies (differential expression, gene association network, gene set enrichment) to cancer studies, such as liver cancer.

4. We also pilot genetic regulatory and interaction network studies when gene-wide expression profiling technique become matured. From earlier model of co-determination network and to recent regulation by using competitive endogenous RNA (ceRNA) concept.

Complete List of Published Work in My Bibliography:

## D. Research Support

## Ongoing Research Support

## Completed Research Support

# PHS 398 Cover Page Supplement

OMB Number: 0925-0001

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

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

## 2. Human Subjects

Clinical Trial? ● No ○ Yes

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

## 3. Permission Statement*

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

● Yes ○ No

## 4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ○ Yes ● No

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

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





Funding Opportunity Number: PA-13-302. Received Date: 2016-03-04T09:02:52.000-05:00

# PHS 398 Cover Page Supplement

## 5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ❍ Yes

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

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

## 6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ❍ Yes ● No

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

Previously Reported*: ❍ Yes ❍ No

## 7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

# PHS 398 Modular Budget

OMB Number: 0925-0001
Expiration Date: 10/31/2018

## Budget Period: 1

Start Date: 12/01/2016 End Date: 11/30/2017

### A. Direct Costs

| | Funds Requested ($) |
|---|---|
| Direct Cost less Consortium Indirect (F&A)* | 250,000.00 |
| Consortium Indirect (F&A) | 6,449.00 |
| Total Direct Costs* | 256,449.00 |

### B. Indirect (F&A) Costs

| | Indirect (F&A) Type | Indirect (F&A) Rate (%) | Indirect (F&A) Base ($) | Funds Requested ($) |
|---|---|---|---|---|
| 1. | Modified Total Direct Cost _ On Campus | 52.50 | 242,449.00 | 127,286.00 |
| 2. | | | | |
| 3. | | | | |
| 4. | | | | |

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

Indirect (F&A) Rate Agreement Date 11/17/2014

Total Indirect (F&A) Costs 127,286.00

### C. Total Direct and Indirect (F&A) Costs (A + B)

Funds Requested ($) 383,735.00





Funding Opportunity Number: PA-13-302. Received Date: 2016-03-04T09:02:52.000-05:00

# PHS 398 Modular Budget

| Budget Period: 2 | | | |
|---|---|---|---|
| Start Date: 12/01/2017 End Date: 11/30/2018 | | | |
| **A. Direct Costs** | | | Funds Requested ($) |
| | | Direct Cost less Consortium Indirect (F&A)* | 250,000.00 |
| | | Consortium Indirect (F&A) | 6,449.00 |
| | | Total Direct Costs* | 256,449.00 |
| **B. Indirect (F&A) Costs** | | | |
| Indirect (F&A) Type | Indirect (F&A) Rate (%) | Indirect (F&A) Base ($) | Funds Requested ($) |
| 1. Modified Total Direct Cost _ On Campus | 52.50 | 229,747.00 | 120,617.00 |
| 2. | | | |
| 3. | | | |
| 4. | | | |
| Cognizant Agency (Agency Name, POC Name and Phone Number) | | | |
| Indirect (F&A) Rate Agreement Date 11/17/2014 | | Total Indirect (F&A) Costs | 120,617.00 |
| **C. Total Direct and Indirect (F&A) Costs (A + B)** | | Funds Requested ($) | 377,066.00 |





Funding Opportunity Number: PA-13-302. Received Date: 2016-03-04T09:02:52.000-05:00

# PHS 398 Modular Budget

Budget Period: 3

Start Date: 12/01/2018 End Date: 11/30/2019

## A. Direct Costs

| | Funds Requested ($) |
|---|---|
| Direct Cost less Consortium Indirect (F&A)* | 250,000.00 |
| Consortium Indirect (F&A) | 6,449.00 |
| Total Direct Costs* | 256,449.00 |

## B. Indirect (F&A) Costs

| | Indirect (F&A) Type | Indirect (F&A) Rate (%) | Indirect (F&A) Base ($) | Funds Requested ($) |
|---|---|---|---|---|
| 1. | Modified Total Direct Cost _ On Campus | 52.50 | 223,598.00 | 117,389.00 |
| 2. | | | | |
| 3. | | | | |
| 4. | | | | |

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

Indirect (F&A) Rate Agreement Date 11/17/2014

Total Indirect (F&A) Costs 117,389.00

## C. Total Direct and Indirect (F&A) Costs (A + B)

Funds Requested ($) 373,838.00





Funding Opportunity Number: PA-13-302. Received Date: 2016-03-04T09:02:52.000-05:00

# PHS 398 Modular Budget

Budget Period: 4

Start Date: 12/01/2019 End Date: 11/30/2020

## A. Direct Costs

| | Funds Requested ($) |
|---|---|
| Direct Cost less Consortium Indirect (F&A)* | 250,000.00 |
| Consortium Indirect (F&A) | 6,449.00 |
| Total Direct Costs* | 256,449.00 |

## B. Indirect (F&A) Costs

| | Indirect (F&A) Type | Indirect (F&A) Rate (%) | Indirect (F&A) Base ($) | Funds Requested ($) |
|---|---|---|---|---|
| 1. | Modified Total Direct Cost _ On Campus | 52.50 | 223,598.00 | 117,389.00 |
| 2. | | | | |
| 3. | | | | |
| 4. | | | | |

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

Indirect (F&A) Rate Agreement Date 11/17/2014

Total Indirect (F&A) Costs 117,389.00

## C. Total Direct and Indirect (F&A) Costs (A + B)

Funds Requested ($) 373,838.00





Funding Opportunity Number: PA-13-302. Received Date: 2016-03-04T09:02:52.000-05:00

# PHS 398 Modular Budget

Budget Period: 5

Start Date: 12/01/2020 End Date: 11/30/2021

## A. Direct Costs

| | Funds Requested ($) |
|---|---|
| Direct Cost less Consortium Indirect (F&A)* | 250,000.00 |
| Consortium Indirect (F&A) | 6,449.00 |
| Total Direct Costs* | 256,449.00 |

## B. Indirect (F&A) Costs

| | Indirect (F&A) Type | Indirect (F&A) Rate (%) | Indirect (F&A) Base ($) | Funds Requested ($) |
|---|---|---|---|---|
| 1. | Modified Total Direct Cost _ On Campus | 52.50 | 223,598.00 | 117,389.00 |
| 2. | | | | |
| 3. | | | | |
| 4. | | | | |

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

Indirect (F&A) Rate Agreement Date 11/17/2014

Total Indirect (F&A) Costs 117,389.00

## C. Total Direct and Indirect (F&A) Costs (A + B)

Funds Requested ($) 373,838.00





Funding Opportunity Number: PA-13-302. Received Date: 2016-03-04T09:02:52.000-05:00

# PHS 398 Modular Budget

| Cumulative Budget Information | |
|---|---|
| **1. Total Costs, Entire Project Period** | |
| Section A, Total Direct Cost less Consortium Indirect (F&A) for Entire Project Period ($) | 1,250,000.00 |
| Section A, Total Consortium Indirect (F&A) for Entire Project Period ($) | 32,245.00 |
| Section A, Total Direct Costs for Entire Project Period ($) | 1,282,245.00 |
| Section B, Total Indirect (F&A) Costs for Entire Project Period ($) | 600,070.00 |
| Section C, Total Direct and Indirect (F&A) Costs (A+B) for Entire Project Period ($) | 1,882,315.00 |
| **2. Budget Justifications** | |
| Personnel Justification | |
| Consortium Justification | |
| Additional Narrative Justification | |





Funding Opportunity Number: PA-13-302. Received Date: 2016-03-04T09:02:52.000-05:00

**PERSONNEL JUSTIFICATION**

**Principal Investigator, (2.4 calendar months)**, will oversee the proposed studies, provide guidance on the scope, implementation, and interpretation of experimental studies, closely monitor experimental progress, and coordinate publication of the results. The PI will also facilitate communication between study team members, Co-investigator , and collaborators

The PI, a , has extensive experience and a documented record of research expertise in cancer molecular biology and the biochemistry and molecular patho/biology of MED12/Mediator, particularly in the context of uterine fibroids. The PI is an expert in the biochemical, cell biological, and transgenic animal methods used in pursuit of these studies.

**Postdoctoral Fellow, (12 calendar months)**, will participate in studies pertaining to Aims 1B and 2B of this proposal. With respect to Aim 1B, will participate on studies designed to investigate the tumor suppressive potential of MED12 in LM. With regard to Aim 2B, will lead studies that include genome-level RNA-seq and ChIP-seq to investigate the mechanistic basis of altered transcriptional programming in MED12 WT and mutant LM stem cells (SC). is an expert in the function and regulation of Mediator, bioinformatics-based analyses of whole genome sequencing and gene expression data, and notably, the isolation, characterization, and manipulation of MM and LM SC. Pertinent to the proposed studies, made important discoveries in my laboratory regarding the identity of MED12-regulated genes through global expression profiling. Most recently, has taken the lead in our successful efforts to isolate and characterize uterine tissue-derived SC using the side population method as well as the Stro-$1^+$/$CD44^+$ cell surface marker-based enrichment protocol developed in the laboratory of our Co-investigator, . is an expert in basic and specialized biochemical, molecular, and cell biological methods necessary to conduct the proposed studies. is expected to continue her exceptional productivity toward completion of the study aims. Budgeted funds commensurate with her full-time effort are requested.

**Graduate Research Assistant, (6 calendar months; 100% of a 50% appointment for 12 months per year**), will participate in studies pertaining to Aims 2A and 2B of this proposal. For Aim 2A, will lead biochemical experiments to investigate the mechanism by which LM-linked exon 2 mutations in MED12 disrupt CycC-CDK8/19 kinase activity. For Aim 2B, will work with on studies to investigate the mechanistic basis of altered transcriptional programming in MED12 WT and mutant LM SC. is an exceptionally talented Ph.D. student who has excelled at both his didactic and practical training. He is well versed in the basic and specialized biochemical, molecular, and cell biological methods necessary to conduct the proposed studies. Pertinent to the proposed studies, he has made important discoveries concerning the impact of LM-linked mutations in MED12 on its CycC-CDK8 stimulatory activity, and he contributed prominently to our preliminary results in this regard. is expected to continue his excellent productivity and contribute significantly to completion of the proposed aims. Graduate students are appointed at 50% time for research work, and will devote his entire research-related effort to this project.

**Graduate Research Assistant, (6 calendar months; 100% of a 50% appointment for 12 months per year**), will participate in studies pertaining to Aims 1A and 3 of this proposal. For Aim 1A, will contribute to studies designed to investigate the tumorigenic potential of Mediator kinase disruption, including experiments involving characterization of genome-edited MM and LM SCs in both cell culture and transplantation xenograft models. For Aim 3, will lead studies to investigate the therapeutic susceptibility of mutant MED12-induced transcriptional reprogramming in fibrotic transformation and uterine leiomyomagenesis. She will investigate whether restoration of CDK8 function or modulation of Wnt/β-catenin and GR pathway reverse the fibrotic phenotype of MED12 mutant LM SC both in vitro and in tumor xenografts. is an outstanding Ph.D. student who has excelled in both her didactic and practical training. She is expected to contribute significantly as part of our team toward completion of the proposed

aims. Graduate students are appointed at 50% time for research work, and will devote her entire research-related effort to this project.

**Research Assistant, (9 calendar months),** will provide technical, general management, and administrative assistance to the PI and study team in support of all aims of the proposed project, including oversight of routine laboratory maintenance and operating procedures. has served with distinction as a highly valued and indispensable member of our study team for the past 10 years, and he is expected to continue his excellent service and productivity in pursuit of the proposed study aims. Budgeted funds commensurate with his 9-calendar month effort are requested.

**Other significant contributors/collaborators on this study are listed below. These individuals will contribute to the project as needed. No salary funds are requested for these individuals.**

**Collaborator, (as needed),** will serve as a collaborator on Aim 2 studies to investigate the pathogenic consequences of transcriptional dysregulaton and altered signaling arising from Mediator kinase disruption in MED12 mutant LM. is a at the . is an expert in the molecular genetics of human tumor susceptibility, with an emphasis on LM, hereditary colorectal cancer, hereditary leiomyomatosis and renal cell cancer, and pituitary adenoma. In this regard, has developed expertise in NGS technologies, including whole genome/exome sequencing as they relate to disease gene identification. Pertinent to the propose studies, laboratory discovered MED12 as a key mutational target in LM, and we have recently established a series of highly productive collaborations with the laboratory to investigate the molecular basis of MED12 in pathogenesis of uterine fibroids. In this regard, our early collaborative efforts have thus far produced several manuscripts [one published in ***Cell Reports*** (highlighted in ***Nature Reviews Cancer***) and another in ***Human Mutation***). will offer computational, biostatistic and bioinformatics-based support, as needed, pertinent to Aim 2 of the proposed studies, including genome-scale efforts to investigate the mechanistic basis of altered transcriptional programming in MED12 WT and mutant LM. We expect our ongoing fruitful collaborations with the laboratory to mature moving forward.

**Collaborator (as needed)** will serve as a collaborator on Aim 1 studies to examine the tumorigenic potential of Mediator kinase disruption and the requirement for MED12-dependent transcriptional reprogramming in fibrotic transformation. is the and , as well as , at is a noted clinician-scientist and leading expert in the areas of estrogen metabolism and hormone-dependent reproductive tract diseases, including breast cancer, uterine fibroids, and endometriosis. Pertinent to the proposed studies, is an expert in the isolation, maintenance, and characterization of human MM and LM SC. Furthermore, the laboratory has been at the forefront of efforts to establish and optimize a murine renal capsule xenograft model for fibroid tumor formation. As documented in his accompanying letter of support, has pledged to commit technical and conceptual support as required regarding the use of LM SC in culture and the mouse kidney capsule xenograft model. Notably, we have a documented record of prior productive collaboration with , strengthening our expectations for meaningful interactions in pursuit of the proposed studies. We anticipate continued productive collaborations with on this project.

**Collaborator (as needed)**, will collaborate on study Aims 1-3 including efforts to decipher the requirement, mechanism, and therapeutic susceptibility of Mediator kinase disruption and transcriptional reprogramming in the pathogenesis of MED12-mutant uterine fibroids. is . is a past and currently serves as a is an esteemed clinician-scientist and expert in the fields of clinical infertility, assisted reproduction, reproductive endocrinology, and endometriosis, including innovative stem cell research using primate models. As part of his clinical practice, has extensive experience in surgical interventions for uterine, endometrial, and other reproductive tract diseases,

including hysterectomy/myomectomy for symptomatic fibroids. As documented in both his accompanying letter of support and our clinical collaborative research plan (see Facilities section), will continue to provide MM and LM tissues recovered during routine hysterectomy/myomectomy as well as pathological analyses to confirm LM status. Our overlapping research interests, complementary expertise, and immediate proximity on the campus will significantly enhance the prospect of productive collaborative interaction between my research team and that of in pursuit of our study aims.

**Collaborator (as needed)**, will oversee all computational biological and biostatistical analyses of data derived from RNA-seq and ChIP-seq experiments relevant to Aims 1-3, including, but not limited to, alignment of sequence reads to the human genome, identification of uniquely mapping reads, binding peak detection, and DNA motif discovery analysis. is a P and is an expert in bioinformatics, computational modeling and biostatistics in the areas of gene expression, DNA copy number, SNP, and data analysis methods development. Under direction, the is at the forefront of developing computational intensive analysis tools at and establishing synergistic collaborations between computational and experimental biologists. As documented in his letter of support, has pledged to commit technical and intellectual resources as needed in support of our proposed studies.

**Collaborator (as needed)**, will oversee next generation sequencing (NGS) efforts related to Aims 1-3, including library preparation and purification, as well as high-throughput NGS sequencing analysis of samples derived from RNA extraction and chromatin immunoprecipitations. is , and directly supervises 2 full-time technical scientists. has extensive experience in molecular and genomics technologies, including NGS, to profile differential gene expression, genetic polymorphisms, transcription factor-binding, and epigenetic markers on a genome-wide scale. As , is currently overseeing projects that include RNA/small RNA-seq, Exome-capture-seq, and ChIP-seq applications. As documented in her letter of support, has pledged to commit technical and intellectual resources as needed in support of our proposed studies.

*Domestic Institution*
**Total Annual Funds Requested to the nearest $1,000: $ 19,000**

**BUDGET JUSTIFICATION**

**Co-Investigator** **(0.6 Calendar months/ 5% effort)**
is a practicing G , ,

. As an active gynecologist, his practice provides comprehensive health care to a large segment of the African American population in , many of whom suffer with uterine fibroids. He has well over 20 years of experience treating women with symptomatic uterine fibroids and extensive experience in the area of uterine fibroid biology research. He has established an extensive collection of well-characterized clinical tissue bank of uterine tissues including phase-matched pairs of fibroid and adjacent myometrial tissues from different ethnic groups. He has also established well-characterized primary cell lines from normal myometrial and uterine fibroid specimens from many of his tissue bank entries. will lend his expertise in replicating his established novel humanoid mouse renal capsule xenotransplantation model of uterine fibroids as well as provide expertise and materials to execute *in vivo* animal experiments as outlined in the proposal. Additionally, will provide his expertise in the development of manuscripts in conjunction with the PI and his research team.
will meet via tele-conferencing with the PI monthly to evaluate the project's overall progress as well as troubleshoot any technical issues that may arise.

**ADDITIONAL BUDGET JUSTIFICATION**

Funds for two Graduate Research Assistants (100% of a 50% appointment for 12 months per year) were budgeted with tuition/fees as a part of the overall compensation package for the Graduate Research Assistants. These funds are excluded from the indirect cost base per our federally negotiated F & A Rate Agreement dated 11/14/2014.

The budget includes a subaward to in the amount of:
Total direct cost: $62,010
GRR F&A cost: $32,245

We are using our Modified Total direct cost base which excludes sub-awards in excess of $25,000, per our federally negotiated F&A Rate Agreement dated 11/14/2014.

# PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

| | |
|---|---|
| 1. Introduction to Application (for RESUBMISSION or REVISION only) | Introduction_R01_HD087417_01A11017715673.pdf |
| 2. Specific Aims | R01_HD087417_01A1_Specific_Aims_Final1017715695.pdf |
| 3. Research Strategy* | R01_HD087417_01A1_Res_Strategy_Final1017715694.pdf |
| 4. Progress Report Publication List | |
| **Human Subjects Sections** | |
| 5. Protection of Human Subjects | R01_HD087417_01A1_Protection_of_Human_Subjecets1017715464.pdf |
| 6. Inclusion of Women and Minorities | R01_HD087417_01A1_Women_and_Minorities1017715465.pdf |
| 7. Inclusion of Children | R01_HD087417_01A1_Children1017715466.pdf |
| **Other Research Plan Sections** | |
| 8. Vertebrate Animals | R01_HD087147_01A1_Vertebrate_Animals1017715467.pdf |
| 9. Select Agent Research | |
| 10. Multiple PD/PI Leadership Plan | |
| 11. Consortium/Contractual Arrangements | |
| 12. Letters of Support | |
| 13. Resource Sharing Plan(s) | R01_HD087417_01A1_Resource_Sharing_Plan1017715509.pdf |
| **Appendix (if applicable)** | |
| 14. Appendix | |





Funding Opportunity Number: PA-13-302. Received Date: 2016-03-04T09:02:52.000-05:00

## INTRODUCTION TO RESUBMISSION APPLICATION

**SUMMARY:** I wish to thank the reviewers for their expert evaluations and valuable recommendations for improvement of our application. In response, our revised application includes new preliminary data as well as technical and conceptual experimental refinements, each of which is noted by lined margins in the proposal body. Major proposal strengths included its significance, outstanding study team, and logical mechanistic approach, deemed "*highly likely to advance the leiomyoma field because it takes a rigorous mechanistic approach to the disease coupled with direct translational applications*". Nonetheless, several concerns arose.

**(1) Aim 1A objective superseded by recent published findings:** Reviewers felt that Aim 1A [to establish the fibrotic transformation potential of LM-linked MED12 mutations] was largely achieved, and its significance thus diminished, by a recent study published after submission of our original application. This work (37) showed that uterine-specific expression of a mutant MED12 transgene elicited fibroid tumors in mice, providing direct genetic proof of disease causality. Nonetheless, the mechanism by which mutant MED12 drives tumor formation is unknown, and its elucidation is critical to translate these breakthrough findings into therapeutic applications. In this regard, we discovered that LM-linked MED12 mutations disrupt CDK8 activity in Mediator, suggesting a novel etiological role for CDK8 as a major and heretofore unrecognized driver of uterine fibroids. (49, highlighted in 51). Accordingly, we have revised Aim 1A of our application to focus on the pathogenic role of impaired CDK8 function in fibrotic transformation. We will leverage our lead in the biochemistry and pathobiology of Mediator to advance the field beyond MED12 to CDK8, an actionable drug target bearing the principal downstream biochemical defect arising from mutations in MED12. The published transgenic mouse model (37) cannot address such mechanistic questions, and the genetic and biochemical tractability of our human stem cell model is thus uniquely advantageous in this regard. We will use both molecular and chemical genetic approaches to examine the contribution of impaired CDK8 kinase activity to fibroid pathogenesis.

**(2) MED12 mutation status in study cohort:** Reviewer 1 raised concerns that the MED12 mutation status of fibroids from our (predominantly Hispanic) patient pool is unknown. In our revised application, we now show that the frequency and spectrum of MED12 mutations in fibroids from Hispanic women are comparable to those of other racial and ethnic populations. Among 88 fibroid tumors from 31 different Hispanic patients thus far analyzed, 56 (or 64%) harbor MED12 mutations, the vast majority (81%) of which correspond to missense mutations in codon 44. Our new data from Hispanic patients (127) corroborate those of two prior studies (128, 129) and support the conclusion based on multiple additional reports that the MED12 mutation frequency in uterine fibroids is similar from patients of diverse racial and ethnic origins (25, 27, 28, 32, 128, 129).

**(3) Feasibility of mouse xenograft model:** Reviewer 2 questioned the reliability of our published xenograft model (19). In fact, we have exploited this kidney capsule model to demonstrate the tumorigenic capacity of Stro-1$^+$/CD44$^+$ LM stem cells (SCs), validating its use for the studies proposed herein. Notably, ex-vivo labeling of SCs cells with Molday Ion Rhodamine B (MIRB), a fluorescent iron oxide-based MRI contrast agent, permits imaging-based tracking of engrafted cells in vivo. Reviewer 2 questioned an apparent increase in MIRB signal intensity with tumor growth (Fig. 9B in our original and revised application), which s/he believed should instead remain static or decrease due to signal dilution as cells proliferate. This issue is simply not relevant to Fig. 9B, which is *not* a time course of tumor growth, but instead a comparison of tumor formation at a specific point (8 weeks) following engraftment of different cell type combinations. As shown in Fig. 9B, LM SCs engrafted along with primary LM cells produce sizable tumors, reflecting the dependency of SCs on primary cells for paracrine signaling in response to circulating estrogen/progesterone. Accordingly, fluorescence signal intensity is high for LM SCs co-injected with primary LM cells. By contrast, fluorescence signal intensity is low for LM SCs alone and immortalized human LM (HuLM) cells, which elicit little and no tumor formation, respectively. This xenograft model thus accurately recapitulates the crucial interaction between LM SCs and primary LM cells required to create an appropriate niche for regeneration of fibroid tissue. The reviewer also questioned the extent to which LM SCs contribute to tumor formation, since a control engraftment of primary LM cells alone was not shown. In our xenograft model, as in others, primary LM cells are non-tumorigenic and behave similarly to HuLM cells, which we included as a negative control (Fig. 9B). Thus, fibroid tumors arise directly from LM SCs. Finally, reviewer 2 questioned the degree to which our xenograft tumors accurately model the histology of uterine fibroids. As shown [Fig. 9B and in (19)], our fibroid xenografts differ significantly from normal myometrium, and express higher levels of ACTA2 and Ki67, clinical markers used to differentiate LM from myometrium in human samples. In fact, our xenograft tumors closely resemble uterine fibroids on H&E staining [for comparative reference, see ***Science*** (2005) 308, 1589-92]. Therefore, we believe our xenograft model, one suitably rigorous to have satisfied prior peer review (19), is sufficiently accurate to evaluate the impact of impaired Mediator kinase function in the pathogenesis of MED12-mutant uterine fibroids.

## SPECIFIC AIMS

Uterine leiomyomas (LM; or fibroids) are monoclonal neoplasms of the myometrium (MM) and represent the most common tumors in women worldwide. Although benign, they nonetheless account for significant gynecologic and reproductive dysfunction, ranging from profuse menstrual bleeding and pelvic pain to infertility, recurrent miscarriage, and pre-term labor. As no long-term non-invasive treatment option currently exists for LM, deeper insight concerning tumor etiology is key to the development of newer targeted therapies. Accordingly, this proposal is impactful as it suggests an etiologic basis for the predominant LM subtype and further offers proof of concept for therapeutic intervention in this specific genetic setting.

LM arise from the genetic transformation of a single MM stem cell (SC) into a tumor-initiating cell (LM SC) that seeds and sustains fibroid growth through asymmetric cell divisions and monoclonal expansion. Heretofore, the genetic drivers thought dominantly responsible for fibroid formation have been largely identified. The most prevalent among these, accounting for ~70% of LM, are recurrent somatic mutations in the gene encoding the MED12 subunit of Mediator, a conserved multiprotein signal processor through which regulatory information conveyed by gene-specific transcription factors is transduced to RNA polymerase II (Pol II). However, the impact of these mutations on MED12 function and the molecular basis for their tumorigenic potential are unknown. Herein, we show that LM-linked mutations in MED12 disrupt its ability to activate Cyclin C (CycC)-dependent kinase 8 (CDK8) in Mediator, leading to reduced site-specific RNA Pol II phosphorylation and global dysregulation of gene expression. Furthermore, we identify genetic programs uniquely dysregulated in MED12-mutant fibroids, leading us to hypothesize that Mediator kinase disruption as a consequence of MED12 mutations elicits transcriptional reprogramming and altered signaling sufficient to drive fibrotic transformation of MM SCs. We further hypothesize that MED12-mutant LM are therapeutically susceptible to reactivation of CDK8 or pharmacologic modulation of uniquely dysregulated downstream signaling pathways. To confirm and extend these hypotheses, we propose the following aims, which we expect to significantly impact personalized treatment of women with LM.

**Aim 1. Establish the pathogenic role of Mediator kinase disruption in MED12-mutant LM.**
Our discovery that LM-linked MED12 mutations disrupt CDK8 activity in Mediator implies a new etiological role for CDK8 dysfunction as an important driver of uterine fibroids. To test this prediction, we will ask if genetic or chemical disruption of CDK8 (or its paralog CDK19) is sufficient to induce MM SCs to undergo fibrotic transformation in vitro and form fibroid tumors in vivo. The deleterious impact of mutations in MED12 on its CDK8-stimulatory activity marks them as loss of function mutations and thus reveals MED12 to be a probable tumor suppressor in MM. To test this prediction, we will ask if WT MED12, through restoration of CDK8/19 activity, can suppress the fibrotic phenotype of MED12-mutant LM SCs both in vitro and in vivo.

**Aim 2. Elucidate the pathogenic mechanism of Mediator kinase disruption in MED12-mutant LM.**
The molecular basis by which mutant MED12 disrupts CDK8/19 activity leading to tumorigenesis is unknown. To address the biochemical impact of mutant MED12, we will deploy a combination of in vitro binding and enzyme kinetic analyses to determine whether and how MED12 alters the catalytic efficiency of CycC-CDK8/19, define the role of MED12 in the overall activation mechanism, and clarify how pathogenic mutations in MED12 disrupt this process. To address the molecular basis by which Mediator kinase disruption drives MED12-mutant fibroid formation, we will employ an integrated genome-scale approach. Using RNA- and ChIP-seq in autologous MM and LM SCs, we will acquire tumor- and mutation-specific transcriptomic and epigenomic profiles. This combined analysis will permit us to correlate pathologic changes in gene expression with alterations in Pol II phosphorylation dynamics and super-enhancer activity, providing unprecedented insight into the basis by which MED12 mutations alter transcriptional programs as a course of tumorigenesis.

**Aim 3. Examine the therapeutic implications of Mediator kinase disruption in MED12-mutant LM.**
We hypothesize that transcriptional reprogramming, as a pathological consequence of Mediator kinase disruption, is also a pathogenic trigger of fibrotic transformation. If true, this leads to several predictions with implicit therapeutic considerations. First, reactivation of CDK8/19 in MED12-mutant LM may prove anti-tumorigenic. To test this, we will ask if pharmacologic restoration of CDK8/19 with peptide activators can transcriptionally program MED12-mutant LM SCs and suppress their fibrotic phenotype. Second, targeted modulation of signaling pathways uniquely dysregulated in MED12-mutant tumors, including the Wnt/β-catenin and glucocorticoid receptor (GR) pathways, may alternatively prove therapeutic. To test this, we will ask whether chemical modulation of these pathways can suppress the fibrotic phenotype of MED12-mutant LM SCs. These studies are the first to implicate GR signaling in the pathobiology of LM, and we will exploit these novel insights to establish proof-of-concept for the use of CDK8 activators and GR agonists in LM therapy.

## RESEARCH STRATEGY

### A. SIGNIFICANCE

***The problem:*** Uterine leiomyomas (LM; fibroids) are benign monoclonal neoplasms of the myometrium (MM) and represent the most common tumor in women worldwide (1, 2). Tumors occur in ~77% of women overall and are clinically manifest in ~25% by age 45 (3). Although benign, these tumors are nonetheless associated with significant morbidity; they are the primary indicator of hysterectomy, and a major source of gynecologic and reproductive dysfunction, ranging from profuse menstrual bleeding and pelvic pain to infertility, recurrent miscarriage, and pre-term labor (2, 3). Accordingly, the annual health care costs associated with LM have been estimated at ~$34 billion (4). Uterine fibroids thus represent a significant societal health and financial burden.

***Treatment barriers:*** Current treatment options for LM are primarily surgical or radiological and range from hysterectomy or myomectomy to minimally invasive options, including uterine artery embolization (UAE) and magnetic resonance-guided focused ultrasound (MRgFUS) (5-7). However, the deleterious impact of these procedures on reproductive function is either clear (hysterectomy) or controversial (UAE, MRgFUS), rendering such options unsuitable for women who wish to retain fertility (5-7). Likewise, medical therapies designed to blunt the stimulatory effects of gonadal steroid hormones on fibroid growth are contraindicated in reproductively active women, and otherwise approved only for short-term use due to long-term safety and other concerns (5, 8-10). Accordingly, no long-term noninvasive treatment option currently exists for LM, and deeper mechanistic insight concerning tumor etiology will be key to develop newer targeted therapies.

***LM pathogenesis:*** The prevailing model for fibroid pathogenesis invokes the genetic transformation of a single MM stem cell (MM SC) into a tumor-initiating cell (LM SC) that seeds and sustains clonal tumor growth, characterized by an increase in cell size and number, as well as abundant extracellular matrix production, under the influence of endocrine, autocrine, and paracrine growth factor and hormone receptor signaling (11-24). The genetic drivers thought dominantly responsible for cell transformation have been largely identified. Among these, somatic mutations in the *Xq13* gene encoding the RNA polymerase II (Pol II) transcriptional Mediator subunit MED12 are by far the most prevalent, accounting for ~70% of LM (25-34). A proportionally smaller fraction of tumors are thought to arise from genetic alterations leading to overexpression of HMGA2 (~20%), disruption of COL4A5-COL4A6 locus (~3%), or biallelic loss of fumarate hydratase (FH; ~2%) (2, 15, 26, 33, 35). Unidentified driver mutations appear to account for the remaining tumor fraction (12, 33, 35). Regarding mutations in *MED12*, all reside in highly conserved exons 1 and 2 and most are missense, with a smaller proportion corresponding to in frame deletions/insertions (26, 28, 36). Along with their high frequency occurrence, two additional genetic findings suggest that MED12 mutations are drivers of fibrotic transformation. First, monoallelic expression of mutant *MED12* is observed in human tumors, indicating selection for a functionally altered allele during tumorigenesis (26, 28, 36). Second, expression of a LM-linked MED12 mutant transgene in mice elicits uterine fibroid formation, providing direct genetic proof of disease causality (37).

***Study premise and relevance:*** Although a significant body of rigorous genetic data unambiguously establishes mutant MED12 as the predominant driver of uterine fibroids, the mechanistic basis for its tumorigenic activity is unknown, yet key to the development of applied therapeutics. Herein, we propose a molecular basis for LM formation through MED12 mutation. We show that LM-linked mutations in MED12 disrupt its ability to activate Cyclin C (CycC)-dependent kinase 8 (CDK8) in Mediator, leading to reduced site-specific phosphorylation of RNA Pol II and global dysregulation of gene expression. Moreover, we identify genetic programs uniquely altered in MED12-mutant tumors, leading us to hypothesize that Mediator kinase disruption as a consequence of MED12 mutations elicits transcriptional reprogramming and altered signaling sufficient to drive MM SC transformation. We further hypothesize that MED12-mutant LM are therapeutically susceptible to pharmacologic reactivation of abeyant CDK8 or targeted modulation of dysregulated downstream signaling pathways. This proposal thus addresses two issues that loom large in the field: the pathogenesis of LM and the identification of novel therapeutics.

### B. INNOVATION

***Conceptually,*** this proposal is innovative on many fronts. First, our discovery that LM-linked mutations in MED12 disrupt CDK8 activity in Mediator provides the first description of a molecular defect associated with these pathogenic mutations, implicates aberrant CDK8 activity in fibroid formation, and thus identifies a new druggable target in LM. Second, our revelation that attenuated CDK8 activity in Mediator elicits aberrant RNA Pol II function and global gene dysregulation implies a novel mechanistic basis for fibrotic transformation through transcriptional reprogramming. Finally, our discovery of reduced glucocorticoid receptor (GR) signaling in MED12-mutant LM suggests a novel route to fibrotic transformation and a viable target for pharmacologic

intervention. We will exploit these novel findings to establish proof-of-concept for the use of CDK8 activators and GR agonists in LM therapy.

***Technically,*** this proposal is innovative through its application of advanced MM/LM stem cell isolation, culture, and xenograft methodologies as well as next generation sequencing technology and state-of-the-art computational data analyses. These combined tools will be used to establish whether and how Mediator kinase disruption through mutant MED12 triggers transcriptional reprogramming sufficient to drive LM formation.

## C. Approach

### 1. Background

***Mediator: an integrative hub for signal-dependent gene regulation:*** The revelation that ~70% of LM harbor mutations in Mediator subunit MED12 implicates dysregulation of RNA Pol II-dependent gene expression in fibrotic transformation. Mediator is a conserved multiprotein interface between gene-specific transcription factors and Pol II (Fig. 1) (38, 39). In this capacity, Mediator channels regulatory signals from activator and repressor proteins to affect changes in gene expression programs that control diverse physiological processes, including cell growth and homeostasis, development, and differentiation (38-40). Structurally, Mediator is assembled from a set of 26 core subunits into three distinct modules termed “head”, “middle”, and “tail” that bind tightly to Pol II in the so-called holo-enzyme (41, 42). MED12, MED13, CycC, and CDK8 (or its paralog CDK19) comprise a four-subunit “kinase” module that variably associates with core Mediator (43). The kinase module is implicated in both activation and repression of transcription, at least in part, by site-specific phosphorylation of Pol II on its C-terminal domain (CTD), a heptapeptide repeat present in multiple copies within the Pol II large subunit (43, 44). Notably, the kinase module is a major ingress of signal transduction through Mediator, and MED12-dependent CDK8 activation is required for nuclear transduction of signals instigated by multiple oncogenic pathways with which MED12 is biochemically and genetically linked (Fig. 1) (36, 39, 45). Furthermore, MED12 is a target of oncogenic mutation in colon, prostate, and renal cell carcinomas (46-48). However, these mutations occur in the MED12 C-terminus and thus lie distant from LM-linked mutations that cluster in its N-terminus, suggesting possible distinct etiological mechanisms (36). Heretofore, the impact of LM-linked mutations on MED12/Mediator function and the molecular basis for their tumorigenic potential remain unknown.

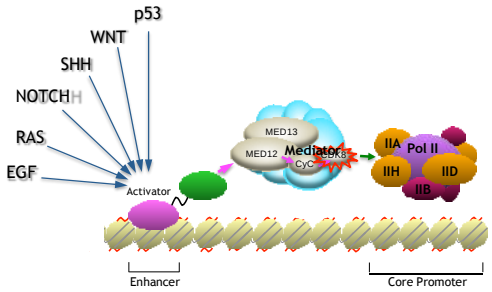



**Figure 1. Mediator function.** Mediator links signal-regulated transcription factors with Pol II. MED12 is an integrative hub for oncogenic signaling through CDK8.

### 2. Preliminary Results

***(1) MED12 activates CDK8:*** Efforts to understand how mutations in MED12 contribute to LM formation would be advanced by insight concerning the nature of its biochemical activity in Mediator. In this regard, we have shown that MED12 is essential to activate CycC-dependent CDK8 within the Mediator kinase module (36, 49). CDK8 is an atypical CDK that escapes activating phosphorylation of a conserved threonine residue in its activation loop by the CDK activating kinase (CAK) (50). Accordingly, CDK8 activation has been proposed to occur by a CAK-independent mechanism, one instead dependent on MED12. Thus, in a system reconstituted from recombinant baculovirus-expressed kinase module subunits, MED12, but not MED13, is critically required to activate an otherwise abeyant CycC-CDK8 complex toward an RNA Pol II CTD substrate (Fig. 2) (49).

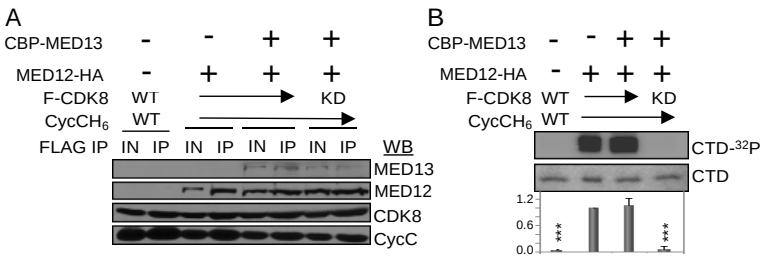



**Figure 2. MED12 activates CDK8.** Baculovirus-expressed kinase module subunits in combinations indicated were subjected to IP with FLAG-specific antibodies. FLAG IPs were processed by WB using the indicated antibodies (**A**) or incubated with [γ-$^{32}$P]-ATP and purified CTD (**B**). WT and KD refer to wild type and kinase dead (D173A) CDK8. IN, 10% of lysate used in IP. Quantified $^{32}$P-CTD levels are expressed relative to the FLAG-CDK8 WT/CycC/MED12-HA IP. Data are the average ± SEM of 3 experiments. Asterisks denote significant differences versus F-CDK8 WT/CycC/MED12-HA IP (Student’s *t* test, *** $p < 0.001$).

***2) LM-linked MED12 mutations disrupt Mediator-associated CDK8 activity:*** Recently, we reported a series of unifying observations that clarify the mechanism by which MED12 activates CycC-CDK8 in Mediator and also reveal the pathogenic nature of LM-linked mutations in MED12 (26, 49, highlighted in 51). First, we found

that MED12 activates CycC-CDK8 through a direct interaction involving the MED12 N-terminus and CycC (49). Notably, we mapped the CycC-binding (and hence CDK8 activation) domain on MED12 to within its N-terminal 100 aa encoded largely by exons 1 and 2 (49). Second, we discovered new LM-linked mutations occurring in *MED12* exon 1 (26); along with those previously identified in exon 2, these findings indicate that all LM-linked mutations in MED12 affect its CycC-binding interface. Finally, we confirmed that pathogenic exon 1 and 2 mutations in MED12 disrupt its interaction with CycC sufficient to impair CDK8 kinase activity both in vitro (Fig. 3A) and, more importantly, in clinically relevant patient fibroids (Fig. 3B) (26, 49). Collectively, these findings identify for the first time a common molecular defect associated with LM-linked mutations in MED12, and implicate aberrant CDK8 activity in the pathogenesis of LM. Notably, we found that the CDK8 paralog CDK19 is also expressed in MM and LM (49). Because these paralogous subunits assemble into the kinase module in a mutually exclusive manner (36), the extent to which the oncogenic activity of MED12 mutations derives from loss of Mediator-associated CDK8 *vs* CDK19 activity is presently unclear, but will nonetheless be addressed in study aims 1 and 2. Taken together, our findings reveal that LM-linked mutations in MED12 disrupt Mediator-associated CDK8 activity without uncoupling CycC-CDK8 from core Mediator (Fig. 3C).

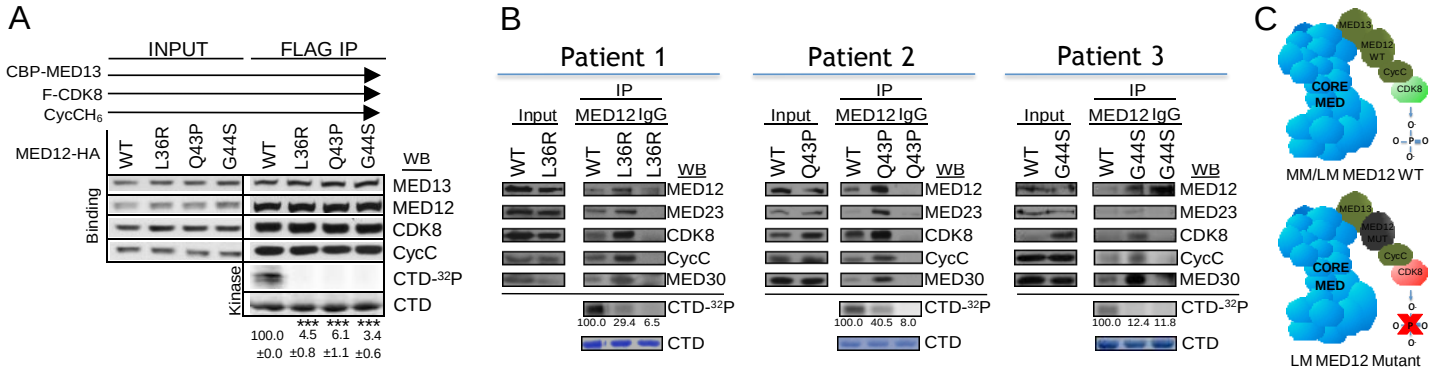



**Figure 3. LM-linked MED12 mutants disrupt CDK8 kinase activity.** (**A**) Baculovirus-expressed CBP-MED12, CycC-$H_6$, FLAG-CDK8, and WT or mutant MED12-HA proteins were immunoprecipitated (IP'd) using antibodies specific for the FLAG epitope tag on CDK8. FLAG-specific IPs were processed by WB or in vitro kinase assay as described in the legend to Fig. 2. INPUT, 10% of protein used in IP reactions. Quantified $^{32}$P-CTD levels are expressed relative to those in WT MED12 IP/kinase reactions. Data are the average ± SEM of 3 experiments. Asterisks: statistically significant differences compared to WT MED12 IP/kinase (Student's *t* test, *** $p < 0.001$). (**B**) Cell lysates from patient-matched MED12 WT and mutant fibroids (3 patients harboring 3 different MED12 mutations) were subjected to IP with control IgG or MED12-specific antibody. IPs were processed by WB analysis using Mediator subunit-specific antibodies as indicated (*top panels*) or in vitro kinase assays using purified CTD (*bottom panels*). Quantified $^{32}$P-CTD levels are expressed relative to those in WT IP/kinase reactions. (**C**) Model for disruption of Mediator-associated CDK8 activity as a consequence of MED12 mutations.

***(3) LM-linked MED12 mutations elicit aberrant RNA Pol II activity:*** In mammalian cells, CDK8-dependent Pol II CTD serine 5 phosphorylation (p-Ser5) marks initiating Pol II close to transcription start sites , while CDK8-independent CTD serine 2 phosphorylation (p-Ser2) characterizes elongating Pol II (52-54). To examine the genome-wide impact of impaired CDK8 activity in Mediator, we therefore acquired ChIP-seq profiles of p-Ser5 (initiating) Pol II on transcribed loci in WT and G44D mutant MED12-expressing cells. Comparative analyses of normalized Pol II ChIP-seq read counts located proximally to (Fig. 4, left panel) or distributed along the length of all annotated transcripts (Fig. 4, right panel) revealed reduced accumulation of Pol II near in G44D mutant *vs* WT MED12-expressing cells. These results reveal that the most common LM-linked MED12 mutation compromises Mediator activity and interferes with cellular RNA Pol II function.

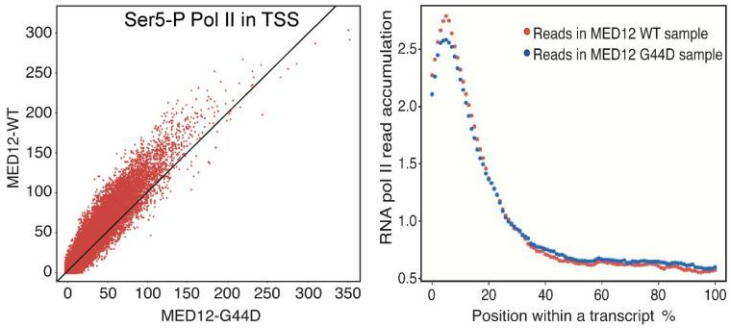



**Figure 4. LM-linked MED12 mutation G44D elicits aberrant RNA Pol II transcription genome wide.** HEK293 cells lines expressing STREP-tagged WT or G44D mutant MED12 (49) were processed for ChIP-seq using antibodies specific for total and p-Ser5 Pol II. **(***Left panel*) Read count in -/+200bp window around all TSS in G44D (x-axis) versus WT (y-axis) MED12-expressing cells. Note relative decrease in initiating Pol II at TSS in G44D-expressing cells. (*Right panel*) Distribution of total Pol II reads along the length of all annotated transcripts. Note that MED12 G44D mutant cells (blue) have less Pol II at promoter-proximal regions compared to MED12 WT cells (red).

***(4) Transcription reprogramming in MED12-mutant LM:*** Aberrant RNA Pol II function resulting from impaired Mediator-associated CDK8 activity could lead to altered gene expression programs that contribute to fibrotic transformation and LM formation. To identify these programs, we comparatively profiled the

transcriptomes of MED12 WT and mutant LM using Affymetrix GeneChip Human Exon Arrays (26). For this analysis, we sampled 42 tumors and corresponding myometrium from 31 patients, including 20 MED12-mutant tumors (16 exon 2-mutant; 4 exon 1-mutant) and 22 MED12-WT tumors (10 HMGA2-overexpressing; 4 FH-deficient; 8 lacking all such aberrations). Hierarchical cluster analysis revealed unique gene expression signatures characteristic of each genetic tumor type (Fig. 5, left), consistent with prior observations that these genetic alterations occur mutually exclusive of one another in LM (33). Collectively, these findings permit the stratification of LM into at least 4 molecular subtypes on the basis of distinct recurrent genetic alterations (i.e., driver mutations) and their corresponding unique gene expression profiles, indicative of separate pathways to tumorigenesis (Fig. 5, right) (26, 33). To identify genetic programs specific to mutant MED12, we subjected the list of genes differentially expressed in MED12-mutant tumors to Ingenuity Pathway Analysis (IPA). These genes are prominently linked to "cancer" ($p$=1.39E-28-5.038E-04), "reproductive system disease" ($p$=9.71E-21-4.71E-04), "gastrointestinal disease" ($p$=1.07E-11-2.65E-04), and "inflammatory responses" ($p$=1.63E-11- 5.03E-04). Top canonical pathways include "colorectal cancer metastasis signaling" ($p$=1.37E-07), "axonal guidance signaling" ($p$=4.83E-06), and "Wnt/β-catenin signaling" ($p$=7.48E-06). Finally, the most prominent "upstream regulators" linked to these genes include dexamethasone/glucocorticoid receptor (GR) (9.42E-16), β-estradiol/estrogen receptor α (ERα) (3.37E-15), TGFβ (1.12E-13), and Wnt/β-catenin (1.54E-13), suggesting dysregulation of transcriptional programs driven by these regulators in MED12-mutant fibroids. Notably, all 4 of these regulators have been linked with MED12/Mediator (55-63), and 3 of these (ERα, TGFβ, and Wnt/β-catenin) with LM (1-3, 14-17, 22, 64, 65). TGF-β is a key regulator of LM fibrosis and growth (66, 67). TGF-β signaling stimulates smooth muscle cell proliferation and promotes fibroid formation through stimulation of ECM-promoting genes and inhibition of matrix-resorbing genes (14, 68). Significantly, MED12 is implicated in suppression of TGF-β signaling (59), and among genes identified herein as dysregulated in MED12-mutant LM, TGF-β regulated genes exhibited altered expression patterns consistent with *activation of TGF-β signaling*. This suggests that LM-linked mutations in MED12 may disrupt its established role in suppression of TGF-β signaling. ERα, as a principal mediator of estrogen action, is thus an important promoter of LM growth (15, 17). Among genes identified herein as altered in MED12-mutant LM, most ERα-regulated genes exhibited altered expression patterns consistent with *upregulation of ERα signaling*. The underlying basis for such enhanced ERα signaling remains unclear, but could involve reduced GR signaling (see below), which inhibits estrogen-dependent signaling in LM cells (69).

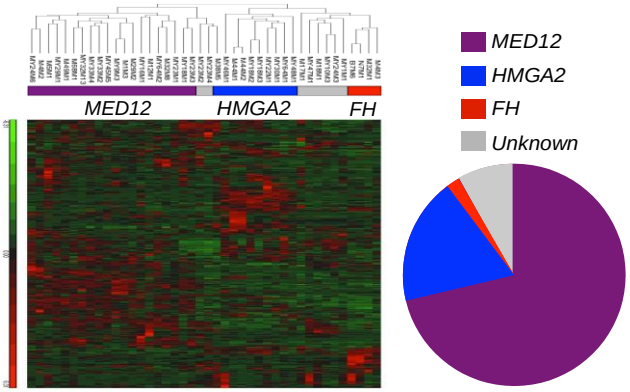



**Figure 5. MED12-mutant fibroids comprise a distinct molecular subtype. (***Left***)** Unsupervised hierarchical clustering (Cosine dissimilarity) of top 1% most variable genes (n=379) differentially expressed in each genetic tumor type relative to corresponding patient-matched myometrium (26). (*Right*) Proportional distribution of driver mutations in LM. *MED12* and *HMGA2* alterations comprise most LM, with a smaller proportion arising from alterations in *FH* or unknown drivers.

Canonical Wnt/β-catenin signaling is implicated in LM growth, and recent studies suggest its involvement as a paracrine effector of estrogen signaling in LM SCs (22, 64, 65). Further, we previously identified MED12 as coregulator of β-catenin (61), and MED12-mutant tumors support elevated levels of Wnt4 expression (30). Notably, among genes identified as altered in MED12-mutant LM, most WNT-regulated genes exhibited altered expression patterns consistent with *upregulation of Wnt/β-catenin signaling*. Studies in Aim 3 of this proposal seek to establish whether Wnt/β-catenin pathway inhibitors attenuate the growth and fibrotic properties of MED12-mutant LM SCs in vitro and in vivo.

Finally, the GR, while not heretofore implicated in the natural history of LM, nonetheless plays an important role in uterine biology, and also antagonizes the effects of estrogen on uterine growth, differentiation, and physiology (70-73). MED12/Mediator has been implicated in regulation of GR activity (57, 74, 75), and most GR-regulated genes, among those dysregulated in MED12-mutant tumors, exhibited altered expression patterns consistent with *downregulation of GR signaling*. Notably, GR signaling has been shown to suppress estrogen-dependent LM cell proliferation (69), suggesting a plausible mechanism to explain enhanced ERα signaling observed in MED12-mutant tumors. Studies in Aim 3 of this proposal seek to address this issue and establish proof-of-concept for the use of GR agonists in LM therapy.

***(5) Reduced MED12 function triggers fibrotic transformation of MM cells in vitro:*** To validate these findings and determine if loss of MED12 function leads to altered signaling in MM, we monitored the impact of

RNAi-mediated MED12 knockdown on the molecular phenotype of primary MM cell cultures. To this end, single cells freshly dissociated from surgically recovered MM tissue were transduced with control or MED12 shRNA-expressing lentiviruses, and transduced cells were subsequently plated for primary culture. Viral transduction efficiencies typically ranged from 60-80%, leading to 50-70% reductions in MED12 mRNA levels depending on viral titer. Strikingly, MED12 knockdown was accompanied by significantly enhanced expression of genes within the TGFβ and Wnt/β-catenin pathways (Fig. 6), concordant with our tumor profiling analyses. Notably, we also observed significantly increased expression of ECM genes known to be activated by TGFβ signaling in LM, indicating that impaired MED12 function elicits hallmarks of fibrotic transformation in vitro (Fig. 6).

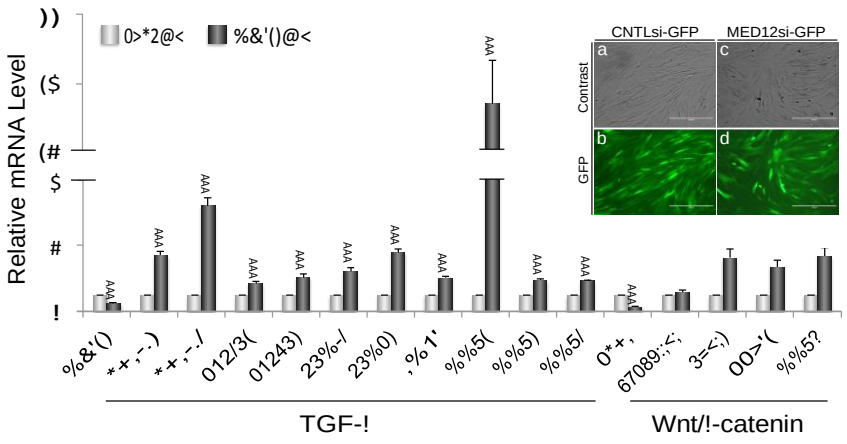



**Figure 6. MED12 knockdown triggers fibrotic transformation of MM cells in vitro.** MM single cell suspensions were transduced with pLFRu-GFP lentiviruses expressing control (CNTLsi) or MED12-specific shRNAs as indicated. Five hours post-infection, cells were plated and 24 hours later subjected to puromycin selection for 4 days prior to RNA harvest and processing by RT-qPCR to monitor expression of the indicated TGFβ and Wnt/β-catenin pathway genes. Note that CTGF, a gene in neither pathway, is downregulated, indicating that observed upregulation of TGFβ and Wnt/β-catenin target genes is not a general phenomenon of MED12 knockdown. mRNA levels were normalized to GAPDH mRNA and expressed relative to their corresponding mRNA levels in CNTL knockdown cells. Data are the mean ± SEM of 3 experiments in triplicate. Asterisks denote statistically significant differences relative to CNTL knockdown (Student's *t*-test: *** $p < 0.001$). Inset: CNTL (**a**, **b**) or MED12 (**c**, **d**) knockdown cells subjected to phase contrast (**a**, **c**) or fluorescence (**b, d**) microscopy.

***A model for uterine fibroid pathogenesis through Mediator kinase dysfunction:*** Based on our findings, we hypothesize that LM-linked mutations in MED12 disrupt Mediator associated CDK8 activity and RNA Pol II function leading to widespread transcriptional reprogramming and altered signaling sufficient to drive fibrotic transformation (Fig. 7). Furthermore, we propose that disabled CDK8 activity and dysregulated downstream signaling pathways will provide an entree for molecularly targeted therapies aimed to supersede these transcriptional defects and suppress the fibrotic phenotype. To test these hypotheses, we propose the following aims to establish the requirement, define the mechanism, and investigate the therapeutic implications of Mediator kinase dysfunction and transcriptional reprogramming in MED12-mutant LM.

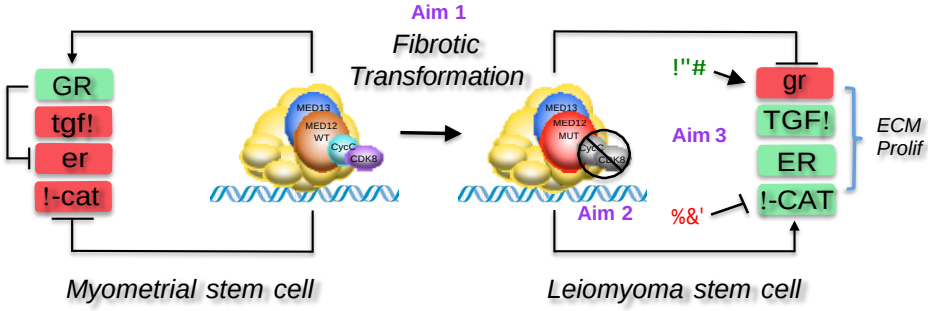



**Figure 7. Molecular basis of MED12 in LM pathogenesis.** MED12 mutations disrupt Mediator-associated CDK8 activity leading to aberrant Pol II function and transcriptional reprogramming (activation of TGFβ, ERα, and Wnt/β-catenin signaling; inhibition of GR signaling) that drives MM SC transformation characterized by altered proliferation and ECM production. Aim 1 addresses the requirement for Mediator kinase disruption and transcriptional reprogramming in fibrotic transformation. We will assess whether chemical or genetic disruption of CDK8 drives fibrotic transformation of MM SCs, establish the role of altered transcription programs in this process, and determine whether restoration of WT MED12 function can restore CDK8 activity and suppress the fibrotic phenotype of MED12-mutant LM SCs. Aim 2 focuses on the mechanism of Mediator kinase disruption and reprogramming. We will elucidate the biochemical basis by which MED12 mutations disrupt CDK8 activity and examine the impact of this defect on global RNA Pol II function, epigenetic status, and gene expression programs. Aim 3 explores therapeutic implications of Mediator kinase disruption and transcriptional reprogramming. We will determine whether restoration of CDK8 function or pharmacologic manipulation of altered signaling pathways [β-catenin inhibition with ICG-100; GR activation with Dex] ameliorates the tumor phenotype.

### *3. Experimental Design*

**Aim 1. Establish the pathogenic role of Mediator kinase disruption in MED12-mutant LM.**

**Aim 1A. Investigate the fibrotic transformation potential of impaired CycC-CDK8 kinase activity.**

***Rationale:*** The high-frequency occurrence and monoallelic expression of mutant MED12 in LM support its role as a tumor driver (25, 27, 28, 30-33, 35, 76), a prospect recently confirmed by the finding that a mutant *MED12* transgene elicits uterine fibroids in a genetic mouse model (37). Nonetheless, the mechanistic basis for the tumorigenic activity of mutant MED12 is unknown. The fact that MED12 mutations disrupt the activity of CDK8

(and possibly CDK19) implies a new etiological role for CDK8/19 dysfunction in fibroid pathogenesis. The following experiments will test this prediction, with important implications for new druggable targets in LM.

***Design:*** To clarify the role of Mediator kinase dysfunction in LM formation, we will test whether disruption of CDK8/19 activity is sufficient to induce otherwise normal MM cells to undergo fibrotic transformation in vitro and form fibroid tumors in vivo. For these studies, we will use MM and LM SCs, which offer advantages over bulk MM and LM primary cells as a model system. First, as the presumptive cell of origin for fibroid tumors, SCs represent the most biologically relevant system for study (77-79). Second, MM/LM SCs are genetically stable, self-renewing, and capable of producing clonal lines, rendering them a propitious in vitro model (18, 19, 21, 24). Finally, LM SCs efficiently regenerate fibroid tumors in mice, even after prolonged culture, as opposed to bulk LM cells that lose their phenotypic and tumorigenic properties in culture (18, 19, 21, 24).

To isolate SCs, we started with LM and adjacent MM tissues from informed consent female patients undergoing surgery for symptomatic fibroids under the clinical care of our study collaborator, (support letter included). Together with we have developed a detailed collaborative clinical research plan in support of the proposed studies (see Facilities section). Briefly, because uterine fibroids are hormone responsive tumors relevant to reproductive age women, participating subjects include only premenopausal cancer-free women of unrestricted race/ethnicity who have received no hormonal treatments (including contraceptives or GnRH analogues) for 3 months prior to surgery. To minimize biological heterogeneity, patients are matched as closely as possible with respect to biometric and clinicopathological features, including age, race/ethnicity, BMI, menstrual phase (proliferative *vs* secretory), and endometrial histology. To reduce tumor heterogeneity, we strive to ensure uniformity with respect to fibroid location (intramural fundal) and size (4-8 cm).

Tissue-dissociated single cell suspensions from patient-matched MM and LM were processed for SC isolation using the side population method. This flow cytometric-based method is used to identify and sort somatic stem cells based on their enhanced ability to efflux intracellular Hoechst dye via the ATP-binding cassette (ABC) family of transporter proteins (18-21). Accordingly, side population (SP) cells correspond to viable propidium iodide-negative and reserpine-sensitive cells with low Hoechst 33342 content (Fig. 8A). Molecular and immunophenotyping of these stem/progenitor-enriched SP cells confirmed them to be both undifferentiated (expressing low levels of hormone receptors and smooth muscle markers) and mesenchymally-derived ($CD90^+$) (Fig. 8B and C).

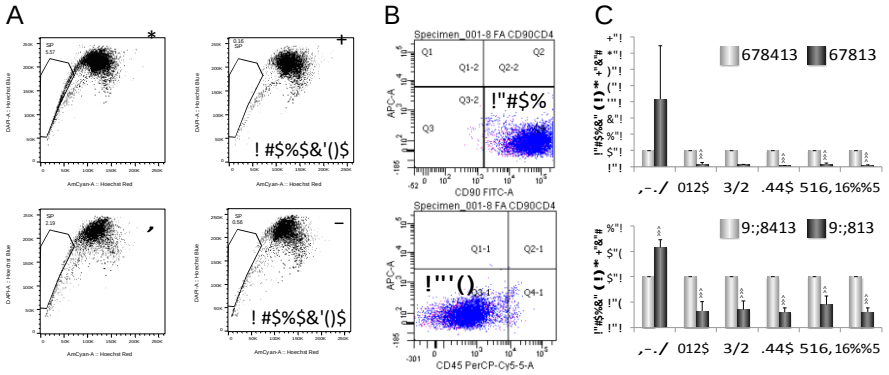



**Figure 8. Isolation and characterization of MM/LM SP.** (**A**) Distribution of SP (framed) and non-SP cells within all Hoechst-stained live cells isolated from MM (plots 1&2) and LM (plots 3&4). Addition of 50μM reserpine (ABC inhibitor) vanquishes the SP fractions. (**B**) Expression of hematopoietic (CD45) and bone marrow mesenchymal (CD90) SC markers in Myo SP cells. Similar results were obtained with Leio SP cells. (**C**) mRNA expression levels of stem cell (ABCG2), hormone receptor (ESR1, PR), and smooth muscle (αSMA, SM22a, CNN1) markers in SP and NSP cells by RT-qPCR and GAPDH normalization. Data are mean ± SEM of 3 experiments in triplicate (****P*<0.01).

MM and LM SP cells were further enriched for SC features using Stro-1/CD44 cell surface markers as described recently (19) by our co-investigator on this application, with whom we have established a close working relationship (87, 127, 130). These $Stro\text{-}1^+/CD44^+$-enriched MM/LM cells have been confirmed to be undifferentiated, multipotent cells of mesenchymal origin, as expected for smooth muscle stem/progenitors (19). Importantly, we also show that $Stro\text{-}1^+/CD44^+$-enriched LM cells efficiently regenerate fibroid tumors in mouse xenotransplanation assays, confirming their tumor SC potential (Fig. 9) (19). Accordingly, these cells represent a suitable SC model for studies described below to examine the pathogenic role of Mediator kinase disruption in MED12-mutant LM.

To determine if Mediator kinase dysfunction drives LM formation, we will investigate whether genetic or chemical disruption of CDK8/19 is sufficient to induce MM SC to undergo fibrotic transformation in vitro and form fibroid tumors in vivo. First, we will employ the CRISPR/Cas9 genome editing system (80) to introduce kinase-inactivating mutations into the endogenous *CDK8* or *CDK19* loci of MM SCs. Human CDK8/19 share 97% aa sequence identity within their corresponding catalytic domains, and an identical missense mutation in

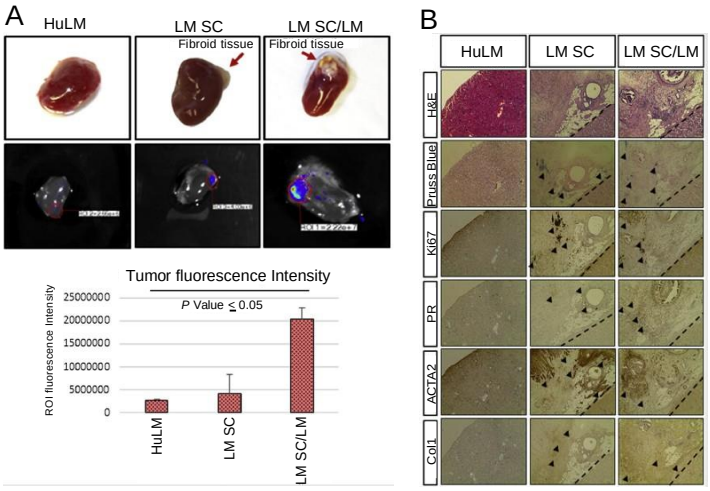



**Figure 9. Regeneration of human fibroid-like tumors by Stro-1$^{+}$/CD44$^{+}$ LM SC.** Hormone-supplemented female mice were injected under the renal capsule with Stro-1$^{+}$/CD44$^{+}$ cells alone (LM SC) or mixed with primary LM cells (LM SC/LM). Immortalized human LM (HuLM) cells are a negative control. **(A)** *Top Panels,* macroscopic visualization of regenerated tumors (red arrows) 8 weeks after cell engraftments. *Middle Panels*, in vivo imaging of MIRB-labeled xenografts using IVIS for 120 seconds (see text for description). The images obtained display a color spectrum: red, strongest signal; blue, weakest. Signal intensity is plotted in the lower graph **(B)** Immunohistochemistry of reconstituted fibroid-like tumors. H&E staining demonstrates characteristic histology of fibroid-like tissue with some adipose cells. Prussian blue dye localizes MIRB-labeled human cells due to iron deposits. Positive immunostaining for Ki67 (proliferation), PR (progesterone receptor), ACTA2 (actin smooth muscle), and Col1A1 (collagen1A1) reveals molecular and structural component biology characteristic of uterine fibroid tumors.

the CDK activation loop of either kinase (c.518A>C, p.D173A) is functionally inactivating (Fig. 2) (36, 88). Therefore, we will independently target *CDK8* or *CDK19* in MM SCs to generate kinase dead (KD) alleles. MM SCs engineered by CRISPR/Cas9 to express either of the two most common LM-linked *MED12* exon 2 mutations (c.131G>A, p.G44D; c.107T>G, p.L36R) will serve as positive controls. Briefly, *CDK8*, *CDK19,* or *MED12* donor plasmids harboring specific point mutations flanked by homology arms of ~1 Kb will be introduced into MM SCs to facilitate homology-directed repair of RNA-guided Cas9-medated DNA double-strand breaks close to *CDK8/19* exon 6 (or *MED12* exon 2) nucleotides targeted for replacement (Fig. 10). Notably, a loxP-flanked neo cassette within a region corresponding to intronic sequences in one homology arm will permit positive selection for targeted clones (and later removal if desired), while an MC-1 promoter-driven thymidine kinase cassette within the donor plasmid will permit negative FIAU selection to eliminate clones bearing random integration events. Independent clonal MM SC cell lines (2 clones each) sequence-verified to express CDK8 KD, CDK19 KD, MED12 G44D, or MED12 L36R will be validated for impaired Mediator-associated CDK8/19 activity by IP/kinase assay as described (Fig. 3) (26, 49). Validated clones will then be processed for both in vitro and in vivo fibrotic transformation potential as described below. Additional controls for these experiments will include non-targeted MM SCs as well as MED12-mutant LM SCs. These studies will be conducted in collaboration with our co-investigator and, leading experts in translational fibroid models (support letters included).

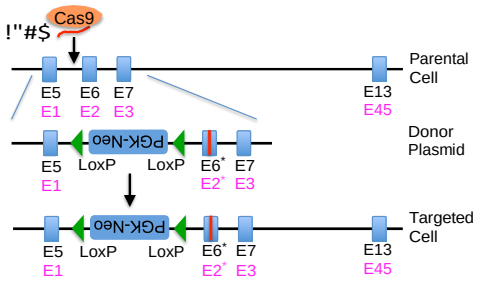



**Figure 10. Targeted knock-in strategy.** *CDK8/19* exons black, *MED12* exons pink.

*(i) Fibrotic transformation in vitro:* Control and CDK8/19-targeted MM SCs will undergo biological and molecular phenotyping for hallmark evidence of fibrotic transformation in vitro, including enhanced proliferation and induced expression of ECM genes (14, 16). To this end, early (between 2-4) passage cells will be assessed for proliferative capacity by viable cell counting, as well as ECM mRNA and protein expression by RT-qPCR and immunoblot analyses, respectively, using our established methods (49, 61, 81-87). Data will represent mean values ± SEM. For single parametric comparisons, significance of mean differences will be assessed with Student's t test. For multiple comparisons, ANOVA, 2-way ANOVA and repeated-measures ANOVA followed by Tukey's or Bonferroni's post hoc tests will be used as appropriate. We will monitor expression levels of TGFβ and ECM marker genes linked to LM, including collagen (types 1, 4, 6), fibronectin, and fibromodulin (14, 16). Additionally, cells will be subjected to unbiased transcriptome profiling by RNA-seq as described in Aim 2B. Cluster and IPA analyses will establish relationships among gene expression profiles and the identity of prominent molecular networks based on gene function and interconnectivity. Should CDK8/19 disruption drive fibrotic transformation of MM SCs in vitro, we would expect CDK8/19-targeted MM SCs, like MED12 exon 2-targeted MM SCs, to exhibit significantly enhanced rates of proliferation and expression of ECM genes compared to control, non-targeted MM SCs. Alternatively, we would expect CDK8/19-targeted MM SCs (like MED12 exon 2-targeted cells) to phenocopy MED12-mutant LM SCs with regard to proliferative rates and gene expression profiles. Accordingly, we would expect common molecular networks to emerge from comparative pathway analyses, suggesting a common basis for transcriptional reprogramming to a pathologic state. Substantiation of these expected results will provide direct evidence that Mediator kinase disruption (like mutant MED12) drives MM SC transformation in vitro.

*(ii) Fibroid formation in vivo:* Control and CDK8/19-targeted MM SCs (1.0 X $10^6$ cells) will be mixed with type I collagen gel and grafted (along with primary MM/LM cells) into the subrenal space of 6 week-old female nonobese diabetic-scid (NOD-SCID) mice (n=6 per group) supplemented with 50 mg progesterone/0.1 mg estrogen. As noted (Fig. 9) (19), this model was used to demonstrate the tumorigenic capacity of Stro-$1^+$/$CD44^+$ LM SCs, validating its utility for the studies proposed herein. Notably, ex-vivo labeling of these cells with Molday Ion Rhodamine B (MIRB), a fluorescent iron oxide-based paramagnetic MRI contrast agent, permits image-based cell tracking in vivo as well as FACs-based tumor cell recovery from injected animals (19). Eight weeks after cell engraftments, mice will be sacrificed and reconstruction of fibroid tissue established by gross appearance and tumor volume ($V=LxW^2x0.5$), as well as histological and IHC analyses to monitor tissue morphology, ECM production, collagen and smooth muscle fiber content, proliferative indices, and hormone receptor status (Fig. 9) (18, 19, 21). MIRB-labeled human cells will be isolated from dissociated xenograft tissue by FACS and subjected to molecular profiling by RNA-seq (or single cell RT-qPCR with BioMark™ if cell numbers are limiting). As observed previously (19), we expect control MM SCs to form xenografts without evidence of fibrotic transformation. By contrast, should CDK8/19 disruption trigger fibrotic transformation, we would expect CDK8/19-targeted MM SCs to form xenografts that grow and reconstitute tumor tissue with histology comparable to those produced by MED12 exon 2-targeted MM SCs and MED12-mutant LM SCs. Finally, among xenograft-recovered cells, we would expect gene expression profiles of CDK8/19-targeted MM SCs to cluster with those from MED12 exon 2-targeted MM SCs and MED12-mutant LM SCs, indicative of a common MED12-dependent path to tumor formation via transcriptional reprogramming.

As an alternative approach to genetic disruption of CDK8/19, we will use a newly characterized, highly specific, and orally bioavailable chemical inhibitor of CDK8/19, CCT251545 (88, 89; ApexBio). MM SCs treated with CDK8/19-inhibitory concentrations of CCT251545 (predetermined by IP/kinase assay; 25-250 nM range) will be evaluated for in vitro and in vivo fibrotic transformation potential as described above (engrafted mice will be orally dosed with compound at 37.5 mg/kg body weight to maintain circulating inhibitor levels). Should the oncogenic properties of mutant MED12 derive from impaired CDK8/19 activity, we expect CCT251545-treated MM SCs to resemble MED12-mutant LM SCs with respect to their biological and molecular phenotypes in vitro and in fibroid xenografts. Collectively, these molecular and chemical genetic studies will establish for the first time whether Mediator kinase disruption elicits fibrotic transformation and LM formation.

**Aim 1B. Investigate the tumor suppressive potential of WT MED12.**

***Rationale:*** LM-linked mutations in MED12 disrupt its CycC-CDK8 stimulatory activity, revealing them to be *loss of function* mutations (Fig. 3) (26, 49). The recent finding that a mutant MED12 transgene drives uterine fibroid formation in mice (37) should not be construed as evidence that MED12 mutations are gain of function, as MED12 is an integral subunit of the multiprotein Mediator. In this genetic model, WT MED12 in Mediator likely undergoes reciprocal exchange with ectopic mutant MED12, resulting in overall impaired Mediator kinase activity. Thus, mutational loss of MED12 function is a likely tumorigenic trigger, revealing MED12 to be a probable tumor suppressor in myometrium. The following experiments will test this prediction.

***Design:*** To determine if MED12 is a tumor suppressor, and establish a biochemical basis for such activity, we will ask whether WT MED12, through restoration of CycC-CDK8 function, can divest MED12 mutant LM SCs of their fibrotic properties in vitro and tumorigenic potential in vivo. To this end, MED12-mutant LM SCs (isolated as in Aim 1A) will be transduced with WT or G44D mutant MED12-expressing lentiviruses (pSMPUW) following our established methods (49, 84, 86, 90). Controls for these experiments will include MED12-mutant LM SCs transduced with pSMPUW backbone-carrying lentivirus, as well as nontransduced MM SCs. Control and virally transduced SCs will be characterized for their proliferative capacity, ECM production, and global gene expression profiles in vitro or engrafted into mice for analysis of their tumorigenic properties as described in Aim 1A. MIRB-labeled human cells will be recovered from dissociated tumor tissues by FACS and subjected to molecular profiling by RNA-seq or single cell RT-qPCR as described in Aim 1A. Rescue of Mediator-associated CDK activity in WT MED12-reconstituted cells will be confirmed by IP/kinase assay also as described in Aim 1A. Should MED12 function to suppress fibrotic transformation in vitro, we would expect MED12-mutant LM SCs transduced with WT, but not G44D mutant, MED12 to exhibit significantly reduced proliferation rates and ECM production levels approaching those of control MM SCs. In vivo, we would expect engrafted LM SCs transduced with WT, but not G44D mutant, MED12 to reconstitute tissue with histology comparable to that produced by control MM SCs with little evidence of fibrotic growth. Among xenograft-recovered cells, we would expect gene expression profiles of WT MED12-transduced cells to cluster with those from control MM cells, indicative of MED12-dependent tumor suppression through transcriptional programming. Collectively, studies in Aim 1 should clarify the contribution of Mediator kinase disruption to the pathogenesis MED12-mutant LM.

***Caveats and Alternative Approaches:*** Should unanticipated technical difficulties preclude our Aim 1A knock-in approach using the CRISPR/Cas9 system, we propose an alternative lentiviral-based knockdown/rescue strategy that we have used effectively in other contexts to study the biological impact of pathogenic MED12 mutations (82, 86, 90). Briefly, MM SCs will be transduced with pFLRu-GFP-based dual-purpose lentiviruses (91, 92), each concurrently expressing both a CDK8/19-specific shRNA and a cDNA-encoded siRNA-resistant CDK8/19 derivative (WT or KD). Accordingly, in a single round of infection, contemporaneous knockdown of endogenous CDK8/19 and expression of a GFP-tagged CDK8/19 WT or KD rescue protein is achievable.

**Aim 2. Elucidate the pathogenic mechanism of Mediator kinase disruption in MED12-mutant LM.**
**Aim 2A. Define the biochemical basis by which MED12 mutations disrupt CycC-CDK activity.**
***Rationale:*** Our studies revealed that LM-linked mutations in MED12 disrupt its CycC-CDK8-stimulatory, but not its CycC-CDK8-binding, activity (Fig. 3) (26, 49). This indicates that MED12 binding is necessary but not sufficient for CycC-CDK8 activation, and reveals an additional MED12-dependent step in the activation process reliant on residues mutated in LM. However, the biochemical basis for this MED12-dependent activation step and how this step is disrupted by MED12 mutations is unclear. Furthermore, whether and how MED12 activates the CDK8 paralog CDK19 is unknown, but nonetheless germane to the pathogenesis of MED12-mutant fibroids, since both paralogs are expressed in MM/LM and assemble into the Mediator kinase module in a mutually exclusive manner. We propose the following experiments to resolve these issues.

***Design:*** We propose that MED12 residues mutated in LM normally trigger a structural reconfiguration in the CDK8 T-loop sufficient to promote an open activation segment, rearrangements typically driven in other CDKs by T-loop phosphoresidues that are missing in CDK8 (50). Absent atomic level structural detail, mechanistic insight into the basis by which MED12 activates CDK8 and how MED12 mutations impact this process may be gleaned by kinetic analyses (93, 94). Therefore, we will perform kinetic measurements using a fluorescence- and peptide substrate-based kinase assay validated for use with CycC-CDK8 (Omnia→ Kinase Assay; Life Technologies). Following standard kinetic measures (95-97) under conditions of variable ATP and CTD peptide substrate concentrations, and in the absence or presence of purified recombinant MED12 WT or mutant derivatives (Figs. 2 and 3A), we will determine the impact of MED12 on the catalytic efficiency of CycC-CDK8, as well as its binding affinity for either or both ATP and peptide substrate. These experiments will establish whether and how MED12 alters the catalytic efficiency of CycC-CDK8, clarify the role of MED12 in the overall activation mechanism, and reveal how pathogenic mutations in MED12 disrupt this process.

While CDK8 has been studied extensively, comparably little is known regarding the structure and function of CDK19. Nonetheless, the two paralogs share extensive sequence homology (80% and 97% aa sequence identity within their respective CycC-binding and catalytic kinase domains domains) suggesting that CDK19 may similarly be activated by MED12. To test this, we will monitor the ability of MED12 to stimulate CycC-CDK19 using our established in vitro kinase assays (Figs. 2 and 3A) (26, 49). Recombinant baculovirus-expressed CDK19 derivatives [WT and KD (D173A)] have been produced and purified for study purposes. As with CDK8, we will monitor in vitro kinase activities of CycC-CDK19 derivatives in the absence and presence of MED12 and MED13. Should these experiments confirm that MED12 activates CycC-CDK19, we will then monitor the impact of LM-linked MED12 mutations on this process using conditions and assays established for CycC-CDK8 (Figs. 2 and 3A). Similar to CycC-CDK8, we predict that MED12 binding is necessary but not sufficient for CycC-CDK19 activation. Confirmation of this prediction will justify kinetic studies described above to determine if and how MED12 alters the catalytic efficiency of CycC-CDK19 and how MED12 mutations disrupt this process. These straightforward yet important studies will be critical to establish the biochemical relationship between MED12 and CDK19, heretofore unknown, and complement biological studies in Aim 1A to delineate the relative contribution of CDK8 versus CDK19 to the pathogenesis of MED12-mutant LM.

**Aim 2B. Determine the impact of MED12 mutations on the LM SC transcriptome and epigenome.**
***Rationale:*** We found that MED12 WT and mutant fibroid tumors differ significantly in their respective gene expression profiles, both of which in turn differ from MM, indicative of extensive transcriptional reprogramming as a course of fibrotic transformation (Fig. 5) (26). Mechanistically, reprogramming could derive from reduced levels of CDK8-catalyzed p-Ser5 (initiating) RNA Pol II that we observe on TSS (Fig. 4). Reduced levels of initiating Pol II could reflect either impaired transcription complex formation and reduced transcription rates or enhanced clearance of promoter-bound Pol II and increased transcription rates (52, 98). Which of these processes operate at individual transcription units across the genome will require global CTD phosphorylation profiling to distinguish initiating from elongating forms of Pol II. Further, Mediator is a functional constituent of super-enhancers (SEs), clustered enhancer elements that drive high-level expression of genes encoding key regulators of cell identity and disease (99-103). Notably, recent work has revealed SEs to be regulated by

Mediator-associated CDK8 activity (104) that we showed is disrupted by LM-linked mutations in MED12 (26, 49). Therefore, genome-wide distribution profiling of H3K27ac and H3K4me1, highly enriched SE marks (99, 100, 103), could reveal a new paradigm for the pathogenesis of MED12-mutant fibroids involving epigenetic dysregulation of enhancer-driven genetic programs that control MM stem cell fate and drive tumorigenesis.

***Design:*** We will employ an integrated genome-scale approach to understand how Mediator kinase disruption drives MED12-mutant LM formation through transcriptional reprogramming. First, we will use RNA-seq to acquire gene expression profiles from patient-matched MM SCs as well as MED12 WT and mutant LM SCs. This will be important to extend our prior datasets derived from whole tumors, and also to ensure acquisition of transcriptomic and epigenetic profiling datasets from identical patient-matched SCs. Thus, the second component of our integrated approach will rely on ChIP-seq to establish genome-wide CTD phosphorylation and SE histone modification profiles in the same patient-matched samples sets used for RNA-seq. This combined analysis will permit us to correlate pathologic changes in gene expression with alterations in Pol II phosphorylation dynamics and SE activity, providing unprecedented insight into the mechanism by which transcriptional circuits are pathologically rewired as a course of fibrotic transformation.

Briefly, Stro-$1^+$/$CD44^+$ SCs isolated from patient-matched MM as well as MED12 WT and mutant fibroids will be processed for RNA extraction and ChIP using our published protocols (49, 61, 81-86). For ChIP, solubilized chromatin will be subjected to IP (5 X $10^5$ cells/IP) using non-specific isotype-matched IgGs or antibodies specific for: (a) Mediator kinase module subunits MED12, CycC, CDK8, CDK19; (b) core Mediator subunits MED4, MED30; (c) p-Ser5, p-Ser2, total Pol II; (d) active enhancer histone marks H3K27ac, H3K4me1; and (e) repressive histone mark H3K27me3. Importantly, this list includes only antibodies that we and others have validated for ChIP (49, 61, 81-86). Processed RNA and ChIP DNA samples will subjected to NGS by, while computational analyses will be conducted by (support letters included).

We expect to observe altered gene expression profiles between MM and LM SCs, and among the latter, clear distinctions between MED12 WT and mutant SCs, as observed in whole tumors (Fig. 5) (26). Gene ontology and IPA analyses will reveal the biological functions, mechanisms, and pathways linked to these genes, as well as their associations with reproductive tract and other diseases. We expect to observe dysregulation of TGFβ, Wnt/β-catenin, GR and ER pathways in MED12-mutant LM SC. Nonetheless, should these studies reveal dysregulation of additional or unique pathways, we will then be guided in our accompanying aims to assess the biological role and pharmacologic sensitivity of these pathways in LM.

At the chromatin level, we expect altered patterns of CTD phosphorylation in MED12 WT and mutant SCs to reveal mechanistic insight regarding the impact of Mediator kinase disruption on Pol II dynamics and differential gene regulation. For example, reduced levels of initiating Pol II with or without a compensatory increase in the pool of elongating enzyme on specific loci could reveal a basis for enhanced or diminished gene expression, respectively. Regarding epigenomic profiling, comparative distribution analysis of Mediator along with active SE histone marks (105) will clarify the impact of CDK8 dysfunction on the structure and activity of these important regulatory elements. Notably, tumor cells often acquire SEs to drive high-level expression of signal-induced oncogenes (106-110), frequently those controlled by the Wnt/β-catenin and TGF-β pathways (100), both of which we found to be upregulated in MED12-mutant fibroids. Thus, we propose that MED12-mutant fibroids are transcriptionally reprogrammed at least in part through the acquisition of Wnt- and TGFβ-responsive SEs that drive high-level expression of oncogenic programs that promote tumorigenesis.

To explore the feasibility of epigenomic profiling in uterine fibroids, we conducted a pilot ChIP-seq experiment to interrogate the genome-wide distribution of H3K27ac in MED12 mutation-negative fibroid tissue from our patient cohort. Binding peaks of H3K27ac occurring within 12.5 kb of one another were stitched together, and these constituent enhancers (1,025 in number) were ranked by their respective H3K27ac signals using the algorithm ROSE (99, 100) to identify 87 SEs, providing the first catalog of these functionally important regulatory elements in the uterine fibroid setting (Fig. 11). Gene set enrichment analysis revealed SE-associated genes to be prominently linked with the following pathways: focal adhesion ($p$=5.41E$^{-11}$), ECM-receptor interaction ($p$=3.02E$^{-7}$), MAP kinase ($p$=3,21E$^{-7}$), insulin signaling (1.55E$^{-3}$), and VEGF signaling (7,11E$^{-3}$), among others. This is consistent with the known pathobiology of uterine fibroids, and reflects the biological significance of SEs in control of cell identity. These feasibility studies validate our ability to derive high-quality ChIP-seq data from patient-derived uterine fibroids, and support studies proposed above to interrogate the epigenetic landscape of MED12 WT and mutant LM-derived SCs.

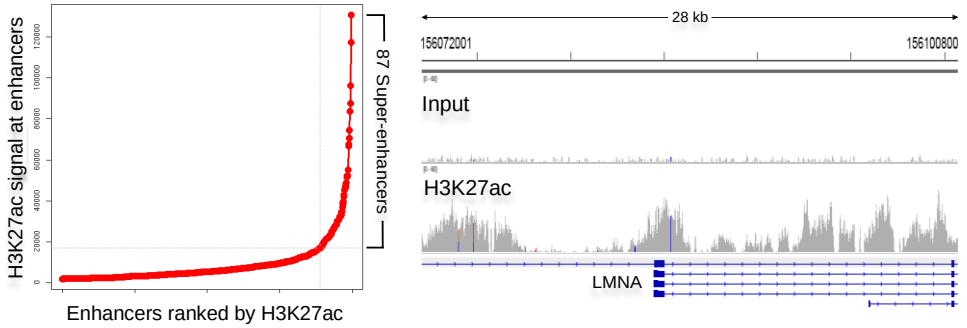



**Figure 11. Uterine fibroid SEs.** (***Left Panel***) Distribution of background-normalized H3K27ac ChIP-seq signal across 1,025 uterine fibroid enhancers, with a subset of enhancers [87 Super-Enhancers (SEs)] marked by exceptionally high amounts of H3K27ac. (***Right Panel***) ChIP-seq binding profile for H3K27ac (compared to control Input DNA) at the *LMNA* (Lamin A/C) locus, which is associated with the top-ranked SE in uterine fibroid tissue.

We propose a systematic plan to validate findings from RNA- and ChIP-seq analyses. First, we will monitor expression levels and CTD/enhancer modification patterns by conventional RT-qPCR and qChIP, respectively, on randomly selected genes in MED12 WT and mutant SCs. Second, we will establish functional relationships between MED12-regulated Pol II dynamics, enhancer histone marks, and gene expression. Thus, for selected genes, we will monitor the impact of RNAi-mediated MED12 depletion on changes in RNA pol II elongation rates, enhancer activity, and expression of linked genes. Together, studies in Aim 2 should clarify the biochemical and genetic bases of transcriptional reprogramming elicited by MED12 mutations.

***Caveats and Alternative Approaches:*** Studies in Aim 2A to define the role of WT and mutant MED12 in the CycC-CDK8/19 activation mechanism will rely on kinetic analyses, as these are based on rate measurements and thus appropriate. However, these studies could be augmented by spectroscopic or calorimetric analyses, among other analytical methods. Should time and resources permit, we will pursue these alternatives to further clarify the mechanism by which mutations in MED12 disrupt its CycC-CDK8/19 stimulatory activity. Regarding epigenomic profiling efforts in Aim 2B, possible concerns related to qualified computational expertise, often a bottleneck in systems level studies, are mitigated by the support of , established experts in genome-level and complex systems-based computational analyses, especially in the LM setting.

**Aim 3. Examine the therapeutic implications of Mediator kinase disruption in MED12-mutant LM.**

**Aim 3A. Determine if reactivation of CDK8 ameliorates the fibrotic phenotype of MED12-mutant LM.**

***Rationale:*** Our studies reveal that LM-linked mutations in MED12 disrupt its CDK8 stimulatory activity leading to aberrant RNA Pol II function (Figs. 3-5) (26, 49). We hypothesize that transcriptional reprogramming, as a pathological consequence of these events, is also a pathogenic trigger for fibrotic transformation. If true, this leads to several predictions with implicit therapeutic considerations. First, direct reactivation of CDK8 in MED12-mutant LM may transcriptionally program tumor cells to reverse their fibrotic phenotype. Second, pharmacologic modulation of signaling pathways uniquely dysregulated in MED12-mutant tumors may alternatively reverse the fibrotic phenotype. We propose the following studies to test these predictions.

***Design:*** We previously mapped the minimal CycC-CDK8 binding and activation domain in MED12 to its N-terminal 100 aa (M12N) (49). Purified recombinant MED12N, but not an otherwise identical polypeptide harboring mutation G44D (M12Nmut), binds and fully activates CycC-CDK8 in vitro (49). To determine if reactivation of CDK8 holds therapeutic potential in MED12-mutant LM, we will assess the ability of M12N to suppress the fibrotic properties of MED12-mutant LM SCs both in vitro and in vivo. To this end, SCs isolated from patient-matched MM as well as MED12 WT and mutant LM will be treated in culture with increasing concentrations (0.1-3 μM) of M12N or M12Nmut, each bearing a 9-aa peptide corresponding to the HIV TAT protein transduction domain (111, 112). At 0.5, 1, 2, 4 and 8 hours post-transduction, cells will be processed and analyzed for: (i) M12N/mut internalization and subcellular localization by immunofluorescence and immunoblot analyses; and (ii) M12N-mediated rescue of CDK8 activity in MED12-mutant LM SCs by IP/Kinase assay. Thereafter, SCs will be treated with peptide activators under conditions optimized for rescue of CDK8 kinase activity prior to molecular and biological phenotyping. Alternatively, should peptide transduction fail as a vehicle for CDK8 rescue, SCs will instead be transduced with M12N/mut-expressing lentiviruses. Peptide (or virally) transduced cells will be characterized for their proliferative capacity, ECM production, and global gene expression profiles in vitro or engrafted into mice for analysis of their tumorigenic properties, all as described in Aim 1A. The extent to which M12N ameliorates the fibrotic phenotype of MED12-mutant LM SCs will provide a measure of its tumor suppressive potential and a biochemical basis for this activity through restoration of CDK8 function. Experimental validation of this prediction will offer proof-of-concept for CDK8 activators, including peptidomimetics and/or small molecules, as therapeutic options in MED12-mutant LM.

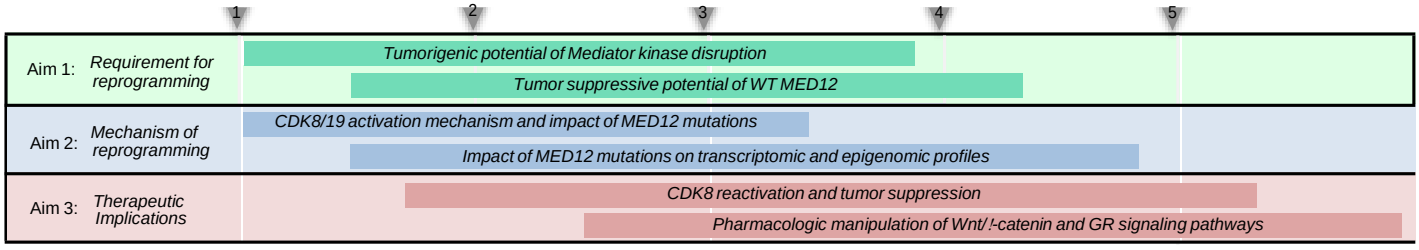
**Aim 3B. Investigate the therapeutic sensitivity of mutant MED12-dysregulated pathways in LM.**

***Rationale:*** We acknowledge the possibility that irreversible genetic or epigenetic changes elicited by MED12 mutations could limit the therapeutic efficacy of CDK8 activators, functioning upstream of these entrenched processes. In this event, pharmacologic manipulation of mutant MED12 dysregulated pathways may offer an alternative therapeutic option. Because the Wnt/β-catenin and GR pathways are activated and inhibited, respectively, in MED12-mutant LM, we will target β-catenin and GR for inhibition and activation, respectively. As shown previously by our collaborator, inhibition of Wnt/β-catenin signaling attenuates LM SC cell growth through disruption of paracrine signaling initiated by neighboring MM/LM cells (22). However, because the MED12 mutation status of SCs used in these studies was not established, the impact of mutant MED12 on paracrine Wnt/β-catenin signaling in LM is currently unknown. We propose that MED12-mutant fibroids are particularly sensitive to Wnt/β-catenin inhibition. Heretofore, the GR has not been considered a prospective therapeutic target in LM. However, our finding that GR signaling is depressed in MED12-mutant LM implies a possible therapeutic benefit for GR agonists. The following experiments will test these novel hypotheses.

***Design:*** We will target β-catenin and GR for inhibition and activation, respectively, using ICG-100 and dexamethasone (Dex). ICG-100 (Selleck Chemicals) is a small molecule inhibitor of the β-catenin/CBP coactivator interaction required for β-catenin transactivation activity (113, 114). Notably, ICG-100 has been shown effective in the inhibition of Wnt/β-catenin-driven fibrosis in mouse models of pulmonary, renal interstitial, and dermal fibrosis (115-117). Dex (Selleck Chemicals) is a synthetic glucocorticoid with potent anti-inflammatory and immunosuppressant properties and thus widespread clinical utility in the treatment of inflammatory and autoimmune disease, cancer, and fibrotic conditions (118-124). These drugs will be evaluated for their respective abilities to suppress the fibrotic properties of MED12-mutant LM SCs in vitro and in vivo. For in vitro analyses, patient-matched WT and mutant MED12 LM SCs will be cocultured in Transwells with primary MM cells (1:1 cell number ratio) in the presence of estrogen (17β-estradiol; $10^{-7}$M) plus progestin (R5020; $10^{-7}$M) to facilitate robust paracrine signaling (22). Cocultures will be treated with vehicle control, ICG-100 (5, 10, 20 mM) or Dex ($10^{-10}$ to $10^{-6}$ M) (115-117, 125, 126). At 24, 48, and 72 hours following drug treatments, cells will be evaluated as described in Aim 1A for their proliferative capacity, ECM production, and pathway-specific gene expression profiles, the latter to confirm expected pharmacologic inhibition or activation of Wnt/β-catenin and GR pathways, respectively. We will also monitor the impact of drug treatments on the tumorigenic capacity of MED12-mutant SCs using fibroid xenografts. Mice bearing 8-week-old cell grafts established as described in Aim 1A from MED12 WT or mutant LM SCs and confirmed by IVIS (Fig. 9) will be randomized into control and treatment groups (n=6 per group). Control mice will be receive vehicle, while treated mice will receive ICG-100 (IP injection; 5 mg/kg daily) or Dex (oral gavage 5 days/week; 3mg/kg body weight) (115-117, 125, 126). Animals will be sacrificed 4 weeks following initiation of drug treatment and evidence of tumor engraftment, proliferation, and fibrotic growth evaluated as described in Aim 1A. We will also monitor expression levels of β-catenin, GR, and target genes of each by immunohistochemistry. Should either drug inhibit the fibrotic properties of MED12-mutant LM SCs in vitro, we would expect that drug treatments, compared to vehicle, would significantly reduce proliferation rates and ECM production levels concomitant with pathway-specific inhibition/activation. Furthermore, compared to vehicle-treated mice, we would expect xenografts in drug-treated animals to exhibit reduced fibrotic properties, growth rates, and/or proliferative indices. Evidence for therapeutic efficacy of Dex in these LM models would be a first, and provide proof-of-concept for the use of selective GR modulators in LM treatment.

In summary we have outlined a rigorous proposal that should permit us to elucidate the molecular basis and therapeutic implications of MED12 in the pathogenesis of uterine fibroids. We expect these studies to significantly impact the personalized treatment of women with this clinically significant disease.

## *4. PROJECT TIMELINE*



## PROTECTIONS OF HUMAN SUBJECTS

### 1. RISKS TO HUMAN SUBJECTS

***(a) Human Subjects Involvement, Characteristics, and Design***

Informed written-consent female patients (N=120) undergoing elective hysterectomy/myomectomy at UTHSCA for symptomatic uterine leiomyomas (LM) will participate in this Institutional Review Board (IRB) expedited study (protocol number HSC20150071N). Due to the characteristic biology of LM, which are hormone responsive and thus principally relevant to reproductive age women, participating subjects will meet the following criteria for inclusion: premenopausal cancer-free women of unrestricted race/ethnicity who have received no hormonal treatments (including contraceptives or GnRH analogues) for 3 months prior to surgery. In addition, subjects undergoing immunomodulatory therapies or those with established gynecologic comorbidities, such as endometriosis, will be excluded.

***(b) Sources of Materials***

Paired myometrial and leiomyoma (intramural, subserosal, and submucosal) tissues from 120 patients will be harvested at the time of surgery for study purposes. In addition to written informed consent, subjects will provide information (via questionnaire) concerning their reproductive history, parity, fibroid symptoms, hormonal use, last menses, and age. Upon study entry, subjects will be assigned a unique study number to permit de-identifiable coding. This code, and clinical data linked to each subject will be stored on a private computer, which is University-encrypted and personal password-protected. Backup files maintained on a secure external hard-drive will be updated automatically on a continuous basis using computer-installed software. Individually identifiable private information will be accessible only by the physician/surgeon (), while de-identifiable information concerning patient biometric data and tumor clinicopathology will be provided to the study PI (). This information will include reproductive history, parity, fibroid symptoms, hormonal use, last menses, age, BMI, menstrual phase (proliferative *vs* secretory), and clinicopathological features including fibroid type, location, size, and characteristics including tumor cellularity, matrix deposition, and vascularity. De-identified myometrial and leiomyoma samples will be processed and analyzed in laboratory as described in the accompanying research plan.

***(c) Potential Risks***

Uterine tissue derived from patient hysterectomies/myomectomies performed under strict standards of clinical care would otherwise be discarded following surgical excision. Therefore, the proposed study does not impose additional surgical risks to subject health or safety. There is the possibility of a minimal risk of patient psychological distress arising from the disclosure of personal information. However, the voluntary nature of informed consent and the strict confidentiality assurance guidelines in place mitigate such concerns.

### 2. ADEQUACY OF PROTECTION AGAINST RISKS

***(a) Recruitment and Informed Consent***

Subjects participating in this study will be undergoing elective hysterectomy/myomectomy for symptomatic LM at University Hospital and affiliated area hospitals, including . and his faculty routinely perform in excess of 300 of these procedures annually. Therefore, no proactive recruitment effort will be required or undertaken for the proposed study. Subject written informed consent will be a requirement for study participation, and given only after consultation and communication with concerning all procedural risks.

***(b) Protections Against Risk***

The study imposes no additional subject health or safety risks beyond those routinely experienced as a course of elective hysterectomy/myomectomy, since uterine tissues derived from such procedures would otherwise be discarded. Strict subject confidentiality assurance guidelines will be followed to ensure minimal risk to privacy. These include the use of coded information to ensure de-identification of patient samples, limited investigator () access to identifiable patient information, and secure

(encrypted and password-protected) electronic storage of subject codes and individual identifiable private information. The application of these strict guidelines should significantly minimize the risk for any potential breach in confidentiality.

## 3. Potential Benefits of the Proposed Research to Human Subjects and Others

Subjects will receive no direct tangible benefits from the proposed studies. However, subjects may derive satisfaction from their informed decision to participate in studies with significant basic and translational implications for LM. Because the proposed studies impose no additional subject health or safety risks beyond those routinely experienced as a course of elective hysterectomy/myomectomy, the risks are certainly reasonable in relation to the knowledge acquired from the proposed studies.

## 4. Importance of the Knowledge to be Gained

Uterine leiomyomas (LM; fibroids) are the most frequent tumors in women worldwide and, although benign, are nonetheless associated with significant gynecologic and reproductive dysfunction. Current treatment options are limited beyond surgery, and the development of alternative effective medical therapies will require a better understanding of the underlying molecular etiology of LM. The studies proposed herein, focused on the etiological role of MED12, mutated in ~70% of LM, address these looming issues and are therefore expected have important implications for personalized treatment of women with LM.

## INCLUSION OF WOMEN AND MINORITIES

Due to the characteristic biology of uterine leiomyomas (LM), which are hormone responsive tumors of reproductive age women, only premenopausal females of unrestricted race/ethnicity will be eligible for this study. No study subject will be excluded based on racial or ethnic criteria. In fact, the participation of racial and ethnic minority females would constitute an important benefit for the study, since race is an established risk factor for LM. In this regard, African American females, in comparison to Caucasian females are reported to experience a 3-fold increase in the overall incidence and relative risk of symptomatic LM, an earlier mean age of onset, and evidence of more severe clinical disease. By contrast the risk and incidence of LM in Asian and Hispanic females does not significantly differ from that of Caucasians. Accordingly, we might therefore expect African American females to be represented among study group participants in numbers proportionally greater than their numbers in the local population.

This study is based on patients undergoing elective hysterectomy/myomectomy for symptomatic LM within the local and surrounding communities. The demographic racial breakdown for this area of is as follows: Caucasian, 67.8%; African American, 6.7%; Asian, 1.6%; Mixed Race, 3.9%, and Other Races, 20%. Mexican-Americans/Hispanics, who may be of any race, constitute 59% of the local population but only 17% nationally. Thus, although we would expect our study to reflect a significant number of U.S. minority subjects, we acknowledge that this fact is not clinically or scientifically meaningful as there is no evidence that Mexican-American/Hispanics, the predominant minority group represented in this study, experience a higher incidence of LM than the general population.

OMB Number: 0925-0002

# Planned Enrollment Report

**Study Title:** Molecular basis of MED12 in the pathogenesis of uterine fibroids

**Domestic/Foreign:** Domestic

**Comments:**

| Racial Categories | Ethnic Categories | | | | Total |
|---|---|---|---|---|---|
| | Not Hispanic or Latino | | Hispanic or Latino | | |
| | Female | Male | Female | Male | |
| American Indian/Alaska Native | 0 | 0 | 0 | 0 | **0** |
| Asian | 2 | 0 | 0 | 0 | **2** |
| Native Hawaiian or Other Pacific Islander | 0 | 0 | 0 | 0 | **0** |
| Black or African American | 10 | 0 | 0 | 0 | **10** |
| White | 36 | 0 | 66 | 0 | **102** |
| More than One Race | 2 | 0 | 4 | 0 | **6** |
| **Total** | **50** | **0** | **70** | **0** | **120** |

Study 1 of 1





Funding Opportunity Number: PA-13-302. Received Date: 2016-03-04T09:02:52.000-05:00

## INCLUSION OF CHILDREN

Due to the characteristic biology of uterine leiomyomas (LM), which are generally slow-growing hormone responsive tumors of reproductive age women, the incidence of symptomatic LM in adolescent females is rare. In fact, only 11 such cases have been reported in the literature. Furthermore, female children (under 21 years of age) who might present with clinical LM are unlikely to undergo hysterectomy in order to preserve reproductive function. For these reasons female children will be excluded from this study.

## VERTEBRATE ANIMALS

### 1. DESCRIPTION

Species: ***Mus musculus***, (Mouse)
Strain: nonobese diabetic-scid mice (NOD-SCID; strain code 394; NOD.CB17-Prkdc$^{scid}$/NcrCrl)
Number: 180 (See point 2 for number justification)

Studies in Aim 1 and 3 of this application seek to investigate the oncogenic nature and therapeutic implications of Mediator kinase disruption linked to MED12-mutant uterine fibroids. We propose the use of mice to address the following questions: *(Aim 1A)* Is CycC-CDK8/19 kinase disruption oncogenic and therefore sufficient to induce myometrial (MM) stem cell transformation in vivo? (*Aim 1B)* Can ectopically expressed WT MED12 suppress (through restoration of CycC-CDK8/19 kinase activity) the tumorigenic potential of MED12-mutant LM stem cells in vivo? (*Aim 3A*) Can restoration of CycC-CDK8/19 function in MED12-mutant LM stem cells reverse their fibrotic phenotype in vivo? (*Aim 3B*) Will pharmacologic manipulation of Wnt/β-catenin or glucorticoid receptor (GR) signaling pathways reverse the fibrotic phenotype of MED12-mutant LM stem cells in in vivo? These questions will be addressed using an established mouse xenograft model for fibroid tumor formation (19).

For these studies, Stro-1$^+$/CD44$^+$ Molday Ion Rhodamine B (MIRB)-labeled MM or LM stem cells (1 X $10^6$ cells) will be suspended into (0.01 ml) rat-tail collagen (type I) solution. Cell pellets will be incubated at 37°C overnight as floating cultures and mixed with an equal volume of high concentrated Matrigel HC supplemented with epidermal growth factor (0.5 ng/ml), basic fibroblast growth factor (2 ng/ml) and insulin (5 μg/ml). Stro-1$^+$/CD44$^+$ MIRB-labeled MM or LM stem cells will be injected under the kidney capsule of 6 week-old female nonobese diabetic-scid (NOD-SCID) mice (n=6 per group) supplemented with estrogen (E)/progesterone (P) (0.1 mg/50 mg, respectively in 60-day or 90-day slow release pellets depending on experimental protocol) implanted subcutaneously 48 hours prior to engraftment. Hormone supplementation is necessary to decrease variability and inconsistency in the growth of hormone responsive tumors. For cell engraftments and hormone supplementation, mice will be anesthetized with xylazine/ketamine and incisions sealed using Histoacryl tissue adhesive. The biological impact of hormone supplementation on the host female reproductive tract will be confirmed by gross appearance and histology, as well as the presence of hormone pellets, at the time of euthanasia. For cell engraftments, kidneys will be externalized and mice will be injected beneath the renal capsule with single-cell suspensions (1 X $10^6$ cells) from Stro-1$^+$/CD44$^+$ MIRB-labeled MM or LM stem cell grafts along with an equal number of MM/LM primary cells. The mice will be sutured with a single horizontal mattress suture consisting of 6-0 polyglactin 910, hydrated with 1 ml of sterile saline, and placed on a heating pad in a housing container until recovery from anesthesia.

For drug treatment experiments described in Aims 1A and 3B, vehicle, CCT251545, ICG-100, or dexamethasone (Dex) will be administered as follows to mice (n=6 per group) supplemented with E+P (60-day slow release dose for CCT251545 experiments, 90-day slow release dose for ICG-100 and Dex experiments): CCT251545 will be administered at a dose of 37.5 mg/kg body weight by oral gavage twice daily, ICG-100 will be administered at a dose of 5mg/kg body weight daily by intraperitoneal (IP) injection, and Dex will be administered at a dose of 3mg/kg body weight by oral gavage 5 days/week (115-117, 125, 126). Administration of CCT251545 will commence immediately prior to cell engraftments (to maintain systemic concentrations of kinase inhibitor) while ICG-100 and Dex will commence 8 weeks after cell engraftments (when tumors are generally ~2-3 mm$^3$).

Tumor volume will be estimated by the formula: $V=LxW^2x0.5$, where L is length and W is width of tumor xenografts. Because LM grow slowly as xenografts, evidence of engraftment and reconstruction of leiomyoma tissue may be more effectively confirmed by histological and immunohistochemical analyses to monitor tissue morphology, ECM production, collagen and smooth muscle fiber content, proliferative indices, and hormone receptor status (18, 19, 21). This will be done following euthanasia of mice at 8 weeks (Aim 1 and 3A studies) or 12 weeks (Aim 3B studies) following cell engraftments.

The Biostatistics and Bioinformatics Shared Resources provided through the Department of Epidemiology and Biostatistics at the University of Texas Health Science Center at San Antonio () will provide fee-for-service statistical support on experimental design (including reassessment of group composition and sample size prior to initiation of a new series of experiments) and statistical analysis for the proposed studies. All exact statistical analyses on counts or ordered and unordered categorical variables will be done using StatXact 8 (Cytel Corp., Cambridge, MA). Tumor assessment will be based on quantitative measures of tumor growth, proliferative indices and morphometric parameters.

**2. JUSTIFICATION**

LM represent the most frequent tumors in women worldwide. Although benign, they nonetheless account for significant gynecologic and reproductive dysfunction. Current treatment options are primarily surgical, and the development of alternative medical therapies will require a better understanding of the underlying molecular etiology of LM. Our recent breakthrough findings concerning the biochemical basis by which MED12 mutations, found in ~70 LM, impact MED12/Mediator function (26, 49, 51) offers new opportunities to clarify the etiology of LM and identify novel pharmacologic agents for personalized treatment of this disease. We found that LM-linked mutations in MED12 disrupt its ability to activate CDK8 in Mediator, leading to diminished site-specific phosphorylation of RNA Pol II and global dysregulation of gene expression. Moreover, we identified genetic programs uniquely dysregulated in MED12-mutant tumors, leading us to hypothesize that Mediator kinase disruption as a consequence of MED12 mutations elicits transcriptional reprogramming and altered signaling sufficient to drive MM stem cell (SC) transformation. We further hypothesize that MED12-mutant LM may be therapeutically susceptible to restoration of CDK8 function or pharmacologic manipulation of pathways uniquely dysregulated in MED12-mutant tumors. As part of our approach to test these hypotheses, we propose to examine whether genetic or chemical disruption of endogenous CDK8 can fibrotically transform MM SCs, and conversely, whether restoration of CDK8 function or modulation of dysregulated signaling pathways can reverse the fibrotic phenotype of MED12-mutant LM SCs. The most important measure of fibrotic transformation in these experiments will be fibroid tumor formation using an established mouse xenograft model (19).

The mouse xenograft model is necessary to achieve the objectives of this proposal for the following reasons: (i) analyses of clinical tumor samples cannot address the causal relationship between MED12 mutations and LM; (ii) presently available cell culture systems cannot faithfully recapitulate the complex and multifaceted nature of tumorigenesis in vivo, including tumor-stroma interactions; (iii) while in vitro models can be used to examine the impact of therapeutic compounds on specific biological pathways and endpoints relevant to LM, including proliferation and ECM production, this system cannot recapitulate the complex physiological milieu required for preclinical studies. Thus an *in vivo* model is required for the proposed studies, and in this regard, the NOD-SCID mouse model represents a current standard for growth and reconstruction of LM tissue in engrafted animals.

Justification for animal numbers: Animal numbers are based on power analyses (paired t-test or one-way ANOVA as appropriate; NCSS PASS 13) using published (19) as well as unpublished xenograft datasets from our co-investigator, , and our collaborator,. Numbers required for xenograft studies are shown below. If statistical significance is not reached with these numbers, which we believe unlikely, then sample sizes will be increased accordingly:

Experimental animal numbers:

Aim 1A:

*Experiment 1:*

| | | |
|---|---|---|
| CDK8/19 KD mutant knock-in MM SC (2 clonal lines each) | x 6 mice/group = | 24 mice |
| L36R/G44D mutant MED12 knock-in MM SC (2 clonal lines each) | x 6 mice/group = | 24 mice |
| Control (CDK8/19 and MED12 WT) MM SC (for 2 clonal lines tested) | x 6 mice/group = | 12 mice |
| Control MED12-mutant LM SC (for 2 clonal lines tested) | x 6 mice/group = | 12 mice |

*Experiment 2:*

| | | |
|---|---|---|
| MM SC + vehicle | x 6 mice/group = | 6 mice |
| MM SC + CCT251545 | x 6 mice/group = | 6 mice |
| Control MED12-mutant LM SC | x 6 mice/group = | 6 mice |
| Control MM SC | x 6 mice/group = | 6 mice |

Aim 1B:

*Experiment 1:*

| | | |
|---|---|---|
| Ectopic WT MED12-expressing LM SC | x 6 mice/group = | 6 mice |
| Ectopic G44D MED12-expressing LM SC | x 6 mice/group = | 6 mice |
| Control MED12-mutant LM SC | x 6 mice/group = | 6 mice |
| Control MM SC | x 6 mice/group = | 6 mice |

Aim 3A:
*Experiment 1:*

| | | |
|---|---|---|
| M12N/M12Nmut transduced MED12 mutant LM SC | x 6 mice/group = | 12 mice |
| Control MED12-mutant LM SC | x 6 mice/group = | 6 mice |
| Control MM SC | x 6 mice/group = | 6 mice |

Aim 3B:
*Experiment 1:*

| | | |
|---|---|---|
| MED12-mutant LM SC + vehicle (2 control groups) | x 6 mice/group = | 12 mice |
| MED12-mutant LM SC + ICG-100 | x 6 mice/group = | 6 mice |
| MED12-mutant LM SC + Dex | x 6 mice/group = | 6 mice |
| Control MED12-mutant LM SC | x 6 mice/group = | 6 mice |
| Control MM SC | x 6 mice/group = | 6 mice |
| | Total mice | = 180 mice |

**3. VETERINARY CARE**

The Laboratory Animal Resources (LAR) program at is under the leadership director, who supervises several full-time clinical veterinarians. In addition, an experienced, full-time mouse pathologist, , will be available to evaluate and interpret pathological results from the animal studies. The program is AAALAC accredited. The veterinarians directly oversee all aspects of animal husbandry and review all animal use protocols as voting members of the Institutional Animal Care and Use Committee (IACUC). All animal use protocols are fully reviewed by the IACUC. Investigators must demonstrate that they have considered suitable alternatives to any procedures that provoke animal pain or distress. Veterinary advice on suitable anesthetic and analgesic regimes is consistently available to investigators. Humane endpoints are established for all potentially painful procedures and investigators are challenged to use the minimum number of animals possible.

Animals will be housed in a modern air-conditioned Specific Pathogen Free (SPF) environment, including microisolator-top cages and filtered-air cabinets, located within the on the campus. LAR veterinarian staff will monitor animals daily, and staff veterinarians will be available for consultation as well as routine and emergency animal care. In addition to LAR veterinary staff, we will also monitor animals daily following cell engraftments and maintain records related to body weight, tumor size/location, and overall animal health. Mice found to be moribund during this period will be euthanized to minimize distress, discomfort, suffering, and pain. All procedures will be approved by the and, accordingly, will conform to the standards set forth in the NIH Guide for the Care and Use of Laboratory Animals.

**4. PAIN AND DISCOMFORT MANAGEMENT**

Cell engraftments into mice will require the injection of 1 x $10^6$ cells in a rat-tail collagen/Matrigel mixture under the renal capsule of 6 week-old ovariectomized female nonobese diabetic scid (NOD-SCID) mice using a 27-gauge needle under the influence of xylazine/ketamine anesthesia. For ovariectomy, animals will be anesthetized via xylazine/ketamine. Once anesthetized, animals will be placed in the supine position within a sterile laminar-flow hood. The abdominal area will be prepped with a10% povidone-iodine solution, and ovaries excised. The mice will be sutured with a single horizontal mattress suture consisting of 6-0 polyglactin 910, hydrated with 1 ml of sterile saline, and placed on a heating pad in a housing container until recovery from anesthesia. Mice will be monitored daily following cell engraftments and records related to body weight, tumor size/location, and overall animal health will be maintained. Mice found to be moribund during this period will be euthanized to minimize distress, discomfort, suffering, and pain.

**5. EUTHANASIA**

At the termination of each experiment, mice will be euthanatized via CO2 inhalation to effect followed by cervical dislocation, a preferred method of choice not only consistent with recommendations of the Panel on Euthanasia of the American Veterinary Medical Association, but one compatible with downstream histological and biological studies.

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February 17, 2016

Re: Letter of Collaboration for R01

Dear :

It is with great pleasure that I write this letter in support of your NIH R01 grant application “Molecular basis of MED12 in the pathogenesis of uterine fibroids”. I agree to serve as Co-Investigator at 5% effort on this highly innovative and important grant. I am well aware of your remarkable work on the molecular basis of MED12 mutations in uterine leiomyomas, and I will be delighted to work with you to pursue this outstanding research question moving forward.

As you know, my laboratory has a long-standing interest in reproductive medicine, with an emphasis on the basic biology and development of novel therapeutics, including nutritional supplement-based, gene, and stem cell therapies for premature ovarian failure, endometriosis, and particularly uterine fibroids. Accordingly, and particularly pertinent to your studies, we have developed exceptional expertise in the isolation, maintenance, and biochemical and biological analysis of human myometrial and uterine leiomyoma stem cells. Moreover, we have appropriately proven expertise in relevant in vitro and in vivo animal systems with which to model uterine fibroids, including the mouse renal capsule xenotransplantation model. As we have discussed, we will be happy to provide you with technical assistance and conceptual insight as needed with respect to your proposed aims, including those related to stem cell culture and manipulation, as well as the use of the mouse xenotransplantation assay to model fibroid growth. I find your proposal to be highly innovative and believe it has tremendous potential to provide significant advancement for the uterine leiomyoma field.

I look forward to collaborating with you on this important and innovative study.

With Best Regards,

February 18, 2016

**Re: NIH R01 Collaboration**

Dear:

I am writing this letter to express my enthusiasm and willingness to collaborate and consult with you on your R01 application entitled *Molecular basis of MED12 in the pathogenesis of uterine fibroids*. I am intrigued and truly impressed by the weight and quality of data that serves as the foundation for your application, including your important new findings that reveal how mutations in MED12, found in nearly 70% of uterine fibroids, impact its biochemical function through disruption of Mediator-associated CDK activity. This groundbreaking discovery clearly opens up many new routes for further basic and clinically applied studies, including those with possible therapeutic potential.

As you know, my research program focuses on estrogen metabolism and hormone-dependent human diseases, including breast cancer, uterine leiomyomas, and endometriosis. Thus, we clearly have much common ground to explore in this area. We also have extensive experience in development and use of both in vitro cell-based and in vivo animal models as it relates to reproductive disease and cancer, including the derivation, culture, and characterization of uterine tissue-derived primary cell lines and, notably, somatic stem cells. Furthermore, as you are aware we have developed an excellent in vivo tumor model based on injection of leiomyoma cells under the renal capsule, which provides for more reliable establishment of tumor xenografts characterized by more consistent growth characteristics. In this regard, we will be happy to assist you in the development and application of this tumor model. In addition, we can provide you with technical and conceptual insight and support regarding the isolation and culture of uterine leiomyoma stem cells to ensure that these studies are implemented and successfully conducted in the most expeditious and technically proficient manner possible.

In summary, we are committed to work with you and offer both material and technical assistance as needed, as well as conceptual insight that might be important to advance the goals of this project. We look forward to a most fruitful collaboration with you on this exciting application.

Best Regards,

08 February 2016

Dear:

This letter will confirm my sincere interest and intent to collaborate with you on your NIH R01 application titled "Molecular basis of MED12 in the pathogenesis of uterine fibroids". Of course, these interactions will continue what has already proven to be a most productive collaboration between our laboratories. I am very excited about the initial results that we have so far generated from these collective efforts, including those that were already published in in *Ce// Reports.* The discovery that exon 1 and 2 mutations in *MED12* linked to uterine leiomyomas disrupt Mediator-associated CDK activity represents the first description of a functional defect associated with these mutations and thus represents a major step forward in understanding the molecular etiology of these tumors. Along with our recent whole genome sequencing and profiling analyses in myometrium and uterine leiomyomas (including both $WT$ and mutant MED12), these findings provide strong support for your hypothesis that Mediator kinase disruption secondary to MED12 mutations triggers transcriptional reprogramming and altered signaling to drive fibrotic transformation of myometrial cells. Clearly, our respective strengths and relevant areas of expertise in molecular genetics and biochemistry dovetail precisely as they relate to this project, and I am confident that our continued allied efforts will lead to important new insights concerning the role of MED12 in uterine leiomyomas as well as other human neoplasms.

As part of our longstanding and ongoing research interests in the molecular genetics of human tumor susceptibility, including uterine leiomyomas, our working group has established a fluid pipeline and an array of next generation genomics- and proteomics-based technologies, including exome, methylome, and whole genome sequencing, expression profiling, and mass spectrometry . We are closely connected to the relevant clinics, and have excellent access to tumor samples as well as the relevant patient record data. We are happy to make available to you these resources moving forward, as well as our own unique insights and relevant conceptual and technical expertise concerning the molecular genetics of uterine leiomyomas.

I look forward to continued productive interactions with you and your research group on this exciting and fast moving project.

Sincerely,

February 11, 2016

Re: R01 Letter of Collaboration

Dear:

I am pleased to write this letter in support of your R01 application entitled "Molecular basis of MED12 in the pathogenesis of uterine fibroids." I will be happy to serve as a collaborator on your grant. I find this proposal highly innovative, and I believe it has tremendous potential to provide a significant advancement for women's health and reproductive dysfunction related to uterine fibroids. As we have discussed in detail, we have many common interests as they relate to women's health and reproductive tract disease, and I am confident that our respective strengths will be complementary and advance the aims of this application.

As part of our clinical practice in the, we maintain affiliations with many area hospitals, including, , and, where my faculty and I annually perform hysterectomies and myomectomies for fibroids on more than 300 patients annually. I will be happy to provide you with myometrial and fibroid tissues obtained from such patients after appropriate informed-consent. We can also provide you with histopathological confirmation of fibroids.

In addition, as part of our basic research interests in reproductive endocrinology, we have established a biodepository for reproductive tract tissues including endometriosis, eutopic endometrium, malignancies, etc. We also have considerable experience in the derivation, maintenance, and routine culture of primary cell lines derived from reproductive tract tissues. Furthermore, we have developed appropriate expertise in the isolation and molecular characterization of stem cells from reproductive tract tissues for investigation in primate models. Should you require it, we can provide you with technical assistance and conceptual insight as it relates to the isolation and culture of such cells.

We are fully committed to working closely with you on this important and innovative study. Our overlapping research interests, complementary expertise, and close proximity on campus will establish productive and meaningful interactions necessary to achieve the aims of proposal.

Sincerely,

January 23rd, 2016

Dear ,

The and the are delighted to collaborate with your group on your R01 proposal titled “Molecular basis of MED12 in the pathogenesis of uterine fibroids” that will use next gen sequencing technology to perform RNA-seq and ChIP-seq experiments.

As we discussed and have proven through your pilot ChIP-seq experiments, we believe that we are in a good position to assist you by providing state-of-the-art sequencing and data analysis services. Our, at the, was formally opened in June 2011 with the arrival of an Illumina HiSeq 2000 and all necessary peripheral instrumentation to carry out genome sequencing (including *de novo* whole genome sequencing, exome sequencing, and candidate gene re-sequencing), transcriptome analysis (including RNA-seq and small RNA seq), and ChIP-seq for the studies aiming to understand genetic variation, expression profiles, and epigenetic regulation of the genome. The facility is staffed with full-time technical scientists and a facility director, and overseen by a faculty advisory committee for and. We can assure you that our facility and its personnel will be fully committed to the success of your project. In addition, as we discussed earlier, the, headed by, is fully committed to provide data analyses of the highest possible quality for your project.

As with our prior collaborations, the will receive RNA and ChIP DNA samples from your lab. We will perform sample QC, library preparation, sequencing and data analysis. We are most confident we will meet/exceed the proposed high-throughput sequencing activities. We wish you success with your grant application, and we look forward to collaborating with you on this exciting project.

Best Wishes,

## RESOURCE SHARING PLAN

### DATA SHARING PLAN

We will work diligently to deploy mechanisms for data sharing with investigators in the scientific community. RNA-seq and ChIP-seq data will be deposited in our database managed by the. Prior to publication, all epigenetic and genetic data will be entered in the Gene Expression Omnibus depository. Raw data and metadata will be exported and shared with other investigators. We will adhere to the NIH Grants Policy on Sharing of Unique Research Resources and make all analytical algorithms, should they be developed during the course of this project, freely and fully available to the research community.