| | | |
|---|---|---|
| PI: **Amm, Hope** | Title: Hedgehog Pathway Activity and Targeting in KCOT | |
| Received: 03/12/2014 | FOA: PA14-042 | Council: 10/2014 |
| Competition ID: FORMS-C | FOA Title: NIH PATHWAY TO INDEPENDENCE AWARD (PARENT K99/R00) | |
| **1 K99 DE023826-01A1** | Dual: CA | Accession Number: 3679693 |
| IPF: 1288803 | Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM | |
| Former Number: | Department: Institute of Oral Health Resea | |
| IRG/SRG: DSR | AIDS: N | Expedited: N |
| Subtotal Direct Costs<br>(excludes consortium F&A)<br>Year 1: 114,239<br>Year 2: 117,383<br>Year 3: 249,000<br>Year 4: 249,000<br>Year 5: 249,000 | Animals: Y<br>Humans: Y<br>Clinical Trial: N<br>Current HS Code: 54<br>HESC: N | New Investigator:<br>Early Stage Investigator: |
| | | |
| *Senior/Key Personnel:* | *Organization:* | *Role Category:* |
| Hope Amm | University of Alabama at Birmingham | PD/PI |
| Mary MacDougall | University of Alabama at Birmingham | Other (Specify)-Mentor |
| Andra Frost | The University of Alabama at Birmingham | Other (Specify)-Co-Mentor |
| Peter Waite | University of Alabama at Birmingham | Consultant |

*Appendices*

Amm ren 2012 jbc kcot hh,Ren 2011 cto ab1k1ceot1,Genentech approved mta

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**
DE023826

**b. Agency Routing Number**

| 2. DATE SUBMITTED | Application Identifier |
|---|---|
| | |

**c. Previous Grants.gov Tracking Number**
GRANT11605609

**5. APPLICANT INFORMATION** **Organizational DUNS*:** 063690705

Legal Name*: University of Alabama at Birmingham
Department: Office of Sponsored Programs
Division:
Street1*: 1720 2nd Avenue S
Street2: AB 1170
City*: Birmingham
County: Jefferson
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 352940111

Person to be contacted on matters involving this application
Prefix: Ms. First Name*: Ashley Middle Name: N Last Name*: Davis Suffix:
Position/Title: Grants and Contracts Officer
Street1*: 1720 2nd Avenue S
Street2: AB 1170
City*: Birmingham
County: Jefferson
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 352940111
Phone Number*: 2059346956 Fax Number: 2059755977 Email: ashleydav@uab.edu

**6. EMPLOYER IDENTIFICATION NUMBER** *(EIN) or (TIN)** 1636005396A6

**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***
Hedgehog Pathway Activity and Targeting in KCOT

| 12. PROPOSED PROJECT | | 13. CONGRESSIONAL DISTRICTS OF APPLICANT |
|---|---|---|
| Start Date* | Ending Date* | AL-007 |
| 09/01/2014 | 08/31/2019 | |

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



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

Prefix: Dr. First Name*: Hope Middle Name: M Last Name*: Amm Suffix:

Position/Title: Post-Doctoral Fellow
Organization Name*: University of Alabama at Birmingham
Department: Institute of Oral Health Resea
Division: School of Dentistry
Street1*: 1720 2nd Avenue S
Street2: SDB 704
City*: Birmingham
County: Jefferson
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 352940111
Phone Number*: 2059965122 Fax Number: 2059965109 Email*: hopeamm@uab.edu

**15. ESTIMATED PROJECT FUNDING**

| | |
|---|---|
| a. Total Federal Funds Requested* | $997,152.00 |
| b. Total Non-Federal Funds* | $0.00 |
| c. Total Federal & Non-Federal Funds* | $997,152.00 |
| 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: Mrs. First Name*: Lynn Middle Name: W Last Name*: Stedman Suffix: MBA

Position/Title*: Director
Organization Name*: University of Alabama at Birmingham
Department: Office of Sponsored Programs
Division:
Street1*: 1720 2nd Avenue S
Street2: AB 1170
City*: Birmingham
County: Jefferson
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 352940111
Phone Number*: 2059345266 Fax Number: 2059755977 Email*: osp@uab.edu

**Signature of Authorized Representative***
Ashley Davis

**Date Signed***
03/12/2014

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

**21. COVER LETTER ATTACHMENT** File Name:1235-K99_Cover_Letter_HMA.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: | University of Alabama at Birmingham |
| Duns Number: | 0636907050000 |
| Street1*: | 1919 7th Avenue South |
| Street2: | SDB 717 |
| City*: | Birmingham |
| County: | Jefferson |
| State*: | AL: Alabama |
| Province: | |
| Country*: | USA: UNITED STATES |
| Zip / Postal Code*: | 352940007 |
| Project/Performance Site Congressional District*: | AL-007 |

File Name

**Additional Location(s)**





Funding Opportunity Number: PA-14-042. Received Date: 2014-03-12T11:37:54.000-04:00

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: 10-18-2013

Human Subject Assurance Number 00005960

**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 A3255-01

**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*** | 1236-Project Summary_HMA_022014.pdf |
| **8. Project Narrative*** | 1237-Project_Narrative_HMA.pdf |
| **9. Bibliography & References Cited** | 1238-Bibliography_resub.pdf |
| **10.Facilities & Other Resources** | 1239-Resources_and_Facilities_HMA.pdf |
| **11.Equipment** | 1240-Equipment_HMA.pdf |
| **12. Other Attachments** | 1241-ListofReferees.pdf |





Funding Opportunity Number: PA-14-042. Received Date: 2014-03-12T11:37:54.000-04:00

**Project Summary/Abstract**

Keratocystic odontogenic tumors (KCOT) patients are treated surgically with more conservative surgical approaches leading to higher rates of recurrence. Research to reduce recurrence of KCOT is very limited and only focused on surgical technique, primarily owing to the severe lack of cellular models for preclinical studies for the identification and testing of therapeutic options. The aims of this project are designed to utilize novel cell models of KCOT to explore hedgehog (HH) pathway signaling, the role of HH receptor polymorphisms in KCOT, and the potential to use HH as a therapeutic target for KCOT and other craniofacial tumors. KCOT can be non-syndromic or syndromic in association with Nevoid basal cell carcinoma syndrome, an autosomal dominant genetic disease characterized by a mutation in the inhibitory receptors of the HH pathway, Patched 1/2 (PTCH1/2). When the receptor in mutated, it is believed to be unable to inhibit Smo and cause ligand-independent HH signaling, cell proliferation, and tumorigenecity. The K99 phase proposes to (1) to determine if the PTCH1 receptor polymorphism Pro1315Leu, identified in 75% of our KCOTs, has a functional significance in HH pathway activity by accessing cell proliferation, HH transcriptional activity, and tumorigenic and invasive properties in cells expressing PTCH with and without the polymorphisms; and (2) examine the biological effects and therapeutic efficacy of HH inhibition on primary KCOT cell populations, using clinically relevant HH inhibitors (GDC-0449 and LDE225, both currently in clinical trials) as well as selectively targeting the Smo protein using siRNA knockdown. During this time the candidate will complete mentored training in molecular biology and courses in clinical translation research and professional development in preparation for the independent R00 phase where it is proposed to develop animal model of KCOT for testing *in vivo* efficacy of HH inhibitors, starting with xenograft models of KCOTs. Information gleaned from this work may be useful in understanding the role of PTCH1 in fundamental HH signaling in human neoplasias and used in the generation of future clinical trials focusing on preventing KCOT recurrence.

**Project Narrative**

Keratocystic odontogenic tumors (KCOTs) are locally aggressive tumors that invade the jaw bone requiring surgery and no therapies are available to prevent recurrence. An association exists between KCOTs and alterations in the hedgehog (HH) signaling pathway and the aims of this project will be to explore HH function in novel KCOT cell models and examine clinically relevant drugs, which target HH signaling, in KCOTs. Information gleaned from this work may be used in the generation of future clinical trials focusing on therapeutic interventions for preventing KCOT recurrence.

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*These authors contributed equally to this work.

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**Resources/Facilities**

**SOD Institute of Oral Health Research (IOHR) and MacDougall Laboratory –** The SOD underwent a $4.8 million renovation of the 7th and 8th floor research space. This has included removal of interior walls, re-directed plumbing/heating fixtures, and completed re-structuring of several interior walls. This renovation has provided more than 26,000 square feet of modern wet laboratory space with offices. These floors house the Institute of Oral Health Research (IOHR), with flexible space for use by all SOD faculty doing biomedical research requiring tissue culture, molecular biology, biochemistry, immunology, and microbiology resources. The floor has administrative offices for the director (**Dr. Mary MacDougall**), ten faculty, six post-doctoral fellows and two administrative personnel, with two conference rooms/library in close proximity. It contains six wet laboratories approximately 800 sq. ft. each with a common lab having easy access to all investigators. The floor has emergency power, central de-ionized water source, and common-use equipment (e.g., autoclaves, glassware washer, incubators, ultra centrifuges, microcentrifuges, scintillation counter, -20° and -80° freezers, lyophilizer, fume hoods, water baths, sonicator, ice machine). There are 6 desk locations for postdoctoral trainees, each with voice/data jack and wireless internet connections. Each postdoctoral trainee has a laptop computer. **Dr. MacDougall's research area** is on the 7$^{th}$ floor in the School of Dentistry (SOD); it has over 800 square feet of space, including a large tissue culture room with two class II biosafety cabinets and $CO^2$ incubators, a microscope room, histology areas, and storage areas. There is an area designated for implantation and dissection for animal work. The laboratory includes all the equipment (see Equipment section for detailed listing) and supplies needed for cell culture, RT-PCR, DNA, RNA, and protein gel electrophoresis, immunohistochemistry, immunofluorescence, and molecular biology techniques (e.g. cloning and plasmid preparation).

**Dr. Frost's Laboratory –** Dr. Frost's research laboratory occupies approximately 1000 square feet, including cell culture facilities within the Wallace Tumor Institute, the newly renovated (2012) multi-million dollar Comprehensive Cancer Center. The laboratory includes all the equipment (see Equipment section for detailed listing) and supplies needed for cell culture, preparation and analysis of cell lysates for their protein, RNA and DNA content, frozen section preparation, immunohistochemistry, immunofluorescence, laser capture microdissection, and molecular biology techniques, such as plasmid preparation and cloning, and transfection and transduction of cultured cells. Immediately adjacent to the laboratory are dishwashers, drying ovens, autoclaves, and radiographic film development facilities.

**Animal Facilities.** The UAB Animal Resources Program (ARP) encompasses 140,000 ft2 of animal housing, procedure and support space in 17 different buildings on campus with an additional 104,000 ft$^2$ under construction. The Southeastern Regional Biocontainment Laboratory provides additional biosaftey level 3 research space (41,000 ft$^2$) for UAB and regional investigators investigating emerging diseases. The UAB-ARP average daily animal census of 82,000 animals is composed of a wide diversity of species including 78,000 mice, 2,000 rats, and 161 nonhuman primates. All animal facilities at UAB are fully accredited by the American Association for Accreditation of Laboratory Animal Care. UAB complies with the NIH policy on animal welfare, the Animal Welfare Act and all other applicable federal, state and local laws. Seventy-five technicians and supervisors provide husbandry care. Four veterinarians and four animal health technicians provide veterinary medical care (24hrs/day, 7 days/week). The Director and Associate Director are board certified by the American College of Laboratory Animal Medicine. The Comparative Pathology Laboratory staffed with two board-certified veterinary pathologists provide diagnostic and research services for the UAB ARP and investigators using animals. Drs. MacDougall and Amm's mice will be housed in the Lyons-Harrison Building adjacent and connected to the School of Dentistry on the same floor as the Institute of Oral Health Research.

**UAB Specialized Research Facilities**

An exceptional feature of UAB is the operation of a system of University-wide Interdisciplinary Research Centers (UWIRC) which provide a robust infrastructure for research and training that transcendsdepartmental structures and clinical specialties. The Centers are provided substantial Institutional financial support to foster their ongoing interdisciplinary and multidisciplinary research efforts. These multidisciplinary centers are available to all UAB investigators and greatly enhance the research opportunities and career development of their trainees. The Center-associated core facilities and enrichment programs are key trainee resources. Dr. Amm will interact primarily with the Comprehensive Cancer Center and Heflin Center for Genetics. Each Center maintains specialized core facilities. These facilities have been developed to facilitate research by making available the latest instrumentation and specialized research laboratories. In many instances, the facilities also serve as important resources in providing pre-doctoral

students and post-doctoral fellows training and experience in the use of instruments and the ability to carry out specialized techniques. Facilities include but are not limited to:

**Comprehensive Cancer Center** Members of the CCC have access to a broad array of common equipment within the Wallace Tumor Institute including Beckman centrifuges and rotors (two ultracentrifuges, three Avanti J-25 centrifuges, J-6 low-speed centrifuge), a phosphorimager, Zeiss Axioplan II motorized microscope with brightfield, darkfield, fluorescence, DIC, digital camera; two Precision bacterial incubators; speed vac and gel dryer, dark room with film developer, two bacterial shakers, scintillation detector, UV spectrophotometer, dishwasher, dish drying oven, autoclaves, plate readers, an Applied Biosystems 7500 Real-time PCR system, and a luminometer. Numerous core facilities are available including biostatistics and informatics, microarrays, and, clinical protocol and data management

**Heflin Genomics Core Facility.** The Genomics Core Facility has three high-priority technological resources located in the Kaul building: microarray analyses, high-throughput sequencing, and high-throughput genotyping, including single nucleotide polymorphisms (SNPs). The Molecular and Genetic Bioinformatics Facility within the core supports the bioinformatics needs of investigators.

**The UAB DNA Sequencing and Analysis Core Facility.** Under direction of Beatrice H. Hahn, MD, the DNA Sequencing and Analysis Core Facility equipment includes the following: an ABI 3730xl Genetic Analyzer with a 96 capillary array; an ABI 3130xl Genetic Analyzer with 16 capillary array to provide a high throughput automated fluorescence-based DNA sequencing using capillary assisted electrophoresis; one ABI 377 sequenator to provide automated fluorescence-based DNA sequencing using a slab-gel electrophoresis system; a Beckman Coulter Laboratory Automation Workstation (Biomek NX S8), which provides automated liquid handling operations for sequencing reaction set-up and clean-up; four GeneAmp PCR Systems (three 9700, and one 9800) Thermocyclers, which support all cycle sequencing and RT-PCR reactions that amplify DNA templates through the use of specific primers and Taq polymerase and cycling temperature steps; a Labnet centrifuge (HERMLE Z300), which supports the purification step of sequencing reactions that removes unincorporated dye-labeled terminators from samples that underwent thermocycler-assisted cycle sequencing; two Automated Environment SpeedVac Systems (Savant models AES1010 and DNA120-115), which dry cleaned sequencing reactions before they are re-suspended in an appropriate buffer and electrophoresed in the DNA sequencing instruments; and an ABI 7p00 Sequence Detection System, which detects and quantifies specific nucleotide sequences by using real-time PCR signal amplificaton as well as allele discrimination capabilities.

**High Resolution Imaging Core.** An imaging core facility including Ca+2 imaging, EM, and three additional confocal microscopes (including a 2 photon microscope) are available in the Shelby building. A Laser Microdissection Laboratory is also available and contains both and laser capture and Zeiss laser cutting devices. The Zeiss device also provides fluorescent imaging.

**Histomorphometry Core Facility.** State-of-the-art histomorphometry and histochemical analysis along with expert interpretation are provided. Investigators also have access to sophisticated techniques such as electron microscopy. Other services available include special stains, immunohistochemistry, and flow cytometry.

**Library Resources.** The Lister Hill Library of the Health Sciences, has a staff of 13 librarians and 37 other staff, a budget of over $3 million, a collection of 300,000 volumes, 2,500 current serials, and the Horizon integrated library system, which is a client-server-based system that provides an online catalog of the Lister Hill Library (LHL) collections. Multiple databases are available either within the library or remotely by an internet connection. The Library also houses the Reynolds Historical Library and the Alabama Museum of the Health Sciences. The Mervyn H. Sterne Library, which is the general library for the University, contains 1,117,701 books and 102,149 bound periodicals and subscribes to 2,722 periodicals pertaining to chemistry, physics, mathematics and the biological sciences as well as to topics in the social sciences, humanities, business, engineering, and education. There are also 1,072,722 microform items representing copies of books, reports, and other materials.

**Computer Services.** Optical fiber with high speed Internet connection and the Alabama Supercomputer Network connect the entire medical campus. The UAB SOD Office of Bioinformatics maintains network servers that connect laboratories to these central facilities. They also provide technical support for desktop and laptop computer.

## EQUIPMENT

**SOD Institute of Oral Health Research (IOHR) and MacDougall Laboratory –** Common-use equipment includes autoclaves, glassware washer, incubators, ultra centrifuges, microcentrifuges, -20°C and -80°C freezers, scintillation counter, lyophilizer, fume hoods, water baths, sonicator, ice machine. The following equipment is available: Nikon Eclipse TE2000-E inverted microscope and Nikon Eclipse 90i microscope each with full color and fluorescent cameras, Nikon dissecting microscope with full color camera, Nikon Diaphot microscope, Lonza 4D-Nucleofector system for transfection by electroporation, Licor Odyssey Infrared imaging system for western blot analysis, GE Nanovue spectrophotometer, Jasco V-630 spectrophotometer, liquid nitrogen cyrosystems, 4 PCR thermalcyclers (Bio-Rad and Eppendorf), Applied Biosystems 7500 qRT-PCR system, BioTek KCJunior absorbance and fluorescence plate reader, Invitrogen PowerEase and XCell SureLock Western blot system, DNA gel electrophoresis equipment, Alpha Innotech Fluorchem gel documentation system, thermomixers, microtome, paraffin embedding station, cryostat, and bacterial shaker. Each appropriate instrument has its own Dell computer, and there four additional computers available.

**Dr. Frost's Laboratory –** The Frost laboratory is equipped with a Techne TC-412 thermal cycler, multiple BioRad gel apparatuses and power packs for agarose gels, polyacrylamide sequencing gels, and Western, Northern and Southern blotting, a magnetic cell sorting system, centrifuges, water baths, pipetting equipment, a pressure cooker, and a fume hood. Two laminar flow hoods, refrigerator/freezers, refrigerators, -20°C freezers, -80°C freezers, four incubators, an Arcturus Pixcell II Laser Microdissection System, an Olympus multi-head light microscope, microtome, a Zeiss fluorescence microscopic with a Spot camera and imaging system, and a cryostat are also located within her laboratory.
The Department of Pathology Research Core located near to the Wallace Tumor Institute where Dr. Frost's laboratory is housed includes a scintillation detector, UV spectrophotometer, drying oven, hybridization ovens, -70°C freezers, preparative ultracentrifuge, sterile laminar flow hoods, cell culture incubators, plate readers, a luminometer, and a phosphorimaging system.
The members of the Comprehensive Cancer Center also have access to a broad array of common equipment within the Wallace Tumor Institute including Beckman centrifuges and rotors (two ultracentrifuges, three Avanti J-25 centrifuges, J-6 low-speed centrifuge), a phosphorimager, Zeiss Axioplan II motorized microscope with brightfield, darkfield, fluorescence, DIC, digital camera; two Precision bacterial incubators; speed vac and gel dryer, dark room with film developer, two bacterial shakers, scintillation detector, UV spectrophotometer, dishwasher, dish drying oven, autoclaves, plate readers, an Applied Biosystems 7500 Real-time PCR system, and a luminometer.

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

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

**PROFILE - Project Director/Principal Investigator**

Prefix: Dr. First Name*: Hope Middle Name M Last Name*: Amm Suffix:

| | |
|---|---|
| Position/Title*: | Post-Doctoral Fellow |
| Organization Name*: | University of Alabama at Birmingham |
| Department: | Institute of Oral Health Resea |
| Division: | School of Dentistry |
| Street1*: | 1720 2nd Avenue S |
| Street2: | SDB 704 |
| City*: | Birmingham |
| County: | Jefferson |
| State*: | AL: Alabama |
| Province: | |
| Country*: | USA: UNITED STATES |
| Zip / Postal Code*: | 352940111 |

Phone Number*: 2059965122 Fax Number: 2059965109 E-Mail*: hopeamm@uab.edu

Credential, e.g., agency login: hopeamm

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

Degree Type: PhD Degree Year: 2010

| | File Name |
|---|---|
| **Attach Biographical Sketch*:** | 1261-Biosketch_030914_HMA.pdf |
| **Attach Current & Pending Support:** | |

**PROFILE - Senior/Key Person**

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

| | |
|---|---|
| Position/Title*: | Associate Dean for Research and Professor |
| Organization Name*: | University of Alabama at Birmingham |
| Department: | |
| Division: | |
| Street1*: | 1720 2nd Avenue S |
| Street2: | |
| City*: | Birmingham |
| County: | |
| State*: | AL: Alabama |
| Province: | |
| Country*: | USA: UNITED STATES |
| Zip / Postal Code*: | 352940007 |

Phone Number*: 2059965122 Fax Number: 2059965109 E-Mail*: macdouga@uab.edu

Credential, e.g., agency login: MacDougall

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

Degree Type: PhD Degree Year: 1984

| | File Name |
|---|---|
| **Attach Biographical Sketch*:** | 1262-MacDougallNIHHope3_2014.pdf |
| **Attach Current & Pending Support:** | |





Funding Opportunity Number: PA-14-042 . Received Date: 2014-03-12T11:37:54.000-04:00

**PROFILE - Senior/Key Person**

Prefix: Dr. First Name*: Andra Middle Name Last Name*: Frost Suffix:

| | |
|---|---|
| Position/Title*: | Professor |
| Organization Name*: | The University of Alabama at Birmingham |
| Department: | Pathology |
| Division: | Medicine |
| Street1*: | 1720 2nd Avenue S |
| Street2: | WTI 320B |
| City*: | Birmingham |
| County: | |
| State*: | AL: Alabama |
| Province: | |
| Country*: | USA: UNITED STATES |
| Zip / Postal Code*: | 352943300 |

Phone Number*: 2059342746 Fax Number: E-Mail*: afrost@uab.edu

Credential, e.g., agency login: AFROST

Project Role*: Other (Specify) Other Project Role Category: Co-Mentor

Degree Type: MD Degree Year: 1985

| | File Name |
|---|---|
| **Attach Biographical Sketch*:** | 1263-biosketch_for_Dr_Frost2_2014.pdf |
| **Attach Current & Pending Support:** | |

**PROFILE - Senior/Key Person**

Prefix: Dr. First Name*: Peter Middle Name Last Name*: Waite Suffix:

| | |
|---|---|
| Position/Title*: | Chairman |
| Organization Name*: | University of Alabama at Birmingham |
| Department: | Oral & Maxilliofacial Surgery |
| Division: | Dentistry |
| Street1*: | 1720 2nd Avenue S |
| Street2: | SDB 419 |
| City*: | Birmingham |
| County: | |
| State*: | AL: Alabama |
| Province: | |
| Country*: | USA: UNITED STATES |
| Zip / Postal Code*: | 352940007 |

Phone Number*: 2059344345 Fax Number: 2059756671 E-Mail*: pwaite@uab.edu

Credential, e.g., agency login: pwaite

Project Role*: Consultant Other Project Role Category:

Degree Type: MD Degree Year: 1983

| | File Name |
|---|---|
| **Attach Biographical Sketch*:** | 1264-Biosketch_PeterWaite_Amm.pdf |
| **Attach Current & Pending Support:** | |

## BIOGRAPHICAL SKETCH

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

| NAME | POSITION TITLE |
|---|---|
| Amm, Hope M. | Postdoctoral Fellow |
| eRA COMMONS USER NAME (credential, e.g., agency login)<br>hopeamm | |

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

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | MM/YY | FIELD OF STUDY |
|---|---|---|---|
| Holy Cross College, Notre Dame, IN | A.A. | 05/99 | Biological Sciences |
| Saint Mary's College, Notre Dame, IN | B.S. | 05/02 | Biological Sciences |
| University of Alabama at Birmingham, Birmingham, AL | Ph.D. | 08/10 | Pharmacology and Toxicology |
| University of Alabama at Birmingham, Birmingham, AL | Postdoctoral | current | Oral Health |

### A. Personal Statement

My long term research goal is to conduct translational research to elucidate cellular signaling within human tumors and cancers for the development of targeted therapies. My previous research focused on identifying the mechanisms of synergy between TRA-8, an antibody to the TRAIL death receptor 5, and chemotherapy in TRA-8 resistant cancer cells. My initial investigations led me to pursue questions about how modulation of the apoptotic, NFκB, Akt and cell cycle-related cell signaling pathways affect TRA-8 sensitivity. In-depth molecular analysis of the chemotherapeutic response demonstrated modulation of the pro- and anti-apoptotic molecules by chemotherapy and combination treatment. This to the incorporation of small molecule inhibitors targeting anti-apoptotic molecules; both compounds led to sensitization of resistant cancer cells. These studies were supported by a Department of Defense Predoctoral Training grant and lead to two first author publications. Each of the agents used, TRA-8 and the small molecules inhibitors are currently in clinical trials, including the study of TRA-8 (CS-1008) for the treatment of triple negative breast cancer. The expertise I developed led to collaborations working with additional targeted therapies, also resulting in publications. Through my doctoral research and training I obtained an excellent background in cancer biology, cell signaling, mechanisms of drug response and toxicity, and pharmacological concepts; including the development of targeted therapies.

The main objective of my postdoctoral training is to advance my scientific knowledge and research abilities, while developing my professional and teaching skills under the excellent mentorship of Dr. Mary MacDougall, to facilitate my transition to an independent investigator in dental academics. My initial postdoctoral studies provided the unique opportunity to develop and characterize primary cell models of epithelial and mesenchymal odontogenic tumors from patient populations poorly-represented in research. Analysis of one cell population showed regulation of hedgehog signaling and reduced cell proliferation by a small molecule inhibitor of this signaling pathway. The aims of this proposal will seek to validate this observation in additional models and further elucidate the role the hedgehog and patched signaling within odontogenic tumors, specifically keratocystic odontogenic tumors (KCOT). I will investigate the efficacy of clinically relevant hedgehog inhibitors for the treatment of KCOT. Through this research I will be able to employ and expand my knowledge of cellular signaling, molecular techniques, and targeted therapies to a relevant and novel model of human tumors while aiding in the development of novel treatments for patients.

### B. Research and Professional Experience

**Employment**

2002-2010 Graduate Assistant/Trainee, Pharmacology and Toxicology, University of Alabama at Birmingham, Birmingham, AL; Mentor: Donald J. Buchsbaum, PhD

2010-Present Postdoctoral Trainee/Fellow, Institute of Oral Health Research, University of Alabama at Birmingham, Birmingham, AL; Mentor: Mary MacDougall, PhD

**Honors**

2014 American Association Dental Research (AADR) Johnson & Johnson Healthcare Products Hatton Awards Competition Finalist
2014 2nd place, UAB School of Dentistry 9th Annual Scholar's Symposium, Postdoctoral
2014 2nd place, 11th Annual UAB Postdoctoral Reseach Day
2014 UAB Office of Postdoctoral Education Travel Award
2013 International Conference on the Chemistry and Biology of Mineralized Tissue (ICCBMT) Young Investigator's Award
2013 Center for the Integration of Research, Teaching, and Learning (CIRTL) Fellow Program Certification
2013 CIRTL Practioner Program Certification
2013 American Association of Dental Research Bloc Travel Award
2013 3rd place, UAB School of Dentistry 8th Annual Scholar's Symposium, Postdoctoral
2013 UAB Office of Postdoctoral Education Travel Award
2012 American Association of Dental Research Bloc Travel Award
2010-2013 Postdoctoral Trainee, UAB Dental Research Academic Training Fellowship (NIDCR Grant # T32-DE017607)
2010-Present Scholar At-Large, UAB IRACDA Mentored Experience in Research, Instruction, and Teaching (MERIT) program
2006-2008 Graduate Trainee, Department of Defense Predoctoral Traineeship (DOD grant # W81XWH061-070)
2002-2003 Graduate Trainee, Department of Defense Breast Cancer Training Program (DOD grant# BCDAMD17-00-1-0119)
2002 *Sigma Xi* Award for Outstanding Research
2002 Sister M. Rosaleen Dunleavy Allied Medical Award
2002 Bachelor's of Science awarded *Magna Cum Laude*
2000-2002 Saint Mary's College Presidential Scholarship
2000 Inducted into Tri Beta, Biological Honors Society

**Experience and Professional Memberships**

2013-Present Member-at-Large, AADR National Student Research Group (NSRG) Executive Committee
2013 Course Director and Instructor, Dental Genetics, UAB School of Dentistry
2013 Instructor, Dental Histology and Oral Mucosa in Dental Anatomy, UAB School of Dentistry
2013 Instructor, Undergraduate Research Skills Workshop, Laboratory Basics, UAB
2013 Course Design and Instructor, Special Topics in Biology: Cancer Biology, Stillman College
2013-Present Ad hoc Reviewer, Pediatric Dentistry
2012-Present Mentor, Undergraduate Research Honors, UAB
2012 Instructor, Cancer Genetics in Advanced Molecular Genetics, UAB
2012 Instructor, Pharmacogenomics in Genetics, UAB School of Dentistry
2012 Instructor, Dental Histology in Dental Anatomy, UAB School of Dentistry
2012 Instructor, Stillman College Biology Journal Club
2012 Ad hoc Reviewer, Clinical Cancer Research
2011-Present Vice-President, American Association of Dental Research National Student Research Group, UAB Chapter
2011-Present Chair and Organizer of UAB Postdoctoral Research Day Committee
2011-Present Member, UAB Postdoctoral Executive Board
2011-Present Representative, Council for Postdoctoral Education
2011 Mentor, Ronald E. McNair Postbaccalaureate Achievement Program
2011 Instructor, Organelle and Membrane Transport in General Biology, Stillman College
2011 Instructor, Bacterial Genetics in Microbiology, Stillman College
2011 Contributor, UAB Postdoctoral Association Quarterly Newsletter

2010-Present Member, American Association for the Advancement of Science (AAAS)
2010-Present Member, International Association of Dental Research
2010-Present Member, American Association of Dental Research
2010 Judge, Graduate Biomedical Sciences Winter Poster Session
2004-Present Member, American Association of Cancer Research
2002-2006 Member, Sigma Xi

**C. Peer-reviewed Publications**

**Most relevant to the current application**

1. **HM Amm**, MD Casimir, DB Clark, P Sohn, M MacDougall. 2014. Matrix Metalloproteinase Expression in Keratocystic Odontogenic Tumors and Primary Cells. Connective Tissue Research. In press.
2. Ren C*, **HM Amm***, P DeVilliers, Y Wu, J Deatherage, Z Liu, M MacDougall. 2012. Targeting the sonic hedgehog pathway in keratocystic odontogenic tumor. Journal of Biologic Chemistry. 287: 27117-25. *These authors contributed equally to this work.
3. Diniz MG, CC Gomes, WH de Castro, AL Guimarães, AM De Paula, **H Amm**, C Ren, M Macdougall, RS Gomez. 2012. miR-15a/16-1 influences BCL2 expression in keratocystic odontogenic tumors. Cellular Oncology. 35: 285-91.
4. Ren C, MG Diniz, C Piazza, **HM Amm**, DL Rollins, H Erlandsen, H Rivera, P Devilliers, OA Mamaeva, M MacDougall. 2011. Differential enamel and osteogenic gene expression profiles in odontogenic tumors. Cells Tissue Organs. 194: 296-301.
5. Bevis KS, LR McNally, JC Sellers, DL DellaManna, **H Amm**, A Londono, JM Straughn, DJ Buchsbaum. 2011. Anti-tumor Activity of an anti-DR 5 monoclonal antibody, TRA-8, in combination with platinum and taxane based chemotherapy in an ovarian cancer model. Gynecologic Oncology. 121: 193-9.
6. Steg A, **HM Amm**, Z Novak, AR Frost, MR Johnson. 2010. Gli3 mediates cell survival and sensitivity to cyclopamine in pancreatic cancer. Cancer Biology and Therapy. 10: 893-902.

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

1. **Amm HM***, Rollins DL*, Ren C, Dong J, DeVilliers P, Rivera H, MacDougall M. Establishment and Characterization of a Primary Calcifying Epithelial Odontogenic Tumor Cell Population. J Oral Pathol Med. In press. Epub Oct 11, 2013
2. **Amm HM,** DJ Buchsbaum. 2011. Relationship between galectin-3 and TRAIL sensitivity in breast cancer. Expert Review of Anticancer Therapy. 11: 1193-6.
3. **Amm HM**, T Zhou, AD Steg, H Kuo, Y Li, DJ Buchsbaum. 2011. Mechanisms of drug sensitization to TRA-8, an agonistic death receptor 5 antibody, involve modulation of the intrinsic apoptotic pathway in human breast cancer cells. Molecular Cancer Research. 9: 403-17.
4. **Amm HM**, PG Oliver, DJ Buchsbaum. 2011. Combined modality therapy with TRAIL or agonistic death receptor antibodies. Cancer Biology & Therapy. 11: 431-49.

**D. Research Support**

**Completed Research Support**

T-90 DE022736-01 Role: Amm (Trainee) 06/1/12 – 08/31/13
Agency: National Institute of Dental & Craniofacial Research
"UAB Institutional Research Training Grant- Dental Academic Research Training Program-DART",
To provide research training for dental students, graduate students and postdoctoral fellows at the UAB SOD.
Principal Investigator: M MacDougall

T-32 DE017607 Role: Amm (Trainee) 06/1/07 - 05/31/12
Agency: National Institute of Dental & Craniofacial Research
"UAB Institutional Research Training Grant- Dental Academic Research Training Program-DART",
To provide research training for dental students, graduate students and postdoctoral fellows at the UAB SOD.
Principal Investigator: M MacDougall

W81XWH-06-1-070 Role: Amm (PI) 09/01/06-08/31/08
Department of Defense Predoctoral Traineeship
To provide support during graduate studies entitled “Mechanisms and therapeutic efficacy of combination TRA-8, anti-DR5 monoclonal antibody, and chemotherapy in breast cancer”
Principal Investigator: HM Amm

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

# RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

**ORGANIZATIONAL DUNS*:** 0636907050000

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

**Enter name of Organization:** University of Alabama at Birmingham

**Start Date*:** 09-01-2014 **End Date*:** 08-31-2015 **Budget Period: 1**

**A. Senior/Key Person**

| | Prefix | First Name* | Middle Name | Last Name* | Suffix | Project Role* | Base Salary ($) | Calendar Months | Academic Months | Summer Months | Requested Salary ($)* | Fringe Benefits ($)* | Funds Requested ($)* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 . | Dr. | Hope | M | Amm | | PD/PI | | 12.00 | | | | | |

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

**Additional Senior Key Persons:** File Name:

**Total Senior/Key Person**

**B. Other Personnel**

| Number of Personnel* | Project Role* | Calendar Months | Academic Months | Summer Months | Requested Salary ($)* | Fringe Benefits* | Funds Requested ($)* |
|---|---|---|---|---|---|---|---|

**Total Number Other Personnel** **Total Other Personnel**

**Total Salary, Wages and Fringe Benefits (A+B)**

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





Funding Opportunity Number: PA-14-042 . Received Date: 2014-03-12T11:37:54.000-04:00

# RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

**ORGANIZATIONAL DUNS*:** 0636907050000

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

**Organization:** University of Alabama at Birmingham

**Start Date*:** 09-01-2014 **End Date*:** 08-31-2015 **Budget Period: 1**

| **C. Equipment Description** | |
|---|---|
| List items and dollar amount for each item exceeding $5,000 | |
| **Equipment Item** | **Funds Requested ($)*** |
| **Total funds requested for all equipment listed in the attached file** | |
| **Total Equipment** | |
| **Additional Equipment:** File Name: | |

| **D. Travel** | **Funds Requested ($)*** |
|---|---|
| 1. Domestic Travel Costs ( Incl. Canada, Mexico, and U.S. Possessions) | |
| 2. Foreign Travel Costs | |
| **Total Travel Cost** | |

| **E. Participant/Trainee Support Costs** | | **Funds Requested ($)*** |
|---|---|---|
| 1. Tuition/Fees/Health Insurance | | |
| 2. Stipends | | |
| 3. Travel | | 2,000.00 |
| 4. Subsistence | | |
| 5. Other: | | |
| **Number of Participants/Trainees** | **Total Participant Trainee Support Costs** | **2,000.00** |

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



Tracking Number: GRANT11605650 Funding Opportunity Number: PA-14-042 . Received Date: 2014-03-12T11:37:54.000-04:00

# RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

**ORGANIZATIONAL DUNS*:** 0636907050000

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

**Organization:** University of Alabama at Birmingham

**Start Date*:** 09-01-2014 **End Date*:** 08-31-2015 **Budget Period: 1**

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

| **G. Direct Costs** | **Funds Requested ($)*** |
|---|---|
| **Total Direct Costs (A thru F)** | **114,239.00** |

**H. Indirect Costs**

| **Indirect Cost Type** | **Indirect Cost Rate (%)** | **Indirect Cost Base ($)** | **Funds Requested ($)*** |
|---|---|---|---|
| 1 . MTDC | 8.00 | 114,239.00 | |
| | | **Total Indirect Costs** | |

**Cognizant Federal Agency** (Agency Name, POC Name, and POC Phone Number) DHHS, Steven Zuraf, (301)492-4855

| **I. Total Direct and Indirect Costs** | **Funds Requested ($)*** |
|---|---|
| **Total Direct and Indirect Institutional Costs (G + H)** | **123,378.00** |

| **J. Fee** | **Funds Requested ($)*** |
|---|---|
| | |

| **K. Budget Justification*** | File Name: 1234-Budget_justification_HMA_resubmission.pdf (Only attach one file.) |
|---|---|

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

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

# RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

**ORGANIZATIONAL DUNS*:** 0636907050000

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

**Enter name of Organization:** University of Alabama at Birmingham

**Start Date*:** 09-01-2015 **End Date*:** 08-31-2016 **Budget Period: 2**

**A. Senior/Key Person**

| Prefix | First Name* | Middle Name | Last Name* | Suffix | Project Role* | Base Salary ($) | Calendar Months | Academic Months | Summer Months | Requested Salary ($)* | Fringe Benefits ($)* | Funds Requested ($)* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 . Dr. | Hope | M | Amm | | PD/PI | | 12.00 | | | | | |

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

**Additional Senior Key Persons:** File Name:

**Total Senior/Key Person**

**B. Other Personnel**

| Number of Personnel* | Project Role* | Calendar Months | Academic Months | Summer Months | Requested Salary ($)* | Fringe Benefits* | Funds Requested ($)* |
|---|---|---|---|---|---|---|---|

**Total Number Other Personnel** **Total Other Personnel**

**Total Salary, Wages and Fringe Benefits (A+B)**

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





Funding Opportunity Number: PA-14-042 . Received Date: 2014-03-12T11:37:54.000-04:00

# RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

**ORGANIZATIONAL DUNS*:** 0636907050000

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

**Organization:** University of Alabama at Birmingham

**Start Date*:** 09-01-2015 **End Date*:** 08-31-2016 **Budget Period: 2**

| **C. Equipment Description** | |
|---|---|
| List items and dollar amount for each item exceeding $5,000 | |
| **Equipment Item** | **Funds Requested ($)*** |
| **Total funds requested for all equipment listed in the attached file** | |
| **Total Equipment** | |
| **Additional Equipment:** File Name: | |

| **D. Travel** | **Funds Requested ($)*** |
|---|---|
| 1. Domestic Travel Costs ( Incl. Canada, Mexico, and U.S. Possessions) | |
| 2. Foreign Travel Costs | |
| **Total Travel Cost** | |

| **E. Participant/Trainee Support Costs** | | **Funds Requested ($)*** |
|---|---|---|
| 1. Tuition/Fees/Health Insurance | | |
| 2. Stipends | | |
| 3. Travel | | 2,000.00 |
| 4. Subsistence | | |
| 5. Other: | | |
| **Number of Participants/Trainees** | **Total Participant Trainee Support Costs** | **2,000.00** |

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



Tracking Number: GRANT11605650 Funding Opportunity Number: PA-14-042 . Received Date: 2014-03-12T11:37:54.000-04:00

# RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

**ORGANIZATIONAL DUNS*:** 0636907050000

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

**Organization:** University of Alabama at Birmingham

**Start Date*:** 09-01-2015 **End Date*:** 08-31-2016 **Budget Period: 2**

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

| **G. Direct Costs** | **Funds Requested ($)*** |
|---|---|
| **Total Direct Costs (A thru F)** | |

**H. Indirect Costs**

| **Indirect Cost Type** | **Indirect Cost Rate (%)** | **Indirect Cost Base ($)** | **Funds Requested ($)*** |
|---|---|---|---|
| 1 . MTDC | 8.00 | 117,383.00 | |
| | | **Total Indirect Costs** | |

**Cognizant Federal Agency** (Agency Name, POC Name, and POC Phone Number) DHHS, Steven Zuraf, (301)492-4855

| **I. Total Direct and Indirect Costs** | **Funds Requested ($)*** |
|---|---|
| **Total Direct and Indirect Institutional Costs (G + H)** | **126,774.00** |

| **J. Fee** | **Funds Requested ($)*** |
|---|---|
| | |

**K. Budget Justification*** File Name: 1234-Budget_justification_HMA_resubmission.pdf (Only attach one file.)

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

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

# RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

**ORGANIZATIONAL DUNS*:** 0636907050000

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

**Enter name of Organization:** University of Alabama at Birmingham

**Start Date*:** 09-01-2016 **End Date*:** 08-31-2017 **Budget Period: 3**

**A. Senior/Key Person**

| Prefix | First Name* | Middle Name | Last Name* | Suffix | Project Role* | Base Salary ($) | Calendar Months | Academic Months | Summer Months | Requested Salary ($)* | Fringe Benefits ($)* | Funds Requested ($)* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 . Dr. | Hope | M | Amm | | PD/PI | | 12.00 | | | 0.00 | 0.00 | 0.00 |

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

**Additional Senior Key Persons:** File Name:

**Total Senior/Key Person** **0.00**

**B. Other Personnel**

| Number of Personnel* | Project Role* | Calendar Months | Academic Months | Summer Months | Requested Salary ($)* | Fringe Benefits* | Funds Requested ($)* |
|---|---|---|---|---|---|---|---|
| | **Total Number Other Personnel** | | | | | **Total Other Personnel** | |
| | | | | | | **Total Salary, Wages and Fringe Benefits (A+B)** | **0.00** |

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





Funding Opportunity Number: PA-14-042 . Received Date: 2014-03-12T11:37:54.000-04:00

# RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

**ORGANIZATIONAL DUNS*:** 0636907050000

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

**Organization:** University of Alabama at Birmingham

**Start Date*:** 09-01-2016 **End Date*:** 08-31-2017 **Budget Period: 3**

| **C. Equipment Description** | |
|---|---|
| List items and dollar amount for each item exceeding $5,000 | |
| **Equipment Item** | **Funds Requested ($)*** |
| **Total funds requested for all equipment listed in the attached file** | |
| **Total Equipment** | |
| **Additional Equipment:** File Name: | |

| **D. Travel** | **Funds Requested ($)*** |
|---|---|
| 1. Domestic Travel Costs ( Incl. Canada, Mexico, and U.S. Possessions) | |
| 2. Foreign Travel Costs | |
| **Total Travel Cost** | |

| **E. Participant/Trainee Support Costs** | | **Funds Requested ($)*** |
|---|---|---|
| 1. Tuition/Fees/Health Insurance | | |
| 2. Stipends | | |
| 3. Travel | | 0.00 |
| 4. Subsistence | | |
| 5. Other: | | |
| **Number of Participants/Trainees** | **Total Participant Trainee Support Costs** | **0.00** |

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





Funding Opportunity Number: PA-14-042 . Received Date: 2014-03-12T11:37:54.000-04:00

# RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

**ORGANIZATIONAL DUNS*:** 0636907050000

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

**Organization:** University of Alabama at Birmingham

**Start Date*:** 09-01-2016 **End Date*:** 08-31-2017 **Budget Period: 3**

| F. Other Direct Costs | Funds Requested ($)* |
|---|---|
| 1. Materials and Supplies | 249,000.00 |
| 2. Publication Costs | 0.00 |
| 3. Consultant Services | |
| 4. ADP/Computer Services | |
| 5. Subawards/Consortium/Contractual Costs | |
| 6. Equipment or Facility Rental/User Fees | |
| 7. Alterations and Renovations | |
| **Total Other Direct Costs** | **249,000.00** |

| G. Direct Costs | Funds Requested ($)* |
|---|---|
| **Total Direct Costs (A thru F)** | **249,000.00** |

**H. Indirect Costs**

| Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | Funds Requested ($)* |
|---|---|---|---|
| 1 . MTDC | 8.00 | 249,900.00 | 0.00 |
| | | **Total Indirect Costs** | **0.00** |

**Cognizant Federal Agency** (Agency Name, POC Name, and POC Phone Number) DHHS, Steven Zuraf, (301)492-4855

| I. Total Direct and Indirect Costs | Funds Requested ($)* |
|---|---|
| **Total Direct and Indirect Institutional Costs (G + H)** | **249,000.00** |

| J. Fee | Funds Requested ($)* |
|---|---|
| | |

**K. Budget Justification*** File Name: 1234-Budget_justification_HMA_resubmission.pdf

(Only attach one file.)

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

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

# RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

**ORGANIZATIONAL DUNS*:** 0636907050000

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

**Enter name of Organization:** University of Alabama at Birmingham

**Start Date*:** 09-01-2017 **End Date*:** 08-31-2018 **Budget Period: 4**

**A. Senior/Key Person**

| Prefix | First Name* | Middle Name | Last Name* | Suffix | Project Role* | Base Salary ($) | Calendar Months | Academic Months | Summer Months | Requested Salary ($)* | Fringe Benefits ($)* | Funds Requested ($)* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 . Dr. | Hope | M | Amm | | PD/PI | | 12.00 | | | 0.00 | 0.00 | 0.00 |

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

**Additional Senior Key Persons:** File Name:

**Total Senior/Key Person** **0.00**

**B. Other Personnel**

| Number of Personnel* | Project Role* | Calendar Months | Academic Months | Summer Months | Requested Salary ($)* | Fringe Benefits* | Funds Requested ($)* |
|---|---|---|---|---|---|---|---|
| | **Total Number Other Personnel** | | | | | **Total Other Personnel** | |
| | | | | | | **Total Salary, Wages and Fringe Benefits (A+B)** | **0.00** |

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





Funding Opportunity Number: PA-14-042 . Received Date: 2014-03-12T11:37:54.000-04:00

# RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

**ORGANIZATIONAL DUNS*:** 0636907050000

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

**Organization:** University of Alabama at Birmingham

**Start Date*:** 09-01-2017 **End Date*:** 08-31-2018 **Budget Period: 4**

**C. Equipment Description**

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

| **Equipment Item** | **Funds Requested ($)*** |
|---|---|
| **Total funds requested for all equipment listed in the attached file** | |
| **Total Equipment** | |

**Additional Equipment:** File Name:

| **D. Travel** | **Funds Requested ($)*** |
|---|---|
| 1. Domestic Travel Costs ( Incl. Canada, Mexico, and U.S. Possessions) | |
| 2. Foreign Travel Costs | |
| **Total Travel Cost** | |

| **E. Participant/Trainee Support Costs** | | **Funds Requested ($)*** |
|---|---|---|
| 1. Tuition/Fees/Health Insurance | | |
| 2. Stipends | | |
| 3. Travel | | |
| 4. Subsistence | | |
| 5. Other: | | |
| **Number of Participants/Trainees** | **Total Participant Trainee Support Costs** | |

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



Tracking Number: GRANT11605650 Funding Opportunity Number: PA-14-042 . Received Date: 2014-03-12T11:37:54.000-04:00

# RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

**ORGANIZATIONAL DUNS*:** 0636907050000

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

**Organization:** University of Alabama at Birmingham

**Start Date*:** 09-01-2017 **End Date*:** 08-31-2018 **Budget Period: 4**

| F. Other Direct Costs | Funds Requested ($)* |
|---|---|
| 1. Materials and Supplies | 249,000.00 |
| 2. Publication Costs | |
| 3. Consultant Services | |
| 4. ADP/Computer Services | |
| 5. Subawards/Consortium/Contractual Costs | |
| 6. Equipment or Facility Rental/User Fees | |
| 7. Alterations and Renovations | |
| **Total Other Direct Costs** | **249,000.00** |

| G. Direct Costs | Funds Requested ($)* |
|---|---|
| **Total Direct Costs (A thru F)** | **249,000.00** |

**H. Indirect Costs**

| Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | Funds Requested ($)* |
|---|---|---|---|
| 1 . MTDC | 8.00 | 249,000.00 | 0.00 |
| | | **Total Indirect Costs** | **0.00** |

**Cognizant Federal Agency** (Agency Name, POC Name, and POC Phone Number) DHHS, Steven Zuraf, (301)492-4855

| I. Total Direct and Indirect Costs | Funds Requested ($)* |
|---|---|
| **Total Direct and Indirect Institutional Costs (G + H)** | **249,000.00** |

| J. Fee | Funds Requested ($)* |
|---|---|
| | |

**K. Budget Justification*** File Name: 1234-Budget_justification_HMA_resubmission.pdf

(Only attach one file.)

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

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

# RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

**ORGANIZATIONAL DUNS*:** 0636907050000

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

**Enter name of Organization:** University of Alabama at Birmingham

**Start Date*:** 09-01-2018 **End Date*:** 08-31-2019 **Budget Period: 5**

**A. Senior/Key Person**

| Prefix | First Name* | Middle Name | Last Name* | Suffix | Project Role* | Base Salary ($) | Calendar Months | Academic Months | Summer Months | Requested Salary ($)* | Fringe Benefits ($)* | Funds Requested ($)* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 . Dr. | Hope | M | Amm | | PD/PI | | 12.00 | | | 0.00 | 0.00 | 0.00 |

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

**Additional Senior Key Persons:** File Name:

**Total Senior/Key Person** **0.00**

**B. Other Personnel**

| Number of Personnel* | Project Role* | Calendar Months | Academic Months | Summer Months | Requested Salary ($)* | Fringe Benefits* | Funds Requested ($)* |
|---|---|---|---|---|---|---|---|
| | **Total Number Other Personnel** | | | | | **Total Other Personnel** | |
| | | | | | | **Total Salary, Wages and Fringe Benefits (A+B)** | **0.00** |

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





Funding Opportunity Number: PA-14-042 . Received Date: 2014-03-12T11:37:54.000-04:00

# RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

**ORGANIZATIONAL DUNS*:** 0636907050000

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

**Organization:** University of Alabama at Birmingham

**Start Date*:** 09-01-2018 **End Date*:** 08-31-2019 **Budget Period: 5**

**C. Equipment Description**

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

| **Equipment Item** | **Funds Requested ($)*** |
|---|---|
| **Total funds requested for all equipment listed in the attached file** | |
| **Total Equipment** | |

**Additional Equipment:** File Name:

| **D. Travel** | **Funds Requested ($)*** |
|---|---|
| 1. Domestic Travel Costs ( Incl. Canada, Mexico, and U.S. Possessions) | |
| 2. Foreign Travel Costs | |
| **Total Travel Cost** | |

| **E. Participant/Trainee Support Costs** | | **Funds Requested ($)*** |
|---|---|---|
| 1. Tuition/Fees/Health Insurance | | |
| 2. Stipends | | |
| 3. Travel | | |
| 4. Subsistence | | |
| 5. Other: | | |
| **Number of Participants/Trainees** | **Total Participant Trainee Support Costs** | |

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



Tracking Number: GRANT11605650 Funding Opportunity Number: PA-14-042 . Received Date: 2014-03-12T11:37:54.000-04:00

# RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

**ORGANIZATIONAL DUNS*:** 0636907050000

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

**Organization:** University of Alabama at Birmingham

**Start Date*:** 09-01-2018 **End Date*:** 08-31-2019 **Budget Period: 5**

| F. Other Direct Costs | Funds Requested ($)* |
|---|---|
| 1. Materials and Supplies | 249,000.00 |
| 2. Publication Costs | |
| 3. Consultant Services | |
| 4. ADP/Computer Services | |
| 5. Subawards/Consortium/Contractual Costs | |
| 6. Equipment or Facility Rental/User Fees | |
| 7. Alterations and Renovations | |
| **Total Other Direct Costs** | **249,000.00** |

| G. Direct Costs | Funds Requested ($)* |
|---|---|
| **Total Direct Costs (A thru F)** | **249,000.00** |

**H. Indirect Costs**

| Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | Funds Requested ($)* |
|---|---|---|---|
| 1 . MTDC | 8.00 | 249,000.00 | 0.00 |
| | | **Total Indirect Costs** | **0.00** |

**Cognizant Federal Agency** (Agency Name, POC Name, and POC Phone Number) DHHS, Steven Zuraf, (301)492-4855

| I. Total Direct and Indirect Costs | Funds Requested ($)* |
|---|---|
| **Total Direct and Indirect Institutional Costs (G + H)** | **249,000.00** |

| J. Fee | Funds Requested ($)* |
|---|---|
| | |

**K. Budget Justification*** File Name: 1234-Budget_justification_HMA_resubmission.pdf
(Only attach one file.)

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

**Budget justification**

K99 phase:
Dr. Hope Amm will dedicate 12 calendar months of her 100% effort to the proposed research project. A fringe benefit rate 34.7% was applied to her salary.

Dr. Mary MacDougall and Dr. Andra Frost will serve as mentors for the proposed research project and will not receive any salary.

Funds are requested for travel to scientific meetings.

The materials and supplies budget will be used for purchasing tissue culture supplies, reagents for quantitative real-time PCR, antibodies and other reagents for immunohistochemistry, small interfering RNA, MTS assay reagents, Gateway cloning kit.

Funds are requested for publication costs.

Facility user fees include using the Genomics Core Laboratories (sequencing of hedgehog pathway components and cloning analysis).

R00 phase:
Dr. Amm will devote 75% effort serving as PI on this project. In line with the guidance, $249,000 per year is requested for the 4 years of the R00 phase and specific budget justification will be provided once an independent faculty position is obtained.

# RESEARCH & RELATED BUDGET - Cumulative Budget

| | Totals ($) |
|---|---|
| **Section A, Senior/Key Person** | |
| **Section B, Other Personnel** | |
| Total Number Other Personnel | |
| **Total Salary, Wages and Fringe Benefits (A+B)** | |
| **Section C, Equipment** | |
| **Section D, Travel** | |
| 1. Domestic | |
| 2. Foreign | |
| **Section E, Participant/Trainee Support Costs** | |
| 1. Tuition/Fees/Health Insurance | |
| 2. Stipends | |
| 3. Travel | |
| 4. Subsistence | |
| 5. Other | |
| 6. Number of Participants/Trainees | |
| **Section F, Other Direct Costs** | |
| 1. Materials and Supplies | |
| 2. Publication Costs | |
| 3. Consultant Services | |
| 4. ADP/Computer Services | |
| 5. Subawards/Consortium/Contractual Costs | |
| 6. Equipment or Facility Rental/User Fees | |
| 7. Alterations and Renovations | |
| 8. Other 1 | |
| 9. Other 2 | |
| 10. Other 3 | |
| **Section G, Direct Costs (A thru F)** | **978,622.00** |
| **Section H, Indirect Costs** | **18,530.00** |
| **Section I, Total Direct and Indirect Costs (G + H)** | **997,152.00** |
| **Section J, Fee** | |





Funding Opportunity Number: PA-14-042 . Received Date: 2014-03-12T11:37:54.000-04:00

# PHS 398 Cover Page Supplement

OMB Number: 0925-0001

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

Prefix: Dr.
First Name*: Hope
Middle Name: M
Last Name*: Amm
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-14-042. Received Date: 2014-03-12T11:37:54.000-04:00

# 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 Career Development Award Supplemental Form

OMB Number: 0925-0001

| | |
|---|---|
| **Introduction (if applicable)** | |
| 1. Introduction to Application (for RESUBMISSION applications only) | 1242-Introduction_031114.pdf |
| **Candidate Information** | |
| 2. Candidate's Background | 1243-Candidate_Background_030614fMM.pdf |
| 3. Career Goals and Objectives | 1244-Career_Goals_and_Objectives_030614fMM.pdf |
| 4. Career Development/Training Activities During Award Period | 1245-Career_Development_030614fMM.pdf |
| 5. Training in the Responsible Conduct of Research | 1246-Training_in_the_Responsible_Conduct_of_Research_HMA_022614.pdf |
| 6.Candidate's Plan to Provide Mentoring (as applicable) | |
| **Statements of Support** | |
| 7. Plans and Statements of Mentor and Co-Mentor(s) | 1247-Mentor Statement Resubmission_final.pdf |
| 8. Letters of Support from Collaborators, Contributors, and Consultants | 1248-Letters_of_Support_HMA.pdf |
| **Environment and Institutional Commitment to Candidate** | |
| 9. Description of Institutional Environment | 1249-Description_of_Institutional_Environment.pdf |
| 10. Institutional Commitment to Candidate's Research Career Development | 1250-Letter_of_Institutional_Support_for_Hope.pdf |
| **Research Plan** | |
| 11. Specific Aims | 1251-Specific_Aims_HMA_031114.pdf |
| 12. Research Strategy* | 1252-Research_Strategy_HMA_final.pdf |
| 13. Progress Report Publication List (for RENEWAL applications only) | |
| **Human Subject Sections** | |
| 14. Protection of Human Subjects | 1253-HUMAN_SUBJECTS_HMA_resubmission.pdf |
| 15. Inclusion of Women and Minorities | 1254-Inclusion_of_Women_and_Minorities_HMA.pdf |
| 16. Inclusion of Children | 1255-Inclusion_of_Children_HMA.pdf |
| **Other Research Plan Sections** | |
| 17. Vertebrate Animals | 1256-Vertebrate_Animals.pdf |
| 18. Select Agent Research | |
| 19. Consortium/Contractual Arrangements | |
| 20. Resource Sharing Plan(s) | 1257-Resource_Sharing_HMA.pdf |
| **Appendix (if applicable)** | |
| 21. Appendix | |

**Citizenship*:**

- ● U.S. Citizen or noncitizen national
- Non-U.S. Citizen with temporary U.S. visa
- Permanent Resident of U.S. (If a permanent resident of the U.S., a notarized statement must be provided by the time of award)
- Permanent Resident of U.S. Pending

**Introduction**

I thank the reviewers for their thoughtful critique of my K99 application. It is especially rewarding that they view this proposed research as **high potential impact** and myself as an **outstanding applicant with great potential**. Below are specific revision as related to each critique; full changes can be found in the revised proposal highlighted with a line in the left margin. The changes have yielded a more scientifically supported, well-rounded training and focused research plan.

**Concerns Related to the Candidate and the Career Development Plan**

**(1) The reviewers expressed concern over the lack of a mentor with clinical expertise in odontogenic tumors.** I have recruited Dr. Peter Waite, Chair of the Department of Oral and Maxillofacial Surgery at UAB SOD, to my mentorship team. We have published one paper together on the development of odontogenic tumor cell models and another on a technique used in the surgical treatment of odontogenic tumors. He personally performed the surgery on three of our tumors and his involvement has increased patient recruitment. He provided a letter of support and endorses the value of preclinical studies for benign tumors with high recurrence rate, such as keratocystic odontogenic tumors (KCOTs).

**(2) The reviewers expressed concern over lack of knowledge regarding clinical treatment of keratocystic odontogenic tumors (KCOT).** I now have bi-monthly mentoring sessions with Dr. Waite and attended the odontogenic tumor sections of the UAB SOD oral pathology course to obtain a better understanding of the biology and treatment of KCOTs. I have included a more detailed description of KCOT clinical treatment in the Research Strategy Background.

**(2) The reviewers expressed concerns regarding limited publications of the topic of KCOT.** Since the first submission, we have published two additional papers, one on the expression of matrix metalloproteinases in KCOTs, the other on the development of a calcifying epithelial odontogenic tumor cells. Additionally, I have won 6 awards for my research on KCOTs (see Dr. Amm's biosketch). I also have an additional KCOT manuscript to be submitted to PLOS ONE on the establishment of the KCOT primary cells used in this application (previous Aim 1, Research Strategy).

**(3) Reviewers asked for a more formal description of the method by which the applicant will be evaluated by her committee.** Each member of the advisory committee will have scheduled meetings the applicant, review data relevant to their area of expertise (outlined in Table 1) and review grants and manuscripts prior to submission (see Table 2; Career Development for expected deadlines and progress indicators). A written annual progress report will also be provided.

**(4) Reviewer 1 noted it was unclear what drew me to study KCOT.** I am passionate about studying the fundamental signaling of tumor cells as a means of developing therapeutics. KCOTs are an ideal model for studying hedgehog activity due to the high proportions of PTCH1 polymorphisms, which has broad application to many human neoplasias.

**Concerns Related to the Research Plan**

**(1) The reviewers raise the concern that the application does not include *in vivo* therapeutic studies, especially considering the number of reports of drugs having an *in vitro* effect, but lacking *in vivo* efficacy.** I agree with the reviewer that animal models will be necessary prior to clinical evaluation of HH inhibitors for the treatment of KCOT. I have added the development of a KCOT xenograft model from low passage cell populations to Aim 2. I have previous experience with animal models of human cancer from my graduate studies in Dr. Donald Buchsbaum's lab.

**(2) The reviewers expressed concern regarding the number of patients, the origin and handling of the primary KCOT cells, and the difference between syndromic and non-syndromic tumors.** I have included a more detailed description of our cells, their origin, and our handling of data in the Research Strategy. We are continuing to recruit patients for these and other studies regarding odontogenic tumors (see preliminary data of ameloblastoma, Figure 7B). In the past month, we have recruited 3 additional odontogenic tumor patients, including one KCOT patient with NBCCS, and have another 2 KCOT patients scheduled in the next 10 days.

**(3) Reviewers expressed concern over the expense and proposition of using a systemic therapy for the treatment of a benign tumor.** Dr. Waite is an advisor/collaborator, in his letter of support he comments on the lack of global standards for KCOT treatment, the challenge of using conservative treatment versus risk of recurrence in KCOT, and his support for using new targeted therapies for the treatment of KCOT. Additionally, we envision developing a local delivery system in gel form to be placed directly in the surgical site (similar to Emdogain or BMP for tissue and bone reformation) for patients with a lower risk of recurrence. Novartis has a going trial using topical LDE225 for the treatment of basal cell carcinoma. A collaboration with Genentech to provide GDC-0449 (approved Materials Transfer Agreement in letters of support) has been established. Similar collaborations with Novartis for LDE225 and LEQ506, a second generation HH inhibitor, are pending.

**(4) Reviewer 1 commented that Aim 1 had already been completed.** I have removed this aim and a manuscript describing these studies is in preparation for submission to *PLOS ONE*.

**(5) Reviewer 1 states "it is a weakness that the application does not include plans for in situ hybridization of Gli."** We will use immunohistochemistry to access the expression and location of Gli-1 (protein level) as the activity of transcription factors requires nuclear location. We have a primary antibody that Dr. Frost has previously used. For reviewing of stained tissue, Dr. Frost is a certified pathologist and will be available to evaluate tissue staining. Also Dr. Patricia DeVilliers, a certified oral pathologist, will be available for consultation and has personally trained Dr. Amm in the identification of KCOTs from primary patient tissues (see letter of support).

**(6) In relation to the experiments described for Aim 1, Reviewer 2 stated "These are important but are limited in nature and lack depth and breadth in terms of mechanism."** We agree that additional experiments would help define the role of PTCH polymorphisms in fundamental hedgehog signaling and tumorigenesis. We have added additional experiments (e.g., localization and hedgehog activation, anchorage independent growth, and invasion assays) to Aim 1 to provide more mechanistic insight to our studies.

**Candidate Information**

I am currently a postdoctoral fellow at the University of Alabama (UAB) School of Dentistry (SOD). **My overarching research career goal is to investigate signaling mechanisms in dental-related tumors within a research intensive dental school, ultimately developing targeted clinical therapies.** The following sections outline how my research questions have led me to this goal, demonstrate my relevant skills and background, and illuminate my career development plan, which will foster my development as an independent investigator.

**Candidate's Background**

**1. Prior Research.** My first experiences with research and lab techniques were at Saint Mary's College. Under the mentorship of Dr. Kara Eberly, I worked as a teaching/lab assistant for her Microbiology course and completed my senior research project. My research focused on understanding cellular toxicity of tumor necrosis factor (TNF)-alpha secreted by macrophages. I stimulated a variety of knock-out macrophage cell lines with lipopolysaccharide and determined the effect of the conditioned media on murine fibroblasts, which were sensitive to TNF-alpha induced cytotoxicity. I also assisted in execution of laboratory experiments and set-up, provided individual and group tutoring, and managed the bacterial culture collection of over 20 organisms necessary for the microbiology class. During these endeavors I learned a board range of laboratory techniques, such as aseptic technique, cell culture, genetic manipulation and treatment of cells, and statistical data analysis. These studies constituted my senior research thesis, and culminated in a poster presentation at a regional meeting and two awards. I also discovered my love of research and teaching within academic research settings.

**2. Graduate/Dissertation Research.** After earning my bachelor's of science in biological sciences from Saint Mary's College, I entered the UAB Department of Pharmacology and Toxicology's Department of Defense funded Breast Cancer Training Program (DOD Grant# BCDAMD17-00-1-0119, Lamartiniere). Spurred by a love of biology, as well as, a family history of breast cancer, I chose to study breast cancer development and therapeutics. The UAB program provided a multidisciplinary program encompassing the entirety of the UAB campus. As a requirement for the program, I completed a year of classes and training regarding toxicology and breast cancer. The next year I expanded my knowledge and field of study and took the requirements for a Ph.D. specialty in Pharmacology as well, all while maintaining a perfect grade point average. I joined the laboratory of Dr. Donald J. Buchsbaum in the fall of 2003 and began my dissertation research entitled "Mechanisms by which TRA-8 anti-death receptor 5 antibody and chemotherapy enhance cytotoxicity in breast cancer." This research explored the mechanisms of synergy between the anti-DR5 antibody and chemotherapy agents doxorubicin (commonly used in breast cancer) and bortezomib, focusing of the reversal of resistance, a real clinical problem. During these studies I worked with numerous drugs, cellular targets, and signaling pathways. In-depth molecular analysis of the chemotherapeutic response demonstrated modulation of the pro- and anti-apoptotic molecules by chemotherapy and combination treatment. In 2006, I discovered targeting the X-linked inhibitor of apoptosis protein (XIAP) sensitized resistant cells to the DR5 antibody. I was awarded a DOD predoctoral fellowship (DOD Grant# W81XWH-06-1-070, Amm) based on these findings to complete my studies. However, the small molecule inhibitor I needed was not available via collaboration with Ascenta Therapeutics until 2010. In the meantime I studied the how modulation of the NFκB, Akt and cell cycle-related cell signaling pathways affected TRA-8 sensitivity, not finding any significant modulation. I was able to publish these results as part of a review manuscript in *Cancer Biology & Therapy*. Returning to work on apoptotic proteins, I used novel small molecule inhibitors of the inhibitor of apoptosis family and the Bcl-2 family to sensitize breast cancer cells to death receptor induced apoptosis (published in *Molecular Cancer Research*). The antibody used in these studies (TRA-8, Tigatuzumab) has since entered clinical trials for the treatment of breast cancer. Ongoing with my studies, were collaborations within and outside of my lab. With Dr. Kerri Bevis, I examined the effect of TRA-8 and chemotherapy on apoptosis in ovarian cancer *in vitro* and *in vivo* (published in *Gynecologic Oncology*). Also, in collaboration with Dr. Adam Steg, Dr. Martin Johnson, and my co-mentor Dr. Andra Frost, I examined the effects of the hedgehog (HH) pathway inhibitor, cyclopamine, on pancreatic cancer cells published in *Cancer Biology & Therapy*. Each of these studies enhanced my knowledge of cellular signaling and pharmacology, including small molecule inhibitor design and testing.

**3. Postdoctoral Research.** I meet Dr. MacDougall through a collaborator while interviewing for postdoctoral positions. She was not actively looking for a postdoctoral fellow, but our discussions about her unique tumor models intrigued me and quickly led to me accepting a position at the UAB SOD as a member of the Dental Academic Research Training (DART) program (NIDCR Grant # T32-DE017607), a comprehensive research-training program focused on the development of an innovative, integrated, multi-disciplinary approach to produce well-trained, skilled, collaborative scientists that are capable to address critical dental, oral and craniofacial research issues led by Dr. MacDougall. I had three other postdoctoral fellowship offers, but this was the best fit for me based on Dr. MacDougall's reputation as an excellent mentor, my interactions with her and the lab during my interview, and my expertise in cancer biology and pharmacology bringing something new to her research group. *My previous research experiences focused on breast cancer therapy; for the next stage of my career, I wanted an opportunity where I could help patients poorly-represented in research, work with primary samples, and complement a new lab with my established skills, while working with an excellent mentor.* I entered a very collaborative environment and was immediately able to start working on the development of novel odontogenic tumor cellular models. I helped develop and characterize cell populations established from an ameloblastoma, a keratocystic odontogenic tumor (KCOT), and a calcifying epithelial odontogenic tumor (CEOT), which was published in *Cells Tissue Organs*. Since entering the program, I have worked on the isolation and

characterization of a variety of rare odontogenic tumors, including additional ameloblastomas, KCOTs, a malignant mesenchymoma, and a central odontogenic fibroma. The malignant mesenchymoma and central odontogenic fibroma are both tumors of mesenchymal cell origin with literature consisting of only a few case reports. One first-author manuscript is scheduled for resubmitted following an RNA sequencing experiment requested by reviewers and another is in preparation for submission to *PLOS ONE*. **Our studies represent the first establishment of primary cell explants from these tumors for the purpose of characterizing and treating these tumors.** These are opportunities I would not have had if I remained in the breast cancer field. I also have a recent first-author publication detailing a CEOT case and establishing cell populations in *Journal of Oral Pathology & Medicine*. My additional studies have examined the expression of matrix metalloproteinases in ameloblastoma cells and tumors, and KCOTs, which is under review in *Connective Tissue Research*. I have ongoing collaborations studying the role of enamel matrix protein, amelotin, in mineralization of odontogenic tumors with Dr. Bernard Ganss (Univeristy of Toronto), and the role of odontogenic ameloblast-associated protein (ODAM) in odontogenic tumors with Dr. Daniel Kestler (University of Tennessee).

The benefit of isolating and characterizing these primary cell models is the preclinical examination of treatment options for patients. KCOTs are a symptom of Nevoid Basal Cell Carcinoma Syndrome, which is related to mutations within the patched receptor with increased activity of the HH signaling pathway. Therefore, it provides an excellent model for examining HH activity and exploring HH as a treatment modality. Our studies demonstrated that KCOT-1 cells express HH signaling components and are sensitive to HH inhibition by cyclopamine. These results were published in *Journal of Biological Chemistry* (Ren, Amm et al., 2012). I propose to continue and expand on these investigations through the research plan outlined in this proposal. This award would give me to focus on this research with protected time, allow me to explore the signaling components important in KCOTs, and the potential value of HH and other small molecule inhibitors with future translational applications. Similar studies that I have conducted suggest the clinical utility of hedgehog inhibitors for the treatment of squamous cell carcinoma and ameloblastoma as well. This work integrates my background in cancer cell biology and pharmacology perfectly with my postdoctoral knowledge of tooth development and odontogenic tumors. It also provides me the opportunity to learn new molecular biology and genetic techniques, and gain training in translational research at UAB under the mentorship of Dr. MacDougall and Dr. Frost, and then as an independent investigator.

Dr. MacDougall encourages me to accomplish my research goals as well as engage in professional development activities. She provided the unique opportunity to participate in the DART program and to become a Scholar-at-large in the NIH IRACDA Mentored Experiences in Research, Instruction, and Teaching (MERIT) program. This program is designed to provide postdoctoral scholars with outstanding research and teaching experiences at a minority-partner institution. I have taught lectures and labs at undergraduate, graduate, and professional school levels, as well as mentored at least 9 undergraduate and dental students within the IOHR. I completed this program in the spring 2013, the experience culminating in my teaching a semester long Cancer Biology course for which I designed all course materials, content, and activities. This was a very valuable experience where I focused on scientific writing and cancer topics, such as diagnosis and staging, signaling activation, and therapeutics. Mentoring and teaching experiences are rewarding and essential to building a successful laboratory. At the proposed time for entering into the K99 phase of this award, I will dedicate 100% of my time to research and professional development free of teaching duties. In addition to my MERIT training, I am currently a Member-at-Large for the American Association of Dental Research (AADR) National Student Research Group (NSRG) and Vice-President of the UAB chapter of AADR SOD Student Research Group. These positions have allowed me interact with leading dental faculty and students nationwide and plan events and research competitions. For two years, I was an active participant in the UAB community as a member of the Postdoctoral Association Executive Board, as well as the organizer and facilitator of the UAB Postdoctoral Research Day oral presentation competition for 2012 and 2013 implementing many beneficial changes, such as individual written feedback for each presenter to improve their presentation skills. In 2014, I acted as a consultant for this competition and was able to compete, earning 2$^{nd}$place for a presentation regarding my research. I believe these activities provided a well-balanced postdoctoral experience and that an independent faculty member must participate in mentoring, teaching and, institutional service, as well as excellent research.

**Career Goals and Objectives**

**My career goal is to develop an independent research program as an investigator at a dental academic institution. My research goal is to conduct translational research to elucidate cellular signaling within human dental-related cancers and tumors for the development of targeted therapies.** *Benign tumors with high recurrence rate will benefit from adjunctive chemotherapy, such as a hedgehog inhibitor, especially if the toxicity is low or less than secondary surgery.* **While pharmacologic targeting of the HH pathway is an extension of my postdoctoral studies, characterization of the PTCH receptor and more clinically viable therapeutics for odontogenic tumors and other human tumors are new lines of investigation on which I can build an independent research program.**

Fulfilling my career and research goals begins with the exploration of HH signaling and targeting in KCOT. My pre-doctoral background in pharmacology and postdoctoral experience in dental-related tumors provides me the necessary skills for this proposal. My research within a dental school environment has been ideal, as it provides beneficial opportunities for teaching, mentoring, and the ability to impact dental students by increasing their exposure and understanding of the scientific process. I greatly value the collaborative nature of the UAB SOD and appreciate how my pharmacology knowledge makes me a beneficial addition. My immediate objectives for the K99 phase are to (1) to gain technical expertise of human mutational analysis; (2) gain additional training in translational and clinical research necessary to transition my studies into patients; and (3) participate in the professional skills development program I have outlined in the career development section.

**Career Development/Training Activities During the Award Period**

In order to achieve my career and research goals as described previously, I will pursue mentoring and didactic courses to enhance my research skills and aid in my professional development. Past training in pharmacology and cancer biology will provide the foundation for future research and proposed career development plan. To better acquaint myself with relevant oral and dental research, I have attended weekly meetings of the Oral and Skeletal Biology journal club, the odontogenic tumor section of the UAB SOD Oral Pathology course, and International Association of Dental Research Annual meetings. I have also attended UAB sponsored workshops on presentation skills, scientific writing, and research ethics.

Building upon my past training, the proposed career development plan will prepare me for independence by addressing the following: (1) mentored training molecular biology techniques necessary to analyze the role of the patched receptor in KCOT; (2) didactic training in clinical and translation research; (3) formal training in laboratory management; and (4) professional skills development activities. This section summarizes the activities I will undertake to obtain my career goals and objectives, and their value to my independent academic research career (Timeline in Table 2).

**1. Mentorship.** My mentors are Drs. MacDougall (primary) and Frost (secondary). Dr. MacDougall is the James Rosen Chair of Dental Research, Associate Dean for Research, Director of the Institute for Oral Health Research (IOHR), and Director of the new UAB Global Center for Craniofacial Oral and Dental Disorders (GC-CODED). Her research focuses on genetic craniofacial diseases and odontogenic tumors. Dr. Frost is a practicing Pathologist and Professor in the Departments of Pathology and Cell, Developmental and Integrative Biology, and Scientist at the UAB Comprehensive Cancer Center (CCC). Her research focus is on the role hedgehog (HH) and Gli-mediated transcription. Both have experience mentoring post-doctoral fellows, with several of Dr. MacDougall's trainees obtaining faculty positions. Through these mentors, I will have access to expertise and technical resources necessary for the success of the proposed research, including the whole faculty and resources of the Institute of Oral Health Research. Dr. MacDougall and Dr. Frost will be available to me with an open-door policy and regularly scheduled meetings with Dr. MacDougall weekly, and Dr. Frost monthly. For additional scientific and professional development support, I have constructed an External Advisory Committee of Drs. Ruppert, Bray, Klug, and Waite (Table 1). I will have scheduled in-person or phone meetings with Dr. MacDougall weekly, Drs. Frost and Waite monthly, Dr. Ruppert bi-weekly, and Drs. Bray, Klug, and Waite bi-monthly. Each person is also available on an as-needed basis to discuss technical, research, or professional issues. The members of my External Advisory Committee complement my mentors with knowledge of genetics, molecular and cancer biology, translational research, and the treatment of craniofacial tumors. **My advisory committee will aid my progress by review of scientific data (as detailed in Table 1), editing of manuscripts, and reviewing of grant and career development materials. My progress will be evaluated by publication of manuscripts, submission of abstracts to international meetings, submission of grants (for deadlines see Table 2) and an annual progress report.**

**Table 1. Mentorship Team**

| Name | Position | Proposed Role | Expertise |
|---|---|---|---|
| M. MacDougall, PhD | Professor and Chair, IOHR | Primary mentor | Dental genetics, Craniofacial biology |
| A. Frost, MD | Professor, Pathologist; Scientist, CCC | Secondary co-mentor | HH and Gli signaling |
| J.M. Ruppert, MD, PhD | Professor, Biochemistry, West Virginia University | Advisor, review Gli expression data | Molecular biology, Gli-mediated transcription |
| M. Bray, PhD | Professor and Chair, University of Texas at Austin | Advisor, review PTCH receptor data | Genetics, Sequencing, Genetic basis of disease |
| C. Klug, PhD | Professor and Leader, CCC Experimental Therapeutics Program | Advisor, review data with HH inhibitors | Translational research, Cell and Cancer biology |
| P. Waite, MPH, DDS, MD | Professor and Chair, Oral & Maxillofacial Surgery | Advisor, recruit patients and review treatment data | Craniofacial surgery, Treatment of odontogenic tumors |

My primary and secondary mentors were chosen based on their expertise and the areas of research proposed in this application. Dr. MacDougall will provide the novel cell models used in this research, reagents and technical training, as well as research and office space. She and members of her laboratory (Drs. H. Erlandsen, C. Lu and O. Mamaeva) have experience with the genetics techniques outlined in the proposal (specifically Aim 1). Dr. Frost will provide the reagents, technical expertise, and equipment necessary for the Gli-transcriptional assay used in each aim (Kwon et al., 2011).

**2. Obtain didactic training in clinical and translational research.** To address my goal of implementing new therapeutics for patients and strengthen my understanding the biology and treatment of KCOT, **I have added Dr. Peter Waite (Chair of the UAB Department of Oral & Maxillofacial Surgery) to my advisor committee.** I will also attend the odontogenic tumor section of the SOD UAB oral pathology course. In addition, I will enroll in the Office of Postdoctoral

Education (OPE) course **"Translational Science."** This course focuses on interdisciplinary approaches to translational science and includes M.D. and Ph.D. scientists. The course is designed to encourage collaboration and project development between bench scientists and clinicians. It includes design and implementation of all phases of clinical and translation research including regulatory and ethical concerns (18 hours of instruction). Teams of scientists and clinicians design a translational project throughout the course and are given feedback from UAB faculty after presenting their ideas.

**3. Obtain formal training in laboratory management.** Creating an independent lab contains many components, to prepare myself I will enroll in the **"Lab Management"** course offered by the OPE, which covers a variety of topics including manage a budget, effective hiring, mentoring, data management, and safe laboratory practices and regulations (18 hours of instruction). A laboratory management plan is prepared and presented in the course with faculty feedback provided. I also participate in the SOD hosted the **Academic Career Club** to council trainees preparing for independent careers in dental academics. Monthly seminars prepare trainees by addressing effective mentoring, leadership, managing team dynamics, constructing a curriculum vitae, faculty benefits, career paths, and information on which dental schools are seeking new faculty. These skills will be essential during the R00 portion of the career development award as it would help me create my own laboratory and successfully manage my independent research.

**4. Professional skills development activities.** Exceptional writing is essential for writing grants and manuscripts to support independent research projects. I will attend the **"Professional Skills"** and External Advisory Committee will be to **establish milestones for my progress** (manuscript submission, research studies (based on expertise detailed in Table1, job applications, etc.). "**Training Program"** offered by the UAB Center for Clinical and Translational Science (CCTS). This is a seminar series to provide development in the areas of research, grant writing, other scientific writing, presentations, and leadership. This monthly series invites faculty from throughout the university to discuss relevant career development topics (15 total hours per year).

In order to cultivate my presentation skills and provide opportunities for networking, I will participate in local and international events and conferences. Locally, I will present posters at the annual competition at the **UAB Comprehensive Cancer Center (CCC) Retreat** and **SOD Scholars' Day Symposium**. I will compete in the oral presentation competition, **Postdoctoral Research Day (PDRD)**. I garnished 2nd place for my presentation in the 2014 PDRD. The K99 mechanism would provide opportunities to attend the annual **International Association of Dental Research/American Association of Dental Research (AADR) General Session** and the **American Association of Cancer Research (AACR) International Conference on Molecular Targets and Cancer Therapeutics**. I will continue to participate in the **Oral and Skeletal Biology Journal Club** (CD721), which covers a wide variety of topics relevant to genetic, developmental, and molecular features of oral and bone biology. It provides opportunities to improve presentation skills, scientific critical thinking, and stimulating scientific conversation. As outlined in my **Training in the Responsible Conduct of Research (RCR),** I will take **"Principles of Scientific Integrity"** (GRD 717) my first semester of the K99 phase.

**Table 2. Timeline of Research and Career Development**

| Year | Activity (% effort) | Fall | Spring | Summer |
|---|---|---|---|---|
| 1 | Research (65%) | Aim 1: Functional significance of Patched receptor | | |
| | Courses (10%) | CD721, GRD717 | Grant Writing, CD721 | |
| | Career Development (15%) | **Submit papers from postdoctoral studies** | | **Draft paper from Aim 1** |
| | Conferences (5%) | CCC Retreat | AADR, PDRD, SOD Scholars' Day | |
| | Ongoing (5%) | Professional Skills Training Program from CCTS | | |
| 2 | Research (65%) | | Aim 2: HH inhibition in KCOT | |
| | Courses (10%) | Lab Management, CD721 | Translation Science,CD721 | RCR Seminar |
| | Career Development (10%) | **Submit Aim 1 Paper** | | **Draft papers from Aim 2 (*in vitro* component)** |
| | Conferences (5%) | AACR, CCC Retreat | AADR, PDRD, SOD Scholars' Day | |
| | Ongoing (10%) | Write job applications, Finalize professional transition plans | | |
| R00 | Research (75%) | | Aim 2: HH inhibition in KCOT (*in vivo* studies) | |
| | Conferences (5%) | AACR | AADR | |
| | Career Development (20%) | | **Submit paper from Aim 2** | **Write R00** |

**Training in the Responsible Conduct of Research**

During both my graduate and postdoctoral training, I have maintained certification in chemical safety, proper use of research animals, institutional review board training, and health insurance portability and accountability act (HIPAA) privacy and security training. I will take the UAB course "Principles of Scientific Integrity" in the fall of 2014. This course has recently been updated and has a new "team based learning" format.

1) Format: I will take the UAB course "Principles of Scientific Integrity" (GRD 717) in the fall of 2014. This three-credit hour course provides instruction on ethical issues and principles in the practice of science through mentoring, reading, and case discussions.

2) Subject Matter: Topics covered in GRD 717 include the nature, extent, and causes of fraud in science; UAB policies on fraud; ideals of good science; the responsibilities of authorship and peer review; potential problems raised by the commercialization of research; conflict of interest; data management; collaborative science; mentor and mentee responsibilities; and ethical issues involved in animal experimentation and in clinical trials, such as protection of human subjects.

3) Faculty Participation: The course director is Dr. Jeffrey A. Engler, Associate Dean for Academic Affairs, UAB Graduate School, and is team taught by at least ten UAB faculty members allowing each to discuss their area of expertise.

4) Duration of Instruction: This course is offered in the fall and spring semester each year. It provides 25 contact hours of instruction with additional out-of-class activities.

5) Frequency of Instruction: The course meets once a week through the semester.

In addition, Dr. Mary MacDougall (primary mentor) and Dr. Andra Frost (co-mentor) will offer mentorship, including discussions about ethical conduct, daily in the laboratory.

In the second year of the K99 phase, I will attend a 70 minute refresher course offered by the Professional Skills Training Program, sponsored by UAB's Center for Clinical and Translational Science. The presentation focuses on ethical data management in research.

**Statements by Mentor and Co-mentor**

It is my pleasure to write this statement in support of **Dr. Hope Amm's** application for a NIDCR sponsored K99/R00 NIH Pathway to Independence (PI) Award. Hope is a gifted post-doctoral fellow that has been training since August 2010 under my guidance at the Institute of Oral Health Research (IOHR) at the School of Dentistry (SOD) at the University of Alabama at Birmingham (UAB). Dr. Andra Frost, the co-mentor, and I will work together with the candidate to direct and execute the K99 phase of the program. We will provide an evaluation of Dr. Amm's progress and review and comment on the application for the independent phase of the award. Dr. Amm has no teaching duties and will spend 100% of her time in research and training activities described in the proposal.

Dr. Amm's career plan is to develop into an independent scientist by gaining experience in translational research and the writing, designing, and implementation of independent research projects for a career in dental academics related to her interest in pharmacology. Through the support of this grant she will be able to acquire sufficient preliminary data for "proof-of-principle" that targeted therapies are viable treatment options for odontogenic tumors and other oral-related cancers. Due to her interest in dental, craniofacial oral health research sparked by the knowledge of new emerging tumor cell models established in Dr. MacDougall's lab she elected to do her research in the IOHR at the SOD. She was supported by the SOD's NIDCR funded T-90 Dental Academic Research Training (DART) Grant and is a Mentored Experiences in Research, Instruction, and Teaching (MERIT) Scholar At-Large. Her involvement in these programs makes her a unique, highly qualified applicant and her interest in mentoring young scientists and dental students conducting research and teaching dental basic courses makes a dental academic position perfect for her advancement.

As evident from Dr. Amm's CV she has considerable experience and expertise in the field of pharmacology and cancer biology. She completed her Ph.D. thesis with Dr. Donald Buchsbaum, an excellent mentor with an outstanding research program in the development of novel therapeutics for cancer, focusing on targeted antibodies. Hope has several publications regarding synergistic mechanisms of an anti-death receptor antibody, TRA-8, in combination with chemotherapy and small molecule inhibitors for the treatment of breast cancer. Since joining Dr. MacDougall's laboratory, Dr. Amm progressed well in her role and has been in charge of multiple related projects. In the laboratory, Hope has shown incredible attention to detail, an ability to learn and perfect new techniques, and work seamlessly with others. The first project she worked on was the isolation and characterization of cell populations from primary odontogenic tumor samples. She has worked with numerous tumor models, including ameloblastomas, keratocystic odontogenic tumors (KCOT), and several rare tumors (calcifying epithelial odontogenic tumors, malignant mesenchymoma, and central odontogenic fibroma). From these projects, Hope has three first-author manuscripts and two co-author manuscripts. Relevant to this application, Hope has two first-author publications using the KCOT cell populations described in this application and another in submission to PLOS ONE. In addition, Hope has first-author manuscript that will be resubmitted soon pending experiments to respond to reviewers' comment regarding the characterization of a malignant mesenchymoma cell population. Next, Hope's investigations on the role of matrix metalloproteinases in ameloblastoma bone invasion and the study of a rare central odontogenic fibroma from a Gorlin's syndrome patient will also produce manuscripts. Hope also has been isolating stem cell-like populations from KCOTs and discovered increased expression of members of the hedgehog signaling pathway in the stem cells. This work earned her an AADR Bloc Travel Award and an oral presentation at the IADR General Session in 2012 in Iquazu Falls, Brazil. Continuations of this project linking the increased hedgehog expression in KCOT stem cell-like populations to increased matrix metalloproteinase expression earned her an oral presentation and Young Investigator's Travel Award to the 11 International Conference of the Chemistry and Biology of Mineralized Tissue (ICCBMT). A manuscript detailing this work is in preparation for Molecular Cancer Research. The proposed

project extends her current projects into a more translational focus with no overlap. She will use the unique resource of a KCOT cell model system she established presented in her joint first-author publication in the Journal of Biological Chemistry exploring the inhibition of hedgehog signaling to inhibit cell viability in a single KCOT cell population. The results in this manuscript constitute some of the preliminary data found in this proposal and reflect Hope's potential. To further support this work, Hope established a collaboration with Genentech, Inc and is working on another with Novartis, Inc. to provide the hedgehog inhibitors described this the proposal. Recently, she has been inhibiting the hedgehog, notch, and Wnt pathways to reduce cell viability in primary KCOT and ameloblastoma cells, and squamous cell carcinoma cell lines. This work has earned her a UAB Postdoctoral Travel Award, a 2nd place win at the UAB Postdoctoral Research Day, and a place as a finalist for the American Association of Dental Research (AADR) Johnson & Johnson Healthcare Products Hatton Awards Competition. At the AADR Annual Meeting in March 2014 she will compete for the opportunity to present at the International Association of Dental Research (IADR) General Session through an all-expense paid trip to Cape Town, South Africa. She is also a Member-at-Large for the AADR National Student Research Group (NSRG) and has been organizing events, competitions, and content for the AADR Annual Meeting. Hope is a gifted and dedicated scientist who has also become a leader amongst first the dental students and postdoctoral scholars at UAB and later at the national level. This K99 award would allow her 100% projected time to focus on the experiments outlined in the proposal. Hope is familiar and skilled at all of the methods proposed here and will have excellent support available at UAB. We strongly believe Hope can successfully perform these experiments and they will help establish her independent research career.

Dr. Amm's primary mentor, Dr. MacDougall, is an internationally recognized craniofacial biologist with expertise in molecular mechanisms associated with tooth formation, extracellular matrix formation, and related human genetic dental diseases. Dr. MacDougall is a tenured Professor in the Department of Oral and Maxillofacial Surgery and a Senior Scientist at the UAB Comprehensive Cancer Center (CCC) with a joint appointment in the Graduate School (GS). She is the SOD's Associate Dean for Research and the Director of the IOHR holding the James R. Rosen Chair of Dental Research. Dr. MacDougall received her B.A. from the University of California at San Diego and her Ph.D. from the University of Southern California (USC), School of Dentistry, through a NIH/NIDCR funded T-32 Craniofacial Biology Graduate Program under Dr. Harold Slavkin. She was the recipient of both NIDCR pre-doctoral and post-doctoral training awards. In 1993, she was appointed Associate Professor in the Department of Pediatric Dentistry at the UTHSCSA Dental School. In 1998, she was promoted to full Professor with tenure and in 1999 she was appointed as the first Associate Dean for Research for the Dental School. She served as Program Director for the NIDCR funded program project grant, "Gene Expression and Regulation during Odontogenesis-GERO". She is the immediate Past President for the IADR, is an External Advisory Board member for several funded NIDCR T-90 and T-32 Training Programs, and is a member of the UAB GS Advisory Committee. She is a past President of the AADR, a past Chair and member of the NIDCR Special Grants Review Committee, has served on the Editorial Boards of the *Journal of Dental Research* and *Archives of Oral Biology*. Her research has been funded by the NIDCR since 1985. In 2001, Dr. MacDougall received the IADR Distinguished Scientist Award for Pulp Biology, in 2003 the AADR National Student Research Group Mentor of the Year Award, in 2005 the IADR Distinguished Scientist Award for Mineralized Tissue, in 2006 become a Fellow of the American Academy for the Advancement of Science (AAAS), and in 2010 received the second awarded AADR Distinguished Mentorship Award. Dr. MacDougall has been a featured mentor on the NIDCR's video "Research A Way of Life" and ADEA's DVD "Academic Careers in Dentistry. Dr. MacDougall served as Program Director of a NIDCR T-35 Short-term Training Program for Dental Students at UTHSCSA (1995-2001) and a NIDCR T-32 Institutional Research Training Grant "Craniofacial Oral-Biology Student Training in Academic Research-CO-STAR" until moving to UAB (June, 2005). She currently serves as Co-PI of the UAB T-35 Short-Term Research Training for Health Professional Students grant (T-35DK07545, Dr. Robin Lorenz PI). She has trained 5 DDS/Ph.D. students, Drs. Knight,

Inozemtseva-Verona, Yen, Dodds, and Lamani, four who hold faculty positions (UTHSCSA, USC, and University of North Carolina respectively). Her first UAB Postdoctoral fellow Dr. M. Passineau received the first K99/R00 grant (supported by NIDCR) at UAB. Currently, she is mentoring one DMD/Ph.D. student at UAB SOD, A. Gullard. Four of her DDS/PhD students have received NIDCR F-30 Individual Training awards: two at UTHSCSA, one at UAB and her current UAB SOD student (A. Gullard). In 2000, she implemented the UTHSCSA Dental School's "Dental Student Training in Academics and Research (D-STAR) Program", designed as an innovative early intervention program to target minority students for careers in dental academics. This program initially funded by the first ADEA Gies Research Scholarship Award, was highly successful in recruiting minority students to dentistry (V. Martinez, J. Ibarra, M. Garcia, B. Cortez, and L. Martinez). In 2006, she implemented a similar minority pipeline program targeting African American students called **PreDART** at UAB SOD funded through an ADEA William Gies Scholarship Award (2006 and 2012).

This award will allow Dr. Amm to strengthen her transition to a career in dental academics and obtain training and expertise in translational research and genetics through interaction with the SOD's IOHR, GC-CODED, Heflin Center for Genetics, and the UAB CCC. To facilitate this transition to translational research, Dr. Andra Frost has agreed to serve as a co-mentor for Dr. Amm. Dr. Frost is a tenured Professor in the Department of Pathology and a Scientist at the CCC. Her research interests encompass both clinical and laboratory-based translational research focusing on understanding the effects of hedgehog signaling/Gli-mediated transcription on breast cancer promotion and progression, with the ultimate goal of identifying new targets for the prevention or treatment of invasive and metastatic breast cancer. Dr. Frost received her B.A. from University of Tennessee in Knoxville and her M.D. from UAB. She did a residency in clinical pathology and a fellowship in surgical pathology and cytopathology at George Washington University prior to her first Assistant Professor position at Georgetown University in 1990. She has consistently been the PI on grants from the NCI, DOD, and Susan G. Komen since 2000. She has a total of 104 published papers, 34 peer-reviewed papers since 2006, of which 6 focus on hedgehog signaling as a therapeutic target in human carcinoma. She has very active collaborations and has been a co-investigator on numerous NIH-funded grants and has been active in the CCC NCI-SPORE in breast cancer. She has a long record of mentoring in clinical medicine and research. She is or has been a research mentor to 6 undergraduate students, 3 graduate students, 3 postdoctoral fellows and 2 pathology residents. She has been a clinical mentor in pathology to many residents, clinical fellows and medical students. Her research trainees have gone onto to research careers in academia (post-doctoral and faculty positions) and industry as well as to careers in medicine, pharmacy and optometry. Dr. Frost has been a member of the Pathology Residency Training and Selection Committees and the UAB Molecular Pathology Training Committee, and the Program Director of the Cytopathology Fellowship Training program. Hope had an active collaboration with one former trainee in Dr. Frost's lab, Dr. A. Steg. Their collaboration focused on the therapeutic effects of inhibition of hedgehog signaling i pancreatic and breast cancer cell lines and resulted in a publication in *Cancer Biology & Therapy*. During this collaboration, Dr. Frost and Hope developed their professional relationship, and Hope knew she was the perfect co-mentor for this phase of her career. Due to Dr. Frost's clinical and translational research expertise and her knowledge of hedgehog signaling and Gli-mediated transcription relevant to this proposal. Dr. Frost will also be providing viral vectors, specialized reagents, equipment, and technical support necessary for the proposed studies.

During her postdoctoral phase Dr. Amm will participate in several unique training experiences including: the **SOD's Scholars Symposium**, **DART Seminar Program**, **Postdoctoral Research Day**, **SOD Deans' and IOHR Seminar Series**, the **SOD Journal Club, Mentorship in the IOHR,** the **SOD Academic Career Club**, **SOD Faculty Career Development Program, Scientific and Grant Writing seminars**, a **Laboratory Management course,** as well as a course in **Responsible**

**Conduct of Research** Furthermore, she will be expected to present her research at national scientific meetings such as the IADR and AADR. The **SOD Scholars' Symposium** is presented each year prior to the IADR/AADR meeting with all SOD dental students, graduate students, residents and faculty attending. A prominent speaker (2012- Dr. Brendan Lee Howard Hughes Fellow) is invited to present cutting edge research and this presentation is integrated with a UAB faculty speaker. Other activities include Lunch and Learning programs, Table Clinics, mini-workshops, and UAB Centers Core facility presentations. The **Postdoctoral Research Day** is an annual oral presentation competition for postdoctoral fellows. Dr. Amm not only will participate in this event, but has been essential in the planning every facet of the event. For 2013, she raised almost $11,000 of support from UAB sponsors and developed new, beneficial changes to the structure of the event. The annual **DART Trainee Seminar** is designed to allow students to present their research to fellow trainees in a formal setting mimicking a postdoc/faculty interview presentation. The **SOD Deans' and IOHR Seminar Series** invite national and international researchers to present their work related to dental academics/curriculum issues and basic and clinical dental, craniofacial and oral health research, respectively. Dr. Amm will also attend select seminars at other schools/U-WIRCs related to her research exposing her to other UAB faculty and students. She will attend the SOD's **Dental and Skeletal Journal Club** presenting current cutting edge research and methodology articles**.** Dr. Amm will also interface with the SOD's **DART trainees** providing **mentorship training** through faculty guidance/instruction for an assigned Ph.D. student or dental student and as the current Vice-President of the Alabama chapter of the AADR National Student Research Group.

**SOD Academic Career Club** (started in 2002) is to support and provide guidance for students wishing a career in dental academics such as Dr. Amm. Assigned faculty mentors are encouraged to meet at least every month with students to discuss the student's present research, didactic activities, and counsel them on future plans. Mentors are expected to involve their advisee in their routine faculty activities, including research and teaching. This is intended to provide a flavor of the academic life for the student. The Club programs occur once monthly (5:30-7:00pm) and include speakers providing information on various aspects of academics, including practical aspects such as how to construct curriculum vitae, faculty benefits, career paths and information on which dental schools are seeking new faculty.

**SOD Faculty Career Development Program.** The Faculty Career Development Program is directed by **Dr. Noel Childers (Chair Pediatric Dentistry**). The overall goal of this program is to improve the recruitment, retention, and career development of postdoctoral trainees and new faculty. Dr. Childers' facilitates new members' enrollments into its three components (Mentoring Program, Junior Faculty Enrichment Program, Career Development Support Program), and tracks their progress through these programs.

**SOD Leadership Workshop.** This program offers instruction on the general principles of leadership including team building, strategic planning, principles of good leadership, and conflict management and career planning. Seminars are based on the UAB BLAZE Leadership Academy. **Drs. Pam Burke/MacDougall**

**Translational Science Course.** This course focuses on interdisciplinary approaches to translational science and includes M.D. and Ph.D. scientists and is designed to encourage collaboration and project development between bench scientists and clinicians. It includes learning about design and implementation of all phases of clinical and translation research including regulatory and ethical concerns.

**Scientific and Grant Writing Seminar Series (SWSS).** The SWSS cover selecting funding mechanisms, writing of individual grant sections, following the administrative process and peer review. Topics include planning, writing, and submitting competitive NIH grant applications; organizing, writing, and critiquing scientific manuscripts; and developing basic skills to prepare and deliver effective presentations.

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**Laboratory Management Skills** (GRN 721, Fall, 3 credit hours). This course is designed to develop skills related to managing a laboratory and personnel. Topics include building a team, effective hiring, data management, laboratory safety, developing good people skills, budget management, and university organization and the tenure process. Course director **Dr. Lisa Schwiebert**.

**Principles of Scientific Integrity** (GRD 717, 8 1.5 hour sessions). This is a semester-long course that provides a survey of ethical issues and principles in the practice of science.

In summary, Dr. Amm will have no formal teaching or service responsibilities for the duration of this K99/R00 award. She will devote her full time (100%) to the research project in the IOHR. During the end of the first year of the mentored phase, Dr. Amm will be strongly encouraged to apply for tenured faculty positions at dental and medical schools to activate the independent faculty phase of this award and thus be able to transition into truly independent research funding. Dr. Hope Amm is an intelligent and determined individual, and a talented scientist. I am very happy to support and recommend her.

Sincerely,

**Description of Institutional Environment**

The research environment at UAB is very well-developed and provides every resource I need to facilitate my development into an independent investigator. UAB has a long history of encouraging interdisciplinary collaborations as a strategy to maximize its research productivity. Multiple faculty appointments in multiple departments are common, and interactions between basic science and clinical units also occur frequently, leading to an unusual ease in translating bench top discoveries to clinical practice. A major factor that has contributed to the interactive research environment at UAB has been the role of the interdisciplinary, interdepartmental research centers (UWIRC) that are the basis for scientific efforts within the University. There are currently 21 University-wide Interdisciplinary Research Centers and 5 Pilot Centers. These multidisciplinary centers are available to all UAB investigators and greatly enhance the research opportunities and career development of their trainees. The Center-associated core facilities and enrichment programs are key resources for Dr. Amm's research and professional development.

**UAB UWIRCs of particular benefit to this application are:**

**Comprehensive Cancer Center (CCC).** One of the nation's leading cancer research and treatment centers under the guidance of Dr. Edward Partridge, the UAB CCC is routinely recognized as being among the nation's best. The UAB CCC is one of the original National Cancer Institute (NCI)-designated cancer centers, one of only 40 in the nation. They have been awarded more than three multi-million Specialized Program of Research Excellence (SPORE) grants focusing on breast, brain, pancreatic, ovarian, and cervical cancers. The CCC faculty have over $45 million annual in grants from the NCI and an additional $65 million from the NIH. The CCC is home to an outstanding faculty of more than 330 physicians and researchers, many of whom are internationally and nationally recognized for their expertise in oncology. One of the primary missions of the UAB Comprehensive Cancer Center is to support cancer research and the faculty conducting that research across the UAB campus. One form of this support is the establishment and sponsorship of shared facilities, which provide access to high-end equipment, cutting-edge technology and expert scientific consultation. By providing these services to our members, the Cancer Center hopes to foster an interactive and collaborative environment that will lead the way into the future of cancer research.

**Center for Clinical and Translational Science (CCTS):** This is a university-wide, interdisciplinary UAB Center for Clinical and Translational Science which is funded through the NIH Clinical and Translational Science Award Program. This center provides support for translational research, includes career development components such as assistance with developing research ideas and writing grant proposals, and offers professional development opportunities such as scientific writing and leadership seminars.

**Global Center for Craniofacial, Oral and Dental Disorders (GC-CODED).** The GC-CODED is a new center that is housed within the School of Dentistry under the leadership of Drs. MacDougall and John Grant. This center brings together basic, translational and clinical research, clinical patient care, training and educational expertise, community outreach and philanthropy from schools across the UAB campus for global discoveries related to craniofacial, oral and dental disorders, including Nevoid basal cell carcinoma syndrome.

**The Heflin Center for Human Genetics.** Under the leadership of Dr. Bruce Koff, is housed in the Department of Genetics and provides invaluable resources for research. The Genomics Core Facility under the leadership of Drs. Molly Bray and Michael Crowley, has three high-priority technological resources: microarray analyses, high-throughput sequencing, and high-throughput genotyping, including single nucleotide polymorphisms (SNPs). The Molecular and Genetic Bioinformatics Facility supports the bioinformatics needs of investigators.

**Genetically-Defined Microbe Core: Retroviral Unit:** The Retroviral Unit, under the guidance of Dr. John Kappes, provides services and expertise in virus vector gene transfer and expression. The Unit constructs viral vectors, particularly lentiviral vectors, with genes of interest and genetically engineered recombinant cell lines and assay systems making this technology broadly accessible to the UAB community.

**Center for Metabolic Bone Disease (CMBD).** CMBD provides key core facilities include a Histomorphometry and Molecular Analyses Core, which provides immunohistochemistry services and support.

**Office of Postdoctoral Education (OPE):** Under the guidance of Dr. Lisa Schwiebert, the OPE provides postdoctoral fellows with the opportunities and skills they need to be successful. The OPE offers numerous leadership and funding opportunities, travel awards and skill development opportunities, as well as a variety of professional development courses including grant writing, laboratory management, and translational research.

UAB SCHOOL OF DENTISTRY
Office of the Dean

March 7, 2014

To NIH Pathway to Independence Award Committee:

I am pleased to write this letter in support of the K99/R00 application of **Dr. Hope Amm**. I know Dr. Amm through her appointment to the UAB School of Dentistry's NIDCR funded T-32 and T-90 Dental Academic Research Training (DART) programs for which I serve as Co-Director. Dr. Amm is a talented research scientist who has distinguished herself both as a graduate student and postdoctoral fellow. She has the talent, motivation, and commitment needed to become a highly successful academic scientist.

Dr. Amm will work with co-mentors Dr. Mary MacDougall, Associate Dean for Research and Director of the Institute of Oral Health Research (IOHR), and Dr. Andra Frost, Professor, Department of Pathology and Comprehensive Cancer Center. Dr. Amm's research will investigate the role of hedgehog signaling in keratocystic odontogenic tumors. Interdisciplinary collaboration is a major strength and hallmark of the School of Dentistry (SOD) and UAB as reflected by Dr. Amm's current research and research outlined in this application.

The SOD provides a strong research expertise in oral-craniofacial developmental biology and genetics. The SOD has a funded T-90 Dental Academic Research Training (DART) Grant and six individual pre-doctoral fellowship awards. In addition, in 2012 the SOD was awarded a seven year $66.8 million dollar grant from the NIDCR for the National Dental Practice-Based Research Network (PI, Dr. Gregg Gilbert), the largest single grant awarded in UAB history. With this new funding, we were ranked 1st for NIDCR funding with $15.1 million dollars. The full-time SOD faculty numbers 63 (15 FTE research focus) and they published 95 books/chapters and peer-reviewed papers in 2012-13. The SOD is pleased that the first K99 awarded at UAB was to Dr. Michael Passineau under the mentorship of Dr. MacDougall. Dr. Passineau has gone on to a faculty position at Allegheny-Singer Research Institute (Pittsburgh, PA) and received an R01 related to his independent research award pioneering new gene therapy approaches for salivary diseases.

There are numerous opportunities for scientific interactions within the SOD and with entities across the UAB campus relevant to Dr. Amm's training goals. These include the SOD's IOHR, the Global Center for Craniofacial, Oral and Dental Disorders (GC-CODED, housed in the SOD), the Comprehensive Cancer Center (CCC), Center for Clinical and Translational Science, Heflin Center for Genomic Sciences (housed in the Department of Genetics), and the Center for Metabolic Bone Disease (CMBD, housed in the SOM).

The SOD fully supports Dr. Amm's application for this K99/R00 award, and will protect her time so that she can develop into a productive independent investigator. Dr. Amm will devote 100% of her time to training and research during the postdoctoral phase of this grant, with the goal of achieving a successful transition from the K99 to the R00 independent research phase through ascertainment of a tenure track faculty position. During the K99 training period, Dr. Amm will be provided administrative/fiscal support for grant budget management by the IOHR. She will have access to IOHR lab resources and equipment and UAB Core Facilities to successfully carry out her research plan. Time will be allotted by Drs. MacDougall and Frost to provide mentorship and career guidance as outlined in the career development plan.

In summary, the SOD, the IOHR and GC-CODED will provide an ideal environment for Dr. Amm to fulfill the goals of this Pathway to Independence Award.

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## Specific Aims

Keratocystic odontogenic tumors (KCOTs) are highly proliferative, locally invasive cystic lesions with a tendency to recurrence after conservative therapies. Previously known as odontogenic keratocysts, KCOTs were reclassified by the World Health Organization to reflect their neoplastic nature, characterized by a high proliferation rate and bone invasion, particularly within the posterior body of the mandible and ascending ramus (Li, 2011; Shear, 2002). KCOTs are also highly associated with Nevoid basal cell carcinoma syndrome (NBCCS), also known as Gorlin's syndrome, occurring in 66 to 92% of these patients (Lam et al., 2009). NBCCS is an autosomal dominant genetic disease characterized by heterogeneous mutations in Patched 1 (PTCH1) or to a lesser extent Patched 2 (PTCH2) or the SUFU gene. Mutations in PTCH1, a member of the hedgehog (HH) signaling pathway, are present in sporadic cases of KCOT, leading to increased activity of HH signaling within the tumor, which is associated with increased proliferation and neoplastic growth (Li, 2011; Sun et al., 2008; Pan et al., 2009). PTCH is a cell surface transmembrane receptor that represses HH pathway signaling. PTCH binds HH ligands (sonic, indian, and desert HH), and in the absence of the ligand, inhibits the smoothened (SMO) receptor that activates the HH pathway and downstream Gli transcription factors (Ren, Amm et al., 2011). Beside its association with NBCCS and KCOTs, HH activation possibly as a result of PTCH mutations has been reported in ovarian, colon, and pancreatic cancer (Liao et al., 2009). PTCH1 polymorphism Pro1315Leu has been detected in basal cell carcinoma and breast cancer (Asplund et al., 2005; Chang-Claude et al., 2003) with a significant association with breast cancer compared to healthy controls. In our preliminary data, we found this polymorphism in 70% of our KCOT patients suggesting it may play a role in KCOT development. We also identified this polymorphism in a central odontogenic fibroma and its recurrent fibroma in a NBCCS patient. This clearly demonstrates the role of PTCH and HH signaling extends beyond KCOTs and applies broadly to other neoplasias. However, the functional significance of this and many other PTCH1 polymorphisms has not been reported. Additionally, targeting the HH signaling pathway with small molecule inhibitors of the SMO receptor shows clinical promise. Two HH inhibitors, LDE225 (Erismodegib) and GDC-0449 (Vismodegib), are currently in Phase II clinical trials for the treatment of basal cell carcinoma and other human cancers (Raju et al., 2012). These therapies are ideal for the treatment of NBCCS-related and PTCH-related tumors like KCOTs.

The goal of this application is to use novel, unique primary cells derived from KCOTs as models for understanding the role of PTCH and HH signaling and for preclinical development of therapies. **Our hypothesis is that hedgehog pathway signaling plays a role in development and signaling within keratocystic odontogenic tumors, and provides valuable targeting for therapeutics.**

**Specific Aim 1: To determine if the PTCH1 receptor polymorphism Pro1315Leu has a functional significance in HH pathway activity and tumorigenesis potential.**
**Specific Aim 2: To determine the effects of HH inhibition in primary KCOT cell populations using HH inhibitors and knockdown of Smo protein.**

**Significance:** The unique, novel cell models developed here would be beneficial for studying KCOTs and ideal for examining the HH pathway. Research has shown HH activation may play a role in the development of a variety of human tumors. Information gained by these aims will expand our knowledge on the functional significance of HH as mediated by PTCH signaling in tumors. The functional significance of certain PTCH receptor polymorphisms on pathway activity and tumor progression will be defined for the first time. Furthermore, these studies will aid in the development of future clinical treatments for KCOT. There are currently no treatment options available to prevent the recurrence of KCOTs and HH inhibitors may provide great clinical benefit to this patient population.

## Research Strategy

### A. Significance

Keratocystic odontogenic tumors (KCOT) patients are currently treated surgically with more conservative surgical approaches leading to higher rates of recurrence. *Research to reduce recurrence of KCOT is very limited and only focused on surgical technique*, primarily owing to the severe **lack of cellular models for preclinical studies for the identification and testing of therapeutic options**. To address this issue, we generated the first human KCOT cell populations, which can be used to study fundamental hedgehog (HH) signaling, the biology of KCOT and potential treatments. The studies proposed in this grant will help define the role of patched (PTCH) polymorphisms in tumorigenesis and invasion and aid in the development of possible clinical treatments for KCOT. We believe avoiding additional surgeries in a population at risk for recurrence (e.g., those with NBCCS or those treated with conservative surgical methods) offsets the cost of clinical HH inhibitors. KCOTs are associated with mutations within the HH pathway and provide an excellent model for studying the role of HH activity in tumorigenesis, which has broad application to hedgehog involvement in many other human neoplasias. We believe the use of HH inhibitors will be beneficial for other craniofacial tumors (e.g. ameloblastoma) and squamous cell carcinomas, providing benefits to many patients.

### B. Innovation

Primary tumors from patients with KCOTs have been used to create the first explant cultures and only cell populations in existence for *in vitro* preclinical studies. We generated **unique, novel cell models of KCOTs** that will be useful in exploring the functional significance of the HH signaling pathway known to be associated with KCOT. We are continuing to recruit new patients for these studies with IRB approval and informed consent, we anticipate recruitment of 15-20 patients over the period of this grant (to date we have ascertained 6 patients). Mutations and polymorphisms in HH pathway components, such as the PTCH receptors, have been proposed to activate this pathway. However, this theory has not been tested for many of the identified PTCH mutations and polymorphisms. We have made a striking discovery that 70% of our tumors have a P1315L polymorphism in the PTCH1 gene. We aim to define the functional significance of this polymorphism on HH pathway activity, cell viability, and tumorigenesis. These studies could lead to new insights into fundamental HH signaling and its role in tumorigenesis, providing knowledge applicable to many human tumors and cancers.

### C. Approach

#### Background

KCOTs are cystic lesions, previously classified as odontogenic cysts, have a high rate of cellular proliferation and are locally invasive, primarily occurring within the mandible (Li, 2011) (**Figure 1**). A wide variability in the rate of recurrence has been reported ranging from 3-62% (Li 2011). Recently calculated **overall recurrence rate is ~22-26%** by two retrospective studies at the University of Iowa (United States) and Pitié-Salpêtrière University Hospital (France) (Finkelstein et al., 2013; Pitak-Arnnop et al., 2010). The rate of recurrence does vary depending of the aggressiveness of treatment with no standard surgical treatment outline among oral surgeons globally. Surgical techniques commonly employed are curettage, marsupialization, enucleation with or without Carnoy's solution (a mixture of absolute alcohol, chloroform, glacial acetic acid, and ferric chloride), or resection (Johnson et al., 2012). Resection is the most radical of treatments with the lowest recurrence rate followed by enucleation with Carnoy's solution. Also, the use of Carnoy's solution at the surgical site is not without mordibity and recurrence, and would not prevent additional tumors in a patient with NBCCS.

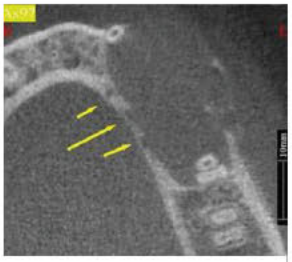

**Figure 1. Radiograph from KCOT patient.** Adapted from www.marcilan.com/pathology/benigntumors/keratocystic-odontogenic-tumor/

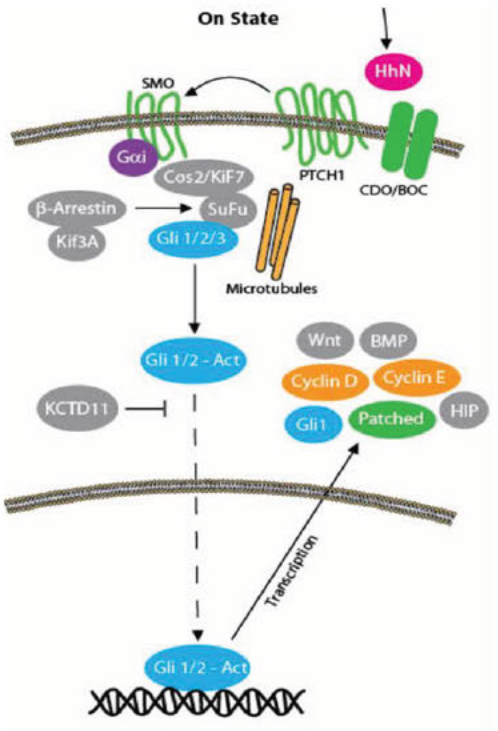


**Figure 2. Active hedgehog signaling.** Adapted from www.Novusbio.com/hedgehogpathway

One well-established means of investigating cellular signaling and preclinical evaluation of therapies is human tumor cells. Our laboratory is the first to establish cell populations from odontogenic tumors (Ren, Amm et al., 2012; Ren et al., 2011, unpublished data) and we have established cell populations from cell outgrowths of tumor from four patients with KCOT (1 syndromic, 3 non-syndromic) to accomplish the aims proposed here. Total cell outgrowths/tumor cell populations represent the heterogeneous nature of human tumors as they are not clonally selected. However, our studies have shown our tumor cell models express relevant epithelial and enamel-derived markers and are

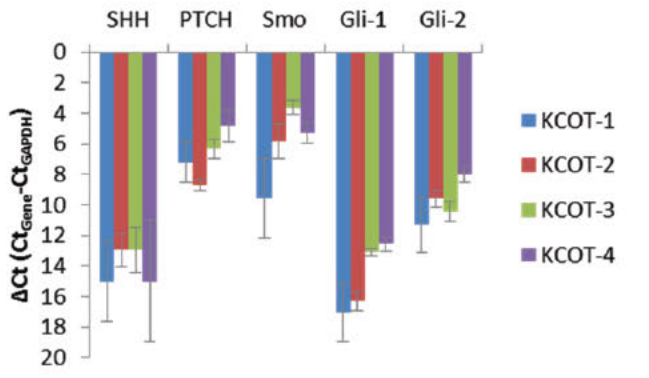


**Figure 3. Expression of hedgehog pathway components in KCOT cell populations.** Expression was determined by qRT-PCR. The housekeeping gene GAPDH was used as normalizing reference. All the experiments were performed in duplicate and repeated three times (error bars denote SD).

capable of contact-independent growth (Ren, Amm et al., 2012; Ren et al., 2011, unpublished data). We have recently recruited an additional NBCCS patient (KCOT-6), and have obtained a recurrent tumor from the KCOT-3 patient. We have not observed statistical differences between the syndromic and non-syndromic KCOTs; however, for any analysis we plan to analyze them separately and compare data. We expect that continued patient accrural will increase the power of our experiments.

Recent studies in the cancer biology field have indicated that early passage primary cell populations and primary tumorgrafts in mice are more likely to retain the genetic features of the human tumor and are better models of human tumors compared to long-term culture, clonally selected cell lines (Hamer et al., 2008; Wang et al., 2013; Garcia et al., 2013).

There is strong association between KCOT and the HH signaling pathway, which is activated when the ligand sonic hedgehog (SHH) binds to the PTCH receptors causing the release of inhibition on smoothened (Smo) (Ren, Amm et al., 2011 and **Figure 2**). GLI-1 and GLI-2 are known activators of HH signaling, and GLI-1 stimulates the expression of itself and PTCH1. Each of these HH pathway components (SHH, PTCH, SMO, GLI-1, and GLI-2) were detected in KCOT-1, 2, 3, and 4 cells by qRT-PCR and protein expression was confirmed by IFC staining (**Figure 3-4**). Expression of these pathway components and transcriptional targets are frequently used to evaluate the presence of HH activity and indicates the KCOT cells have GLI-dependent activity (Ruiz i Altaba et al., 2007; Steg et al., 2010; Steg et al., 2012). We also examined the expression of *GLI1* in a KCOT stem cell-like population. A small subset of tumor cells known as cancer stem cells, stem cell-like cells, or tumor-initiating cells may give rise to tumor recurrence, such as that reported in KCOTs. Stem cell-like populations were isolated from primary KCOT cell population using a tumor sphere forming assay (Bartolomai et al., 2010; Krishnamurthy and Nor, 2013) (**Figure 5**). KCOT-2, KCOT-3, and KCOT-4 tumor spheres, as compared to attached parental cells, had significantly increased *GLI1*, a transcriptional target of HH signaling (**Figure 5**). In addition, GLI-1 is known to mediate the migration and invasion of human cancer cells through modulation of matrix metallo-proteinases (MMP; Kwon et al., 2011; Chen et al., 2012; Liao et al., 2009). We know from our own studies that KCOT cells and tumors express a variety of MMPs, including those regulated by HH (Amm et al., 2014). Due to the invasive nature of KCOTs into the jaw bones and the possible role of GLI-1 in MMPs expression, we investigate the expression of certain MMPs in the tumor stem-like populations. MMP2, MMP11, and MMP14 expression was increased in KCOT-2, 3, and 4 spheres compared to parental cells (**Figure 5**). This may indicate the HH activity plays a role in the local invasion of KCOTs into the surrounding tissue and bone. As confirmation, stem cell-like populations were isolated by aldefluor sorting based on alcohol dehydrogenase (ALDH) activity. ALDH high cell populations of KCOT-2 and KCOT-3 had increased expression of *GLI1* compared to ALDH low populations (**Figure 6**). **The increased expression of *GLI1* and genes possibly regulated by GLI-1 in the KCOT stem cell-like populations in comparison with the primary tumor cell populations suggests a role for increased HH signaling in the recurrence and invasion of KCOT and provides rationale for targeting HH signaling to reduce KCOT tumor recurrence.**

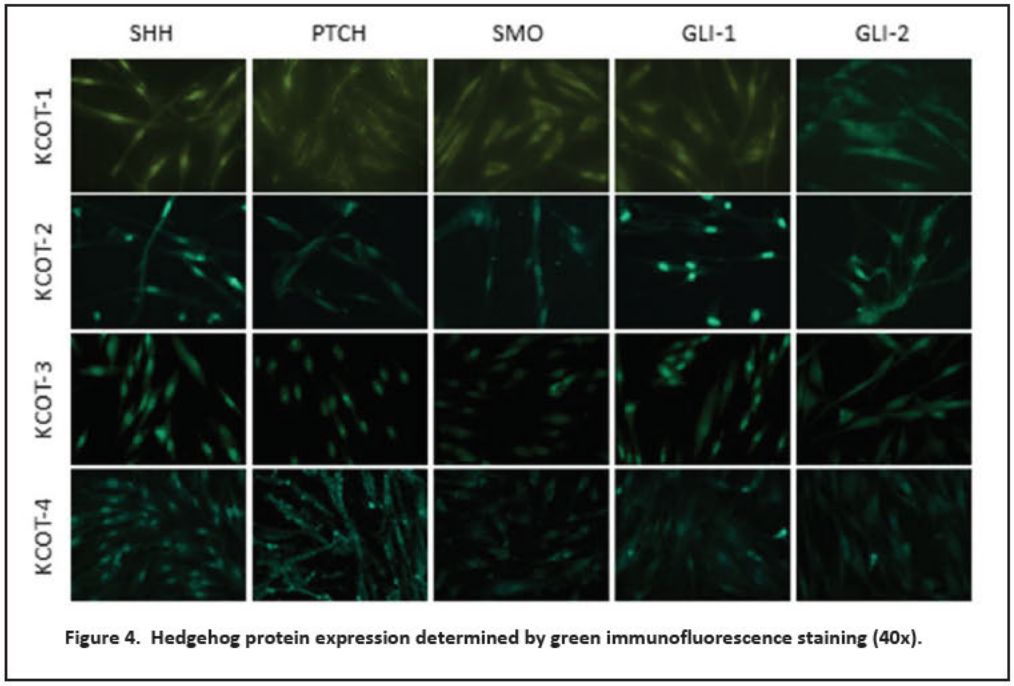


**Figure 4. Hedgehog protein expression determined by green immunofluorescence staining (40x).**

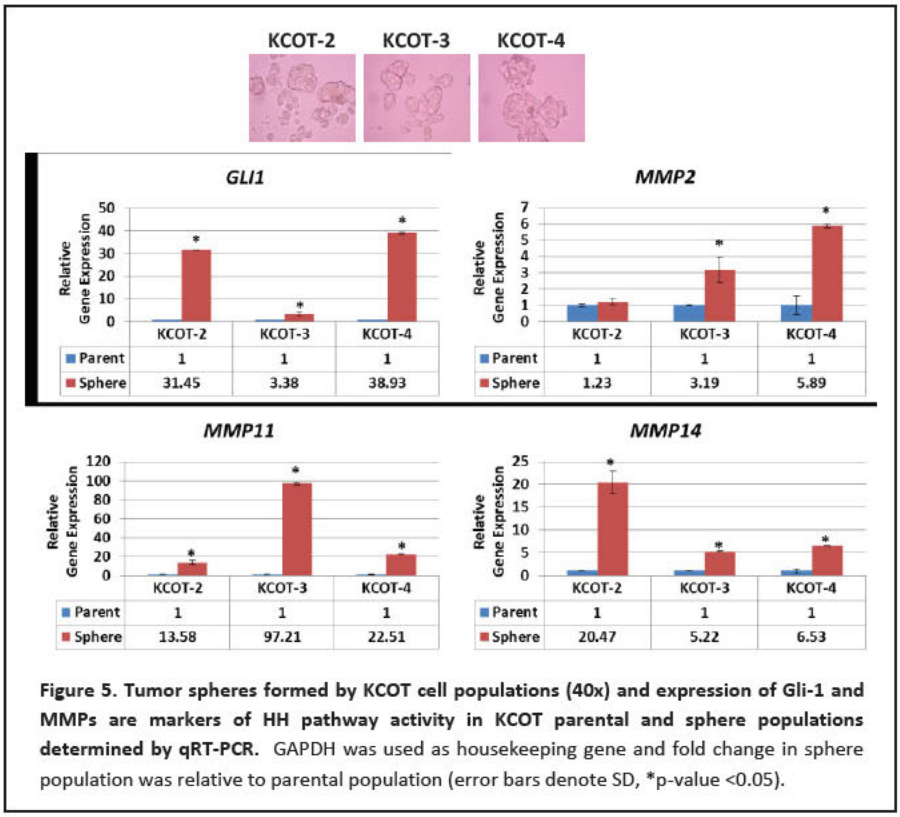


**Figure 5. Tumor spheres formed by KCOT cell populations (40x) and expression of Gli-1 and MMPs are markers of HH pathway activity in KCOT parental and sphere populations determined by qRT-PCR.** GAPDH was used as housekeeping gene and fold change in sphere population was relative to parental population (error bars denote SD, *p-value <0.05).

**Specific Aim 1: To determine if the PTCH1 receptor polymorphism Pro1315Leu has a functional significance in HH pathway activity and tumorigenesis potential.**

**Rationale:** The association between NBCCS (syndromic KCOTs) and mutations affecting the HH pathway has been established (Aszterbaum et al., 1998; Johnson et al., 1996; Lam et al., 2009; Ponti et al., 2012). Patched heterozygous knockout mice (*ptc* +/-) have many symptoms typical of NBCCS, including high incidence of medullablastomas, basal cell carcinoma (BCC)-like lesions, and mandibular jaw cysts similar to KCOTs (Ohki et al., 2003). The expression of Gli2, one of the HH-regulated transcription factors in mice, leads to the development of KCOTs from the epithelial rests of Malassez (Grachtchouk et al., 2006). Non-syndromic KCOTs have also been associated with many PTCH1 mutations, which have been detected in several of the 23 PTCH1 exons (Barreto et al., 2000; Gu et al., 2006; Ohki et al., 2004; Pan et al., 2009; Ponti et al., 2012; Song et al., 2006; Sun et al., 2008). Li (2011) provides a synopsis of known mutations in NBCCS and non-syndromic KCOT patients, which occur in 16 of the 23 PTCH1 exons and include missense mutations, nonsense mutations, frameshifts, duplications, and exon skipping. None of these mutations have been identified in our patients or tumors. Previous literature establishing a connection between PTCH1/2 mutations and KCOT development has focused on the relationship between the PTCH1 mutation and proliferation rates with Ki67 (Kadlub et al., 2013; Pan et al., 2009). These studies correlate PTCH1 mutations with increased proliferative rate in KCOT. However, many other factors can lead to increased proliferative rates in cancer and tumor cells. In other models, a functional analysis of the PTCH1 mutation Gly509Val and a truncation mutant (1130X) in *Drosophila* showed these mutations caused increased HH activity and expression of HH targets, PTCH and the growth factor decapentaplegic (Hime et al., 2004). Another truncation mutant (Gln688X) was shown to induce cell transformation and increased GLI-1 activity when transfected into HEK293T or NIH3T3 cells (Barnes et al., 2005). While a potential role of PTCH in KCOT is suggested, it remains unknown if PTCH plays a direct role in KCOT development and pathophysiology.

Given the strong association between the HH pathway and KCOT, we performed bi-directional sequence analysis to detect polymorphisms in the PTCH1 receptors. Sequence analysis of each PTCH1 exon revealed several intron and exon polymorphisms (**Table 3**). One of these polymorphisms, **Pro1315Leu in exon 23**

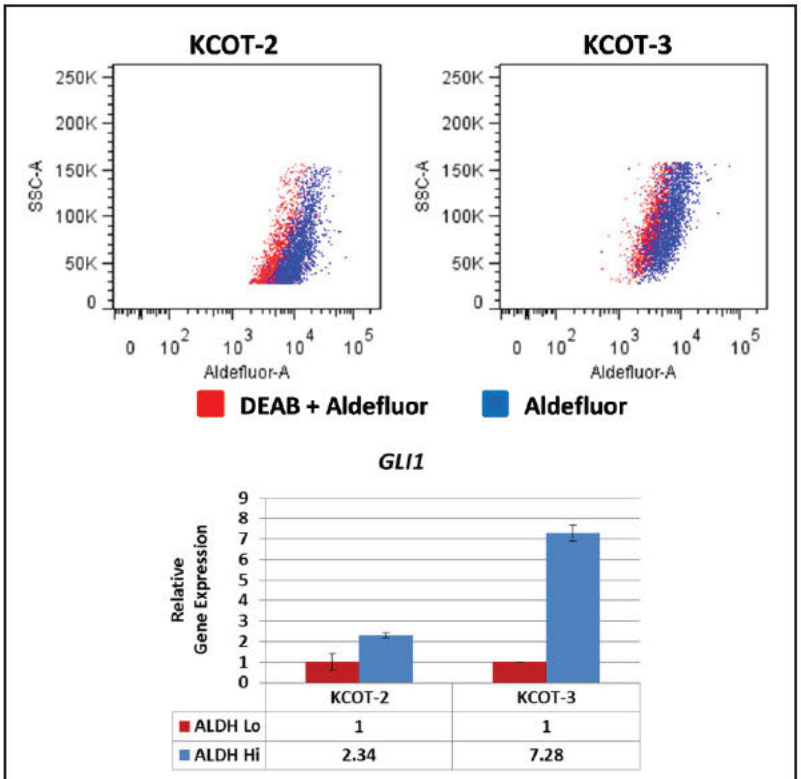


**Figure 6. Flow cytometric analysis of ALDH activity and expression of *GLI-1* in KCOT-2 and KCOT-3 cell populations.** Cells were stained with Aldefluor as a marker of ALDH activity, with DEAB as a negative control. The ALDH populations were isolated from cells with the highest and lowest aldefluor activity. Gene expression was determined by qRT-PCR. The housekeeping gene GAPDH was used as normalizing reference. Fold change in ALDH Hi population was determined relative to ALDH Lo population (error bars denote SD).

**(rs357564)**, is of particular interest as it has been associated with increased severity in breast cancer (Chang-Claude et al., 2003). Recently, the Pro1315Leu polymorphism was identified during PTCH1 mutational analysis of human malignant mesothelioma samples (Lim et al., 2013). This polymorphism was predicted by computational analysis with PolyPhen2 software to decrease the functional activity in PTCH1. This polymorphism occurs in the C-terminal region of PTCH, which has been shown to be essential in the inhibitory activity of PTCH (Johnson et al., 2000). Deletion of the C-terminal region of PTCH leads to increased ligand-independent HH signaling, similar to the activity suspected in human tumor cells. **We identified the Pro1315Leu polymorphism in 70% of our patients.** We expect that the Pro1315Leu PTCH1 polymorphism will increase proliferation, increase anchorage-independent growth, and increase migration and invasion potential in transfected cells. In addition, we expect that the PTCH1 polymorphism will activate HH activity and GLI1-mediated transcription as measured by Smo activation and GLI-1 reporter assay.

| Sample ID | c.395-36 T>G intron 3 | c.747-55 T>C intron 6 | c.1504-8 T>C intron 11 | c.1686 C>T p.A562A Exon 12 | c.2560+9 G>C intron 15 | c.2560+101 C>T intron 15 | c.2561-80 G>C intron 16 | c.2887+21 A>G intron 17 | c.3944 C>T p.P1315L Exon 23 | c.4033 C>T p.R1345C Exon 23 | c.4344+38 A>G intron 23 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| KCOT-1 | yes, homo | no | no | no | no | no | no | no | yes, homo | yes, hetero | yes, hetero |
| KCOT-2 | yes, homo | no | no | no | no | no | no | no | yes, hetero | no | no |
| KCOT-3 | yes, homo | no | no | no | no | no | no | no | yes, hetero | no | no |
| KCOT-4 | yes, homo | yes, hetero | yes, hetero | yes, hetero | yes, homo | yes, hetero | yes, homo | yes, homo | no | no | no |

**Justification & Feasibility:** We identified polymorphisms in PTCH1. In KCOT-1, 2 and 3, a polymorphism in exon 23 (Pro1315Leu), which codes for the essential C-terminal region of PTCH, was found (**Table 3**). In this aim, we will explore the functional significance of this polymorphism. The MacDougall and Frost laboratories have extensive experience and the facilities to perform the cloning and functional assays, which will facilitate the completion of these studies.

**Experimental Approach:** Cloning of PTCH polymorphism from KCOT cells. PTCH1 will be cloned from cDNA of KCOT cells carrying the Pro1315Leu polymorphism with DNA recombination sequences compatible with the Invitrogen Gateway Cloning system (described by Esposito et al., 2009). Site-directed mutagenesis (Stratagene) will be used to make the Leu1315Pro variant for comparison. Multiple vectors are available for expression in mammalian cells. The pcDNA-DEST53 vector creates a N-terminal GFP tag, which will be used to measure and optimize transfection efficiency. Additional vectors (pDEST26 with N-His tag, pcDNA3.1/hV5 with N-V5 tag, pDEST27 with N-GST tag) can be used to find the best transfection and expression of protein using antibodies to specific tags. The polymorphism of interest is in the C-terminus of the human protein. Therefore, vectors with N-terminal tags will be used to avoid interference with the C-terminal sequences. Once the gene of interest is incorporated into expression/destination vector it will purified, sequenced at the UAB Heflin Center for Human Genetics, and used for transfection of PTCH into ST-003 enamel organ epithelial (EOE) cells, late ameloblast-like cells (CRN cells), and HEK293T cells.

Functional significance of PTCH polymorphisms in non-transformed cells. Expression vectors containing the Pro1315Leu and Leu1315Pro variants of PTCH1 will be verified by sequencing and transfected into ST-003-EOE, CRN, and HEK293T cells. ST-003-EOE cells were isolated from the EOE of an unerupted, supernumerary tooth surgically removed for clinical reasons (Ren et al., 2011). CRN cells were isolated from the crown scraping on a surgically removed tooth representing the remnants of the ameloblasts during tooth development. The EOE and CRN cells have a similar developmental lineage (derived from the dental lamina) as supposed for odontogenic tumors, and provide a good resource for testing the functional significance of PTCH1 mutations and polymorphisms (Ren, Amm et al., 2012). In our laboratory we have established a protocol that allows for as high as 50% transfection efficiency of EOE cells using the Lonza 4D-Nucleofector system with special buffer (**Figure 7**).

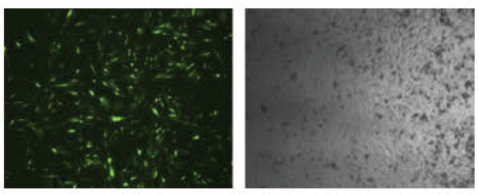

**Figure 7. GFP expression in transfected enamel organ epithelial cells.** Immunofluorescence staining and bright field image 48 h after transfection(10x).

Expression of PTCH1 variants will be confirmed by Western blot, immunofluorescence to confirm membrane localization of PTCH1, and cell proliferation will be analyzed by MTT assay on days 1, 3, 5, and 7 (CellTiter 96, Promega). To determine if the PTCH1 polymorphism affect the *tumorigenic potential* of cells, transfected cells will be grown in soft agar to determine any changes in anchorage-independent growth. Soft agar colony formation is measured by seeding cells in medium containing 10% FBS and 0.3% agarose (UltraPure low melting point agarose, Invitrogen, Carlsbad, CA). Cells are plated over a layer of solidified medium containing FBS and 2% agarose in six-well plates. Cultures were maintained in a humidified, 37°C incubator and media was changed every 3 days for 4 weeks before colonies are photographed and counted. We know that KCOT-3 and KCOT-4 cells are capable of anchorage-independent growth similar to control MDA-MB-231 cells, a human metastatic breast cancer cell line (Lui et al., 2013) (**Figure 8**) and would like to determine if the PTCH1 polymorphism induces tumorigenic capacity in normal cells.


**Figure 8. Anchorage-independent growth of KCOT cells as a measure of tumorigenecity.** KCOT-3 and KCOT-4 cells (5,000 cells/well) formed colonies when suspended in complete media and agarose similar to control MDA-MB-231 cells, a human metastatic breast cancer cell line.

Due to the invasive nature of KCOTs into the jaw bones, we will determine the effect of PTCH1 polymorphism on invasion and migration of the cells. For transwell *migration assays*, $1x10^5$ cells are plated in 24-well inserts (8-µm pore size, BD Biosciences) (Kwon et al., 2011). Cells are incubated in medium with 2% FBS on the transwell inserts (i.e., top well), and medium containing 10% FBS was placed in the bottom well. After 24 h, cells on the upper surface of the transwell filter are removed, the filters stained with hematoxylin and eosin, and the number of cells that migrated through the filters are counted in 5, random microscopic fields per filter. For *invasion assays*, the same experimental procedures as for transwell migration assays are used except that the 24-well inserts are coated with growth factor-reduced Matrigel (8-µm pore size, BD Biosciences). To determine the effect of the PTCH1 polymorphism on *HH activity*, we will use a GLI-dependent promoter luciferase reporter assay to detect basal levels of HH transcriptional activity and the change in GLI-dependent activity related to PTCH1 variants. The necessary materials, equipment, protocol, and technical expertise will be provided by co-mentor Dr. Frost (Mukherjee et al., 2006). The pGL3B/8xGLIBS-lc-luc vector contains eight tandem GLI-binding sites and a lens crystallin promoter. This vector is co-transfected with the renilla luciferase vector (pRL-TK, Promega) with Lipofectamine 2000 (Invitrogen) and the assay is performed with the Dual-luciferase Reporter Assay (Promega). Negative control cultures will be transfected with the pGL3-Basic vector and firefly luciferase will be normalized to the renilla luciferase activity. Expression of GLI-1 will be used a positive control using the pLJD-HA-GLI-1 retroviral construct (with neomycin/G418 resistance gene) and its corresponding empty vector control (provided by Drs. Andra Frost and Michael Ruppert) with selection using G418 for at least 2 weeks (Kwon et al., 2011). To determine if effects on HH actiivty are the result of decreased inhibition of Smo, we will use immunofluorescence to determine the location of Smo, which translocates to the primary cilia when activated. We have an acetylated alpha-tubulin antibody that allows us to visualize the location of the primary cilia (Yuan et al., 2010; Ren, Amm et al., 2012). For each of these experiments the KCOT cell populations will be for comparison and GLI-1 overexpressing MDA-MB-231 breast cancer cells will used as positive controls (From Dr. Frost, Kwon et al., 2011).

**Expected Results, Potential Pitfalls, and Alternative Approaches:** We expect that the Pro1315Leu PTCH1 polymorphism will activate HH activity and GLI1-mediated transcription, as well as increase the tumorigenic and invasion capacity of normal non-tumor cells. However, due to the large size of the PTCH1 gene, it may be difficult to clone. In this circumstance, we can have the plasmid expressing the wildtype sequence of PTCH1 synthesized and use site-directed mutagenesis kit (Stratagene) to create a vector expressing PTCH1 with the Pro1315Leu polymorphism. Another option is the production of lentivirus virus vectors with our PTCH1 genes of interest in collaboration with the UAB Genetically-Defined Microbe Core: Retroviral Unit. Lentiviral vectors provide on the best transfection efficiencies. It is possible the identified polymorphism (Pro1315Leu) has no functional significance on HH pathway activity and cell proliferation rates. The role of PTCH1 on HH activity in KCOT can still be assessed using a C-terminal truncation of PTCH1 (1130X). The C-terminus is known to control HH inhibition by PTCH1 (Johnson *et al*., 2000), and overexpression of truncated PTCH1 in KCOT and normal cells would identify the role of PTCH1 in HH signaling, which has broader application to understanding the role of PTCH1 in HH signaling and tumorigenesis.

**Specific Aim 2: To determine the effects of HH inhibition in primary KCOT cell populations using HH inhibitors and knockdown of Smo protein.**

**Rationale:** We and others have highlighted the utility of treating KCOTs with HH inhibitors (Goldberg et al., 2011; Li, 2011; Mendes et al., 2010; Ren, Amm et al., 2012; Zhang et al., 2006). However, despite the recommendation of the KCOT research community, few attempts at treatment have been made due to lack of available preclinical models, which we have now established. There has only been one clinically reported use of GDC-0449 (Vismodegib, Genentech), a HH inhibitor in a NBCCS patient with KCOTs (Goldberg et al., 2011). Following 12 weeks of daily treatment, the patient had complete regression of his BCCs and after 2-years of therapy nearly complete remissions of the KCOT lesions and no appearance of additional lesions. Even though it was not the goal of his treatment, this demonstrates a possible clinical utility for HH inhibitors to treat KCOT. We have a 14-years-old patient who has had three surgeries with significant cost and morbidity. Our cell models are an excellent preclinical method to test the efficacy of HH inhibitors against KCOTs given the suggested clinical benefit. Each of the KCOT cell populations has been shown to express relevant HH proteins indicative of HH signaling (**Figures 3-4**). PTCH mutations and the expression of HH signaling components have been reported in clinical cases of KCOT. HH inhibitors are designed to target Smo to compensate for loss of PTCH inhibition.

**Justification & Feasibility:** We have previously shown the Smo antagonist cyclopamine inhibits viability of KCOT-1 cells, and decreases PTCH1 and Smo protein in a dose-dependent manner, indicating a reduction in HH signaling (**Figure 9A** and Ren, Amm et al., 2012). Now we have additional confirming data demonstrating cyclopamine also reduces the viability of KCOT-3 and 4 cells in a dose-dependent manner (**Figure 9A**). HH inhibitors may have a clinically benefit in additional models of craniofacial tumors; cyclopamine decreased the viability of primary ameloblastoma cell populations and squamous cell carcinoma cell lines (**Figure 9B and C**) providing broader significance for the studies proposed here. I have extensive experience with the pharmacologic approaches proposed here and the pharmacological targeting of the HH pathway for the treatment of cancer (Amm et al., 2011; Steg et al., 2010). In addition, Dr. Andra Frost has expertise in HH signaling focusing on GLI activity. I will incorporate her expertise and methods into my studies (Mukherjee et al., 2006; Steg et al., 2010; Kwon et al., 2011). Due to the possible off-target, non-specific effects of cyclopamine (Meyers-Needham et al., 2012), we propose using additional HH inhibitors that have been used to treat other human cancer types preclinically and in Phase I and II clinical trials. LDE225 (Erismodegib) from Novartis Oncology and GDC-0449 (Vismodegib) from Genetech are two HH inhibitors under development for the treatment of BCC, medulloblastoma, and other types of human cancers (Raju et al., 2012). GDC-0449 was approved by the U.S. Food and Drug Administration for the treatment of BCC (Cirrone et al., 2012). Reductions in the HH-target gene, GLI-1, have been shown in biopsy specimens from patients treated with GDC-0449 (Tang et al., 2012). LDE225 was shown to decrease PTCH1, GLI-1, and GLI-2 in ovarian cancer cells and reversed resistance to the chemotherapy agent paclitaxel (Steg *et al.*, 2012). These inhibitors are clinically relevant, commercially available, and will be used to determine the effects of HH inhibition on KCOT cells.

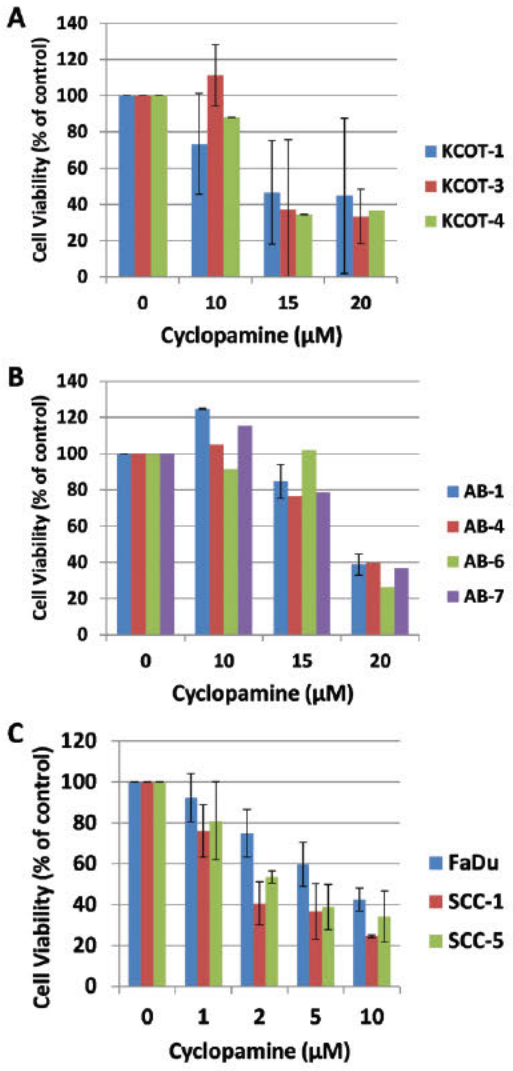


**Figure 9. Dose-dependent response of (A) primary KCOT, (B) primary ameloblastoma, and (C) squamous cell carcinoma cells to treatment with cyclopamine (HH inhibitor).** Cells were incubated with drug for 96 hours prior to the determination of cell viability by MTT assay. Error bars indicate SD of triplicate measurements of three independent experiments

**Experimental Approach:** Treatment of KCOT cell populations with Smo antagonists. Dose-dependent effects on cell viability of cells treated with LDE225 and GDC-0449 will be determined using MTT assay as previously described (Ren, Amm et al., 2012). To a 96-well plate, 2,000 cells/well will be treated with increasing concentrations of LDE225 (5, 10, 15, and 20 μM, Novartis Corporation) or GDC-0449 (5, 10, 15, and 20 μM, Genentech, Inc.) in triplicate for 96 hours. Viability will be measured based on 490 nm absorbance (BioTek) using the MTT assay (Sigma). To determine the efficiency of these inhibitors on HH inhibition, treated samples will be examined for reductions in HH target genes and proteins (PTCH, GLI-1, GLI2) using qRT-PCR (primers and Sybr Green, SABiosciences; ABI Prism 7500, Applied Biosystems) and Western blot analysis (apparatus, Invitrogen; antibodies, Santa Cruz Biotechnology). Additionally, we will use a GLI-dependent promoter luciferase reporter assay to detect basal levels of HH transcriptional activity and detect modulations in activity following HH inhibitors (see description in Aim 1). For each KCOT cell population, we freeze low passage cell stocks (2-6 passages) and use cells within 15 passages for experiments. We also allow each population to be passaged up to 25 passages to be assured of their continued growth, but do not use these cells for signaling or drug treatment experiments. The established cell population, ST-003-EOE, will be used as a control (Ren, Amm et al, 2012). These cells are from the same origin as suspected for the KCOT cells, but are normal, non-tumor cells. They provide a good control for the effects of HH inhibition on a non-tumor cell population of dental origin.

Direct knockdown of Smo to confirm dependence of proliferation on HH signaling. Small interfering RNA (siRNA) targeting of Smo will be used to decrease mRNA and protein levels of the HH signaling activator Smo (siRNA, Sigma; primers, SABiosciences; antibody, Santa Cruz Biotechnology). Various transfection reagents (Invitrogen Lipofectamine 2000, Mirus TransIt-TKO), of which I have experience with, are available to optimize transfection confirmed by qRT-PCR and Western blot. Once successful knockdown has been established, the effects on cell proliferation will be determined in the KCOT and ST-03-EOE cell populations by MTT assay (CellTiter 96, Promega).

Development of xenograft and therapeutic model of keratocystic odontogenic tumors: Application of *in vitro* findings to *in vivo* models is essential for evaluation of any drug for tumor therapy. To determine the therapeutic efficacy of HH inhibitors against KCOTs, 4-6 week old female mice (n = 4) will be subcutaneously inoculated in the flank with $1x10^6$ KCOT tumor cells starting with cells with or without matrigel. The

number of cells will be increased if necessary to establish xenografts ($5x10^6$, $1x10^7$, or $3x10^7$ cells per flank). Athymic nude mice and severe combined immunodeficiency (SCID) mice will be used to increase likelihood of success. Following established tumor growth, mice will be randomly divided into groups receiving different treatments: untreated or HH inhibitor (GDC-0449 or LDE225). The first HH inhibitor to be used in animal models will be determined by which produces the greatest cytotoxicity and HH inhibition *in vitro* (as measured by reductions in GLI1 and PTCH1 levels, and reduced GLI1 activity). Groups of mice will be treated once daily by gavage with vehicle, GDC-0449 (100 mg/kg), or LDE225 (60 mg/kg). Drugs will be prepared fresh daily (GDC-0449 in 0.5% methyl cellulose/0.1% Tween 80 in sterile water; LDE225 in 0.5% methyl cellulose/0.5% Tween 80 in sterile water) (Steg *et a*l., 2012; Ramaswamy *et al*., 2012). Tumor size (surface area equal to product of two diameters) will be monitored three times a week. Mathematical models will be fitted to tumor growth data to estimate tumor doubling time. Mice will be treated for 6 weeks or until tumor doubling time is reached prior to sacrifice and tumor collection. All tumors will be excised, weighed and stored for mechanistic analysis. Eight mice per treatment group will be used. This sample size is sufficient to guarantee 80% power in a two-sample t-test with a significance level of 0.01 (Buchsbaum et al., 2003). The UAB Center for Clinical and Translational Science and Biostatistics and Bioinformatics Shared Facility provide support for statistical analysis (study design, sample size and power calculations, and data analysis.

To confirm HH inhibition *in vivo*, half of each xenograft (treated and untreated) will be homogenized in RIPA lysis buffer and resuspended at a consistent protein concentration. Western blot analysis of HH signaling components (GLI1, PTCH1, Smo) will be conducted to determine if Smo antagonists inhibit HH signaling *in vivo.* Half of each xenograft will be fixed in formalin and paraffin-embedded to confirm presence of tumor and IHC analysis on cell proliferation (Ki67) and apoptosis (TUNEL assay) markers. I generated subcutaneous tumor animal models and orthotopic tumor models, and dissected and processed xenograft tissue during my graduate studies in the lab of Dr. Donald Buchsbaum (Buchsbaum *et al*., 2003; Bevis *et al*., 2011; unpublished studies). The Frost laboratory and my collaborator Dr. Adam Steg both have experience using gavage techniques for the oral treatment of mouse models and will be able to provide support and training for these studies (Steg *et al*., 2012).

**Expected Results, Potential Pitfalls, and Alternative Approaches:** We expect that Smo inhibition and knockdown will reduce cell viability of KCOT primary cells and reduce HH targeted transcription (*PTCH* and *GLI1*) and GLI1 transcriptional activity. However if LDE225 or GDC-0449 fail to inhibit the growth of KCOT cells, I can target the GLI transcription factors directly with siRNA and compounds such as the GLI antagonists, GANT58 or GANT61 (Raju and Pham, 2012). This strategy may work if cells have certain Smo mutations, known to cause resistance, or the GLI expression seen is due to HH-independent signaling (Metcalfe et al., 2011; Raju et al., 2012). Also, resistance to anti-HH therapies has been reported due to PI3K signaling (Metcalfe et al., 2011). Our primary cell populations allow an excellent model for testing other therapies or HH inhibitors in combination with other drugs (e.g. PI3K inhibitors BKM120 or GDC-0941).

Another alternative approach is the targeting of other developmental pathways that play a role in ameloblast-mediated tooth development and may play a role in odontogenic tumorigenesis. For example, dysregulation of Notch by overexpression of receptors or Ras-activation has been associated with human leukemias, GLIomas, breast and pancreatic cancers (Miele et al., 2006). We previously reported that KCOT-1 cells express components of the Notch pathway; Notch1/2/3, Jagged-2, and Delta1 (DLL1) (Ren Amm et al., 2012). Goncalves *et al.* (2012) reported the expression of Notch1 in histologic sections of KCOT. Notch expression (Notch1/2/3, Jagged-1/2, DLL1) has also been associated with ameloblastomas, another odontogenic tumor subtype, believed to have similar origins as KCOTs (Kumamoto and Ohki, 2008; Muraki et al., 2011). Gamma secretase inhibitors are currently under development to target Notch signaling in human tumors and may provide another approach to targeting KCOT (Meng *et al.,* 2011). Aberrant Wnt signaling has also been indicated in ameloblastomas based on detection of nuclear β-catenin (Siriwardena *et al*., 2009; Tanahashi *et al.*, 2008). Other studies identified the expression of Wnt ligands (Wnt-1, 2, 3, 5a, 7b, 8a, 8b, 10a, 10b) in ameloblastomas (Siar et al., 2012; Sukarawan et al., 2010). Studies examining Wnt and its signaling are limited in KCOTs, with one study suggesting lower expression of β-catenin in syndromic cases of KCOT compared to non-syndromic (Hakim *et al*., 2011). The role of Wnt in tumorigenesis has been characterized in many human tumors (e.g., colon, breast, and pancreatic carcinomas), but remains largely uncharacterized in KCOTs and provides an additional opportunity to identify pharmacologic targets in KCOT.

We expect that knockdown of Smo will reduce viability of KCOT cells. However, primary cells can be difficult to transfect with chemical transfection agents alone. An alternate strategy is the use of short hairpin RNAs. We have viral vectors and electroporators available to aid in transfection of cells.

We expect HH inhibitors will reduce volume of KCOT xenografts and impede tumor HH signaling *in vivo*; the development of animal models of KCOT will aid in the translational of our studies into patients. If we are unable to establish xenograft models in athymic nude or SCID mice, another option is NOD-SCID mice, which are deficient for T and B cell lymphocytes and natural killer cells. Later studies would develop an orthotopic model by injection of KCOT cells into the tibia (to model the occurrence of KCOT within the mandible). We could also implant patient tumors directly from surgery. Recent studies conducted at UAB have successfully directly implanted primary tumors into the flank of SCID mice for propagation of the human tumor in an *in vivo* environment (Garcia *et al.*, 2013; Dr. Charles Landen, unpublished data).

**HUMAN SUBJECTS**
The Institutional Review Board of the UAB will review this application**.**

a. **Human Subjects Involvement, Characteristics, and Design**
Human subjects involved in this project will or have already provided tissue specimens collected during surgery for clinical needs. The subject population includes patients diagnosed with odontogenic tumors. The removal of the tumor is part of treatment prescribed by a doctor and not part of the research project. Subjects to date range from ages 11 to 52. No vulnerable “special classes of subjects” will be involved in this study.

b. **Sources of Materials**
Materials are obtained specifically for research purposes and are coded with number identifier. Research material is coded cell populations maintained in liquid nitrogen storage or coded tissue in paraffin embedded blocks. The age, race, and medical record number have been collected. The PI and primary mentor will have access to identifiable private information about human subjects for the purpose of obtaining matched samples from the Department of Pathology. Any specimens stored or data collected are coded. The private information is protected on an encrypted hard drive and in a locked office.

c. **Risks to the Subjects:**
There are no significant risks to the subjects participating in this study. The risks associated with the removal of the tumors are not a part of this study and were explained by the doctor.
**Adequacy of Protection against Risks:**
**Recruitment and Informed Consent:** Patients are/were recruited through the clinic at the UAB with IRB approval Approved consent forms are/were be obtained and signed for each individual recruited by one of the approved investigators. This form explains the aims of this project, the procedures involved, risk involved, benefits gained by participation, compensation, and a physical injury statement. These signed consent forms will be kept as a permanent record by the investigator.
**Protection Against Risks:** Confidentiality will be insured by assigning a number to each specimen at the time of recruitment after obtaining consent to participate in the study.
**Potential Benefits of the Proposed Research to Human Subjects and Others:** There are no direct benefits to the research participants. However, this study may help us better understand keratocystic odontogenic tumors. There are no significant risks to the subjects participating in this study.
**Importance of the Knowledge to be Gained:** This study may help us better understand keratocystic odontogenic tumors, and establish primary cell models for preclinical study. There are no significant risks to the subjects participating in this study.

**Inclusion of Women**

Subjects were selected based on the criteria that they were diagnosed with an odontogenic tumor requiring surgical removal. Analysis of such tissues and cells isolated from specimens will allow the genes associated with the pathogenesis of odontogenic tumors to be identified. No exclusion of any sex/gender or racial group will be done. The enrollment was started at the time of our IRB approval. Half of subjects were female.

**Inclusion of Minorities**

Subjects were selected based on the criteria that they were diagnosed with an odontogenic tumor requiring surgical removal. Analysis of such tissues and cells isolated from specimens will allow the genes associated with the pathogenesis of odontogenic tumors to be identified. No exclusion of any sex/gender or racial group will be done. The enrollment was started at the time of our IRB approval. Half of subjects were Hispanic.

OMB Number: 0925-0002

# Planned Enrollment Report

**Study Title:** Protein Expression in Tumors of the Craniofacial Complex

**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 | 0 | 0 | 0 | 0 | **0** |
| Native Hawaiian or Other Pacific Islander | 0 | 0 | 0 | 0 | **0** |
| Black or African American | 1 | 1 | 0 | 0 | **2** |
| White | 2 | 2 | 0 | 2 | **6** |
| More than One Race | 0 | 0 | 0 | 0 | **0** |
| **Total** | **3** | **3** | **0** | **2** | **8** |

Study 1 of 1





Funding Opportunity Number: PA-14-042. Received Date: 2014-03-12T11:37:54.000-04:00

**Inclusion of Children**

We included children diagnosed with odontogenic tumors when consent was granted by the guardian. Subjects were recommended to have tumor surgically removed by their doctor. There were two children included in the study above the age of 10.

## Vertebrate Animals

### 1. Proposed Work:

**A. Development of xenograft model of keratocystic odontogenic tumors:** 4-6 week old female athymic nude and severe combined immunodeficient (SCID) mice will be inoculated with low passage, primary keratocystic odontogenic tumor cells (KCOT-1, 2, 3, and 4). For establishing the animal model, varying numbers of cells will be inoculated into animals ($1x10^6$, $5x10^6$, $1x10^7$, or $3x10^7$ cells)). 4 mice per group x 4 groups x 4 cell populations = **64 mice.** If lower cell numbers yield usable tumors, experiments with higher cell numbers will not be needed.

**B. Therapeutic model of odontogenic tumors:** Once the appropriate mouse strain (athymic nude or SCID mice) and cell number is determined, animals will be inoculated with KCOT cells and tumors will be allowed to establish. Following established tumor growth, mice will be randomly divided into groups receiving different treatments: untreated or HH inhibitor (GDC-0449 or LDE225). Groups of mice will be treated once daily by gavage with vehicle, GDC-0449 (100 mg/kg), or LDE225 (60 mg/kg). Drug will be prepared fresh daily (GDC-0449 in 0.5% methyl cellulose/0.1% Tween 80 in sterile water; LDE225 in 0.5% methyl cellulose/0.5% Tween 80 in sterile water). Tumor size (surface area equal to product of two diameters) will be monitored three times a week. Mathematical models will be fitted to tumor growth data to estimate tumor doubling time. Mice will be treated for 6 weeks or until tumor doubling time is reached prior to sacrifice and tumor collection. All tumors will be excised, weigh and stored for mechanistic analysis. Therapy studies require a minimum number of mice per group to be accepted as statistically meaningful. Eight mice per treatment group will be used. The sample size is sufficient to guarantee 80% power in a two-sample t-test with a significance level of 0.01 (Buchsbaum et al., 2003). Extra mice to replace those with low tumor uptake (4 mice) will be appropriately euthanized if not used.
8 mice per group x 4 cell models x 3 treatment groups + 4 extra mice = **100 mice**

### 2. Animal Use Justification:

The use of animal models is essential for the study of *in vivo* efficacy of therapeutics for human tumors.
Currently there is no computer model, mathematical equation, nor *in vitro* system that can accurately recapitulate the complex interactions between tumor cells, the stromal tissue, and drug distribution of the *in vivo* system. Please see 'Proposed Work' (above) for a detailed breakdown of the number of animals to be used.

### 3. Veterinary Care:

The University of Alabama (UAB) Animal Resources Program (ARP) provides complete veterinary care and diagnostic services, including daily monitoring of animals for signs of pain and distress. The ARP staff includes three full-time clinical veterinarians and a large team of animal health technicians.

### 4. Procedures to limit animal discomfort and injury:

Every effort will be made to decrease or eliminate pain and discomfort to experimental animals. To minimize infection all procedures will be performed using sterile techniques. Animals will be monitored on a daily to twice-daily basis as tumors near their endpoint. Animals will be euthanized if a tumor ulcerates or interferes with the ability to acquire food or water, or if an animal losses more than 15% of its baseline weight.

**5. Method of euthanasia:** Animals will be euthanized through $CO_2$ inhalation followed by cervical dislocation. This method is consistent with the recommendation of the Panel on Euthanasia of the American Veterinary Medical Association (AVMA).

**Resource Sharing**

The University of Alabama at Birmingham, Dr. MacDougall's Laboratory and I will adhere to the NIH Grants Policy on Sharing of Unique Research Resources including the "Sharing of Biomedical Research Resources: Principles and Guidelines for recipients of NIH Grants and Contracts" issued in December of 1999 (http://ott.od.nih.gov/NewPages/Rtguide_final.html). Once our research findings are published, all reagents will be made available to the scientific community upon request. Once a request for reagents has been received, we will make every effort to send out reagents in a timely manner at the cost of shipping to the receiving party. Specifically, material transfers would be made with no more restrictive terms than in the Simple Letter of Agreement or the UBMTA and without reach through requirements. Should any intellectual property arise which requires a patent, we would ensure that the technology remains widely available to the research community in accordance with the NIH Principles and Guidelines document.