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 2ndplace 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**

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.

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.

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.