Hope M. Amm, PhD **Personal Statement** **November 15, 2018**

I am now a Tenure-track Assistant Professor at the University of Alabama (UAB) School of Dentistry (SOD) in the Department of Oral and Maxillofacial Surgery. **My career goal is to develop a clinical, multi-disciplinary research program as an independent investigator. My research goal is to conduct translational research to develop targeted therapeutics and diagnostics for patients with craniofacial tumors.** UAB and my department provide an excellent environment for clinical and translational research. I have established my own laboratory and currently mentor a postdoctoral scholar, a graduate rotation student, and undergraduate students.

My first experiences with research were as an undergraduate at Saint Mary's College, where my research focused on understanding TNF production by macrophages following bacterial exposure. I learned a broad range of laboratory techniques, such as aseptic technique, cell culture, and statistical data analysis. This culminated in a poster presentation at a regional meeting and two research awards. These experiences and an interest in cancer biology drove my desire to pursue research full-time, leading me to join the Breast Cancer Training Program in the Department of Pharmacology at UAB. My dissertation research with Dr. Donald Buchsbaum focused on drug resistance and the mechanism by which an apoptosis-inducing antibody could enhance the cytotoxicity of chemotherapy against breast cancer. In collaboration with Ascenta Therapeutics, I used novel small molecule inhibitors of anti-apoptotic proteins to sensitize breast cancer cells to death receptor-induced apoptosis (*Mol Cancer Res, 2010*) and was awarded a DOD pre-doctoral fellowship to complete my studies. **Both the antibody and small molecules studied are currently in clinical trials, and will hopefully clinically impact patient care.** I also developed many collaborations, resulting in co-authored publications. For example, as part of one collaboration, I examined the effects of hedgehog (HH) pathway inhibition on pancreatic cancer cells (*Cancer Biol Ther, 2010)*. With another group, I studied the interactions between proteasome and hedgehog inhibitors in ovarian cancer (*Oncotarget* 2014). These additional studies enhanced my knowledge of cellular signaling and pharmacology, with a focus on designing and optimizing small molecules and antibodies for the clinical setting. I have continued to seek out new collaborations, and several new oncology projects are underway. One project is examining the health disparities in oral cancer patients in Alabama. As part of another project, Dr. Jessica Scoffield (Department of Microbiology) and I are examining the oral microbiome of oral cancer patients. These collaborations help to expand my research program and contribute to several different fields.

For my postdoctoral training, I accepted a position on the Dental Academic Research Training (DART) program (NIDCR T32-DE017607) with Dr. Mary MacDougall. This opportunity allowed me to work directly with surgeons and patients. We developed a project exploring novel therapeutics against the hedgehog signaling pathway using unique tumor models derived directly from patient samples collected during surgical resection. I was awarded a K99/R00 (NIDCR K99-DE023826) based on these studies. Moreover, those studies resulted in seven publications, and an additional publication was recently submitted. I also had numerous opportunities to present my work at national and international conferences, and received awards for several of these presentations. **Our studies were the first to establish primary cell populations for the purpose of characterizing these tumors, and should improve the clinical treatment and imaging of these debilitating tumors.**

My short-term goal is to investigate the potential of using an epidermal growth factor receptor (EGFR) antibody to perform intraosseous imaging prior to surgical interventions for aggressive odontogenic tumors. This will allow surgeons to better define the tumor area and margins, with the goal of reducing tumor recurrence. We have successfully established a steady patient flow at UAB and have developed a new translational model for odontogenic tumors using patient-derived xenografts (PDX). We were awarded an Oral and Maxillofacial Surgery Foundation Research Support Grant and have shown (see Research Accomplishments) that a fluorescently-labeled EGFR antibody specifically labels ameloblastoma tumor tissue *in vivo*. We are now transitioning to a radiolabeled anti-EGFR antibody to detect tumors intraosseously via PET scanning. I am currently developing an orthotopic mandibular model and *in vivo* bone tibial models. I will collaborate with the Oral and Maxillofacial Surgery Department to recruit additional patients with odontogenic tumors. Following IRB-approved informed consent, I will collect resected human tumor pieces and directly implant them in murine models, allowing us to examine the specificity of the radiolabeled antibody for human tumor tissue. This work integrates my background in cancer cell biology and pharmacology perfectly with my postdoctoral knowledge of tooth development and odontogenic tumors. It provides me with the opportunity to conduct clinical translational research with the goal of generating the data necessary to support the initiation of a Phase I clinical trial which could positively impact this patient population. I have already completed a UAB sponsored Clinical Investigator Training Program to prepare myself for the initiation and running of a clinical trial. We have also started collaborations with Wayne State University and Emory University to establish multiple sites and increase the patient numbers.

I am excited to continue my academic career in clinical research. **The Loan Repayment Program would provide stability and significantly enhance my future research success, allowing me to accomplish my long-term career and research goals of developing targeted therapeutics and diagnostics for patients with craniofacial tumors as an independent investigator.**

My career goal is to further develop my independent research program at a research-intensive dental academic institution. My research goal is to conduct translational research to elucidate the cellular signaling within human dental-related tumors for the development of targeted therapies and improved diagnostic and surgical tools. While building upon my previous accomplishments, the proposed career development plan will prepare me for greater success as I submit manuscripts and grants and become more engaged in the global research community. I hope to promote further contribute to the field by attending and presenting at scientific meetings and mentoring students and fellows.

Since being awarded the LRP, I have obtained a K99/R00 Pathways to Independence Grant from the National Institute of Dental and Craniofacial Research (NIDCR) and was a Co-Principal Investigator on an Oral and Maxillofacial Surgery Foundation Research Support Grant to support my research endeavors and career development. Towards my goal of independence, I completed interviews and negotiations for a Tenure-track Assistant Professor position. In March 2017, I transitioned to an independent position at the UAB School of Dentistry (SOD) in the Department of Oral and Maxillofacial Surgery and have established my own laboratory. I have since been appointed as member of the SOD Research Advisory Committee, was an organizer of the SOD Scholar's Day Research Symposium, and have been a lecturer on Oral Cancer and Health Disparities for the UAB Cancer Center Partnership Research Student Training Program (PRSTP). I am a member of the UAB Comprehensive Cancer Center, the Global Center for Craniofacial, Oral, and Dental Disorders (GC-CODED), and the UAB Minority Health Disparities Research Center (MHRC). I was appointed a Graduate faculty member for the Graduate Biomedical Sciences Cancer Biology Theme and have had three graduate rotation students. I completed a MHRC Health Disparities Research Training Program for junior faculty. I was also recently appointed to the UAB Council for Postdoctoral Education. These opportunities and activities have helped increase my recognition, engagement, and reputation while establishing new collaborations at UAB, and have contributed to the development of my independent research program.

I am currently mentoring a Postdoctoral Scholar who was recently appointed to the Dental Academic Research Training NIDCR-funded T90 grant. I also currently mentor 6 undergraduates, a graduate rotation student, and a post-baccalaureate volunteer, all of whom are actively working on oral tumor projects in my lab. I strongly encourage my students to present their research findings whenever they have an opportunity. Jonathan Chestang, a high school student who worked on a histology project described in my LRP, received first place and a UAB scholarship for his presentation at the UAB Center for Community OutReach Development (CORD) Summer Science Institute. One of my current undergraduate students, Aubrey Johnson, also worked on aspects of this project, and we are currently preparing a manuscript for which she will be a co-author. Another student, Stefan Kovac, was awarded a UAB Honors College Presidential Summer Fellowship to work on a new project in my lab this summer. He presented locally at our SOD Scholar's Day and at a state-wide Science and Technology Open House. He was also awarded a full travel award and will be attending the Annual Biomedical Research Conference for Minority Students (ABRCMS) this month. We are currently preparing an abstract for when he attends the American Association of Cancer Research (AACR) annual meeting in the spring. I believe that providing my trainees with opportunities and making sure that they have concrete accomplishments (e.g., abstracts/presentations and publications) are important for the progression and support of my research endeavors.

I am an active participant in my local, national, and international research communities. At the UAB SOD, I am Co-Director of two courses, Dental Genetics and Dental Histology, and also lecture on several topics as part of the first-year Fundamentals course. In the summer, my lab contributes to the Summer Health Professions Education Program under the direction of our Interim Dean, Dr. Michele Robinson. As part of that program, my lab leads students through DNA extraction from saliva and polymerase chain reaction to amplify a specific gene. They are also taught how to detect genetic mutations, some mutations associated with craniofacial diseases, and a little about the research we conduct. On a national level, I presented an update on my LRP research at the AADR 47th Annual Meeting in a session on epithelial tumors, and was the Session Chair for a session entitled "Oral Premalignant and Malignant Lesion and Risk Factors." I am currently a board member and the treasurer for the Alabama chapter of the American Association of Dental Research (AADR). I have served as an abstract reviewer, and as an oral and poster session chair for the AADR annual meeting. I am the Councilor for the Student Training and Research (STAR) Network for the International Association of Dental Research (IADR), which provides research opportunities and travel support for students interested in dental-related research. I was recently asked by the Oral Medicine and Pathology Scientific Group officers to be the first "Social Media Officer," who will be tasked with increasing communication amongst members and involving more junior faculty and student members in the organization. I am excited for the opportunity, the freedom to make this new position my own, and the ability increase my exposure to an international research audience. I believe the above appointments and responsibilities show a progression in my

independence and recognition locally, nationally, and internationally, as well as my dedication to the field. The ability to obtain funding and the success of my students demonstrates my pursuit of excellence in my research endeavors, as well as my desire to serve the research community.

In addition to the above-referenced manuscript about histology, a first-author paper written with my former mentor, Dr. Mary MacDougall, is currently under review by *Human Pathology*. I am also the first author (with my colleague, Dr. Anthony Morlandt) of an invited chapter on the genetics of oral cancer, which will appear in *Improving Outcomes in Oral Cancer – A Clinical and Translational Update*. The project that is the focus of my LRP application is a collaborative project among myself, the oral surgeons in my department (Drs. Morlandt and Yedah Ying), Dr. Jason Warram from the Department of Otolaryngology, Dr. Eben Rosenthal (now at Stanford University), and the ameloblastoma patients at UAB. We are currently preparing a manuscript for *Oral Oncology* about the first phase of this project. For the upcoming work (as described in the research activities), Dr. Suzanne Lapi, the Director of the UAB Comprehensive Cancer Center Advanced Imaging Facility, has agreed to join the collaboration. We plan to submit an NIH R21 application in February 2019, which was developed based on the preliminary data described in the research plan. The ultimate goal of these projects is to improve patient care and reduce the recurrence rates for ameloblastoma. To facilitate these goals, I have recently completed a Clinical Investigator Training Program (CITP), which prepares new investigators to set up, start, and implement clinical trials. This program included information about investigator-initiated and industry-sponsored trials, principal investigator responsibilities, preparing a budget, establishing an Institutional Review Board (IRB) for Human Use protocol, hiring staff, establishing feasibility, record maintenance, data management, consent processes, monitoring and site-visits. They also educated us on all of the resources available at UAB for establishing our own clinical trials.

My short-term goal for this project is to investigate the potential of using a radiolabeled epidermal growth factor receptor (EGFR) antibody to provide preoperative imaging for surgical interventions to treat aggressive odontogenic tumors. I have developed a new translational model for odontogenic tumors using patient-derived xenografts (PDX), and have active collaborations with the Oral and Maxillofacial Surgery Department to recruit patients with odontogenic tumors. We have demonstrated that a fluorescently-labelled anti-EGFR antibody specifically binds to ameloblastoma tumor tissue *in vitro* and *in vivo*. This strategy was valuable in proving that the anti-EGFR antibody is capable of labeling ameloblastomas *in vivo*; however, it has proven to be insufficient for imaging tumors within bone. Therefore, the next step is to develop intraosseous models (tibial and mandibular) to optimize a radiolabeled antibody for imaging human tumors in bone via PET scanning. This research is providing me the opportunity to conduct clinical translational research with the goal of providing the data necessary to support the initiation of a Phase I clinical trial which could positively impact this patient population.

In summary, I am dedicated to continuing my academic career in clinical research. **The Loan Repayment Program would provide stability and significantly enhance my future research success, making it easier for me to accomplish my long-term career and research goals of developing targeted therapeutics and diagnostics for patients with craniofacial tumors as part of my work as an independent investigator.**

The Loan Repayment Program (LRP) has provided financial support and stability for myself and my family as I have pursued my career and research goals. Since being awarded the LRP, I have successfully obtained a K99/R00 Pathways to Independence Grant from the National Institute of Dental and Craniofacial Research (NIDCR) and am a Co-Principal Investigator on an Oral and Maxillofacial Surgery Foundation 2015 Research Support Grant to support my research endeavors and career development. In March 2017, I accepted a faculty position as a tenure-track Assistant Professor at the University of Alabama at Birmingham (UAB) School of Dentistry in the Department of Oral and Maxillofacial Surgery. This decision was based on support from my future Chair, my established collaborations within the department, and the population of patients being treated at UAB. I have since established my own laboratory space and resources. My lab now includes one postdoctoral scholar, a graduate rotation student, and several undergraduate research students. I also have established collaborations within the Department of Otolaryngology, the UAB Comprehensive Cancer Center, and the Department of Microbiology.

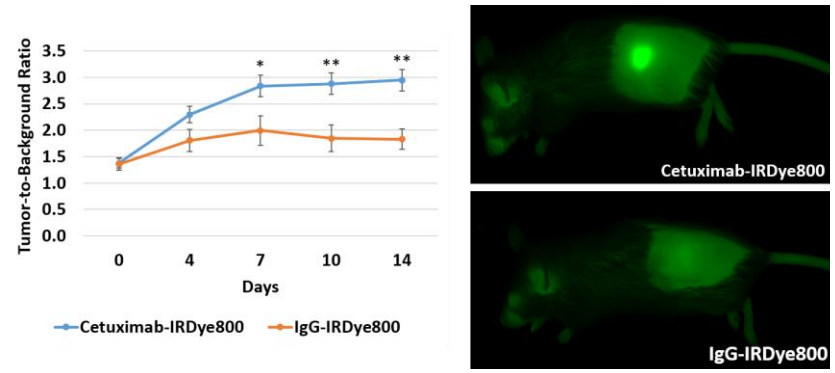


Figure 1. ***In vivo*** **labeling of ameloblastoma (AB-20) tumor tissue.** There was a significantly higher tumor-to-background ratio in the cetuximab-IRDye800-treated animals compared to the IgG-IRDye800-treated animals (*p < 0.05, ** p <0.01). Error bars are the standard error. Shown are representative images obtained using the Pearl imaging system, with the tumor and background areas defined (large ellipse = area with fur removed).

The hypothesis underlying the LRP project is that epidermal growth factor receptor (EGFR) expression in aggressive odontogenic neoplasms may be used to specifically label cells *in vitro* and *in vivo*. It was previously demonstrated that intravenous administration of a fluorescently-labeled anti-EGFR antibody successfully identified microscopic tumor fragments in multiple *in vivo* preclinical models, with limited toxicity. Clinically, this approach may be valuable to guide the surgical removal of aggressive odontogenic neoplasms using real-time intraoperative optical imaging, improving local tumor control while preserving healthy normal tissue. We used qRT-PCR to show that that ameloblastoma (AB) and keratocystic odontogenic tumor (KCOT) cell populations expressed similar mRNA levels of EGFR compared to squamous cell carcinoma (SCC) cell lines (see **Research Activities)**. To assess the ability of cetuximab-IRDye800CW (cetux-IR) to bind and label aggressive odontogenic tumor cells *in vitro*, we stained cells with control IgG-IRDye800CW (IgG-IR) or cetux-IR and imaged them with the Pearl and Odyssey imaging systems. AB-6, AB-17, KCOT-2, KCOT-3, KCOT-9, KCOT-10A, and KCOT-10B cells were all positively labeled with cetux-IR (green fluorescence) with little to no staining by the negative control IgG-IR (data not shown). This demonstrates the ability of cetux-IR to bind to odontogenic tumor cells. Aim 1 was updated accordingly.

We developed subcutaneous murine models of ameloblastoma using patient-derived xenografts (PDX) of surgical specimens. In total, tumor pieces from nine ameloblastomas (AB-20, AB-21, AB-22, AB-23, AB-24, AB-29, AB-30, AB-33, AB-34) were implanted subcutaneously in the flanks of immunodeficient mice. Tumor pieces from patient AB-20 were implanted into 12 mice. The resulting xenografts were imaged *in vivo* on days 0, 4, 7, 10, and 14 following intravenous injection of 200 µg of cetux-IR or IgG-IR. The tumor-to-background ratios (TBRs) produced by cetuximab were significantly higher than those produced by IgG by day 7, and remained significantly higher on days 10 and 14 (**Fig. 1**). On day 14, the skin covering the tumor was removed, and mice were imaged again to represent a pre-resection patient. The TBRs following skin flap removal increased

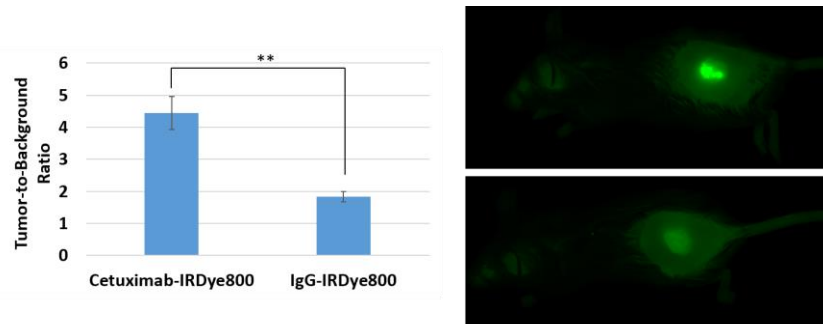


Figure 2. ***In vivo*** **labeling of ameloblastoma (AB-20) tumor tissue pre-resection.** The skin flap overlying the implanted tumor tissue was removed and animal were imaged. There was a significantly higher TBR in the cetuximab-IRDye800-treated animals compared to the IgG-IRDye800-treated animals (** p <0.01). Error bars are standard error. Representative images obtained using the Pearl imaging system.

only in the cetuximab group, and were significantly higher compared to the IgG control (p value = 0.0028; **Fig. 2**). This confirmed that cetux-IR can successfully bind to the AB tissue *in vivo*.

To determine the ability of cetux-IR to detect microscopic disease, tumor fragments (5, 1, and 0.5 mg) were placed on the cleared wound bed and imaged. We were able to detect all pieces of tumor, including the 0.5 mg piece, showing the ability to detect even small amounts of tumor (**Fig. 4**). This indicates that cetux-IR would be able to detect residual disease in surgical margins *in vivo*. Following this imaging study, the tumors were resected and paraffin-embedded. Hematoxylin and eosin (H&E) staining demonstrated the presence of ameloblastoma tumor tissue (**Fig 4**), and imaging studies performed using the LI-COR Odyssey system revealed the specificity of cetux-IR for tumor tissue compared to the surrounding normal tissue.

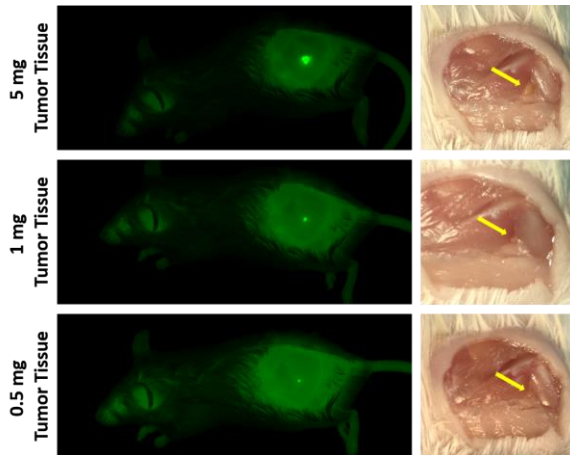


Figure 3. Small amounts of tumor tissue are detectable when labeled with cetuximab-IRDye800. Post-resection tumor tissue was weighed and placed into the wound bed for imaging.

Tissue pieces from AB-22, AB-23, and AB-24 were also implanted in mice; however, following the fluorescence analysis, no significant difference in the TBR was seen between the cetuximab and IgG groups. Paraffin-embedding and H&E staining showed that the implanted tissue was stromal in nature and did not contain ameloblastoma. This provides a valuable control demonstrating that the cetuximab-IRDye800 binds preferentially to tumor cells rather than human tissue implanted in mice or tumor-adjacent stroma. To ensure that we were implanting tumor tissue in subsequent studies, we adjusted our protocol to include a pathologist, Dr. Todd Stevens (UAB Pathology). For subsequent tumors, following surgical removal, small sections of the resected tissue were removed, a core was taken from the middle of the piece, frozen-sectioned, and H&E stained. The pathologist was able to confirm the presence of ameloblastoma in areas adjacent to the pieces that were implanted in mice (AB-29, AB-33, AB-34).

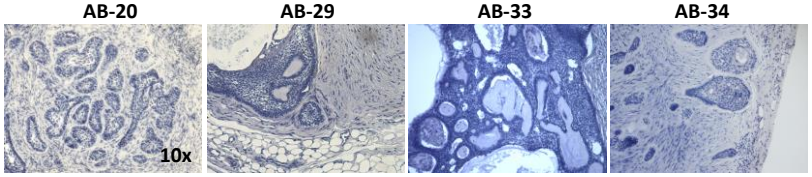


Figure 4. H&E staining of PDX samples showing ameloblastoma tumor tissue (10x).

Tumor pieces from AB-29, AB-33, and AB-34 were implanted in mice, injected with cetuximab-IRDye800 or IgG-IRDye800, and imaged as described above. The TBRs for these tumors were not significantly different between cetuximab and IgG for days 0, 4, 7, 10 or 14. When the skin flap was removed (similar to the mandibular free flap clinically used for these tumors), the TBRs produced by cetuximab-IRDye800 for these samples were significantly higher than those produced by IgG-IRDye800 (**Fig. 5**; AB-29, p value = 0.03; AB-33, p value = 0.013; AB-34, p value = 0.027). This confirmed that cetuximab-IRDye800 can successfully bind to ameloblastoma tissue *in vivo*, is specific for tumor cells, and may provide a valuable tool for intraoperative tumor imaging. However, ameloblastomas naturally occur within the bones of the jaw. During the development of an intraossesous model, we discovered that the fluorescence of the cetuximab-IRDye800 was not greater than the auto-fluorescence of the tibial or mandibular bone. Therefore, we are developing new intraossesous models (tibial and mandibular) of ameloblastoma to determine the ability to detect ameloblastoma via PET scanning with a radiolabeled anti-EGFR antibody (89Zr-panitumumab). Panitumumab is a fully human, monoclonal antibody. Preliminary data (see **Research Activities**) indicate that 89Zr-panitumumab is capable of binding ameloblastoma tissue as well as cetuximab.

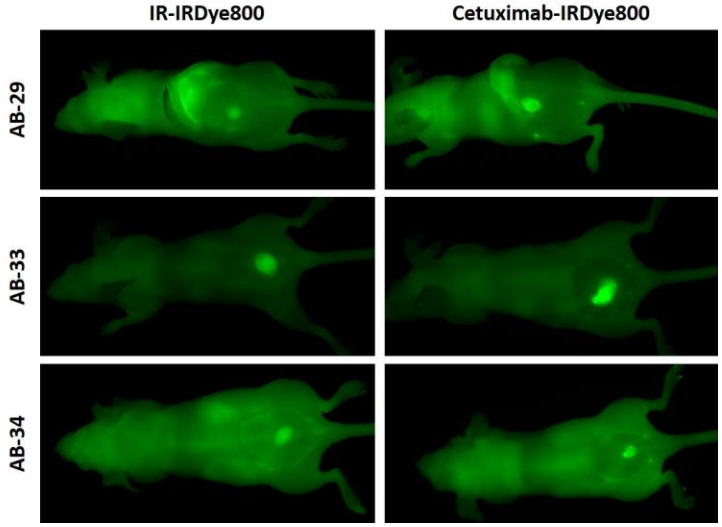


Figure 5. *In vivo* labeling of ameloblastoma tumor tissues pre-resection. The skin flap overlying the implanted tumors was removed and animals were imaged. Shown are representative images obtained using the Pearl imaging system.

Research Activities

A. Significance

Aggressive odontogenic neoplasms, including ameloblastomas, demonstrate locally aggressive and destructive behavior, primarily in the posterior mandible [1, 2]. Although surgical treatment has been advocated for these neoplasms, their location makes complete resection difficult, leading to residual disease and high rates of disease recurrence (3-62%). Alternatively, over-aggressive resection may be carried out to reduce residual disease, resulting in greater disfiguration for the patient [3, 4]. The ability to accurately assess tumor margins with pre-operative imaging could result in the preservation of healthy tissue and improvements in long-term local tumor control, reducing the risk of potentially life-threatening recurrence while also decreasing the need for extensive reconstruction. There are currently no biomarkers available for this tumor type, and no diagnostic strategies beyond standard biopsy. We hypothesize that the epidermal growth factor receptor (EGFR) can serve as a valuable biomarker for ameloblastomas, and can be targeted to specifically label tumor cells, making it possible to assess tumor margins pre-operatively using non-invasive imaging, allowing clinicians to provide better surgical care for their patients.

B. Innovation

This proposal focuses on the development of an accurate biomarker for ameloblastomas, and provides an assessment of bone invasion for a patient population vastly under-represented in the existing research. We have already generated nine novel patient-derived xenograft mouse models of ameloblastoma and are continuing recruitment. Based on our findings, we are developing an immuno-positron emission tomography (PET) agent that can be used to non-invasively image tumors. This non-invasive imaging can help determine the area of resection to obtain clear margins while reducing the resection of healthy tissue, and thus has the potential to directly impact patient care.

C. Background and Approach

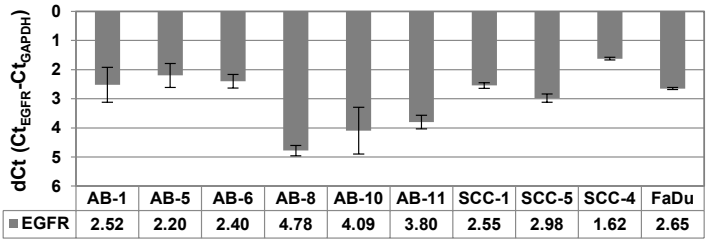
Ameloblastoma demonstrates locally aggressive and destructive behavior, with ~96% of cases occurring as intrabony tumors, primarily within the posterior mandible [1]. A number of surgical treatment modalities have been advocated for ameloblastoma, including subtotal tumor excision with peripheral ostectomy and segmental mandibulectomy with margins [3, 4]. These surgical approaches are similar to those used for oral cases of head and neck squamous cell carcinoma (HNSCC). The wide variability in treatment paradigms has frustrated attempts to assess the long-term tumor control rates, which range from 12-40% [5-7]. Recurrent ameloblastoma may arise in patients with subtotal tumor ablation, and is associated with significant long-term risks. Moreover, aggressive odontogenic tumors may recur many years after surgical treatment, further confounding efforts to identify the optimal treatment [4, 8-10]. Although most recurrences are local, ameloblastoma recurrences have also been identified in the lungs, long bones, and cervical lymphatics [11]. Recurrences may be life threatening, owing to the development of multiple unresectable satellite lesions with direct extension to the airway, skull base or intracranial fossae [12]. Ameloblastoma of the maxilla presents a significant risk of recurrence, given the relatively thin surrounding cortical plates, which offer little barrier to tumor extension [4]. Adjuvant radiation therapy has been recommended for positive gross and microscopic residual disease [13]. However, radiation therapy introduces many morbidities, including osteoradionecrosis, xerostomia, trismus, and the development of radiation-induced osteogenic sarcomas [14].

Conversely, over-aggressive resection with wide margins up to 3 cm for ameloblastoma has been advocated, although margins of 1 to 2 cm are currently considered adequate for curative resection [4]. In one study, the use of a 1 cm margin beyond the radiographically-visible disease was associated with a 29% recurrence rate, so the true extent of resection that is needed remains unclear [6]. Over-resection of healthy tissues results in unnecessary facial deformity, malocclusion, neurosensory disturbances, and speech and swallowing dysfunction. In many cases, radical resection requires extensive reconstruction, including microvascular composite free tissue transfer [15]. The management of recurrences following reconstruction may require the sacrifice of a bone graft or soft tissue constructs, requiring additional reconstructive procedures and leading to donor site morbidity.

It is currently difficult for surgeons to evaluate tumors intraoperatively, leading to positive margins in 30% of head and neck cancer resections [16]. Odontogenic tumor margins are assessed with qualitative visual inspection or radiographs of the resected specimen [17]. Frozen section histology has been used to evaluate the soft tissue adjacent to intrabony tumors to confirm the diagnosis; however, the assessment of bony resection margins requires decalcification and sectioning, which requires up to 14 days [18].

Accurate preoperative assessment of tumor margins via targeted imaging could result in the preservation of healthy tissue and improvements in the long-term local tumor control, thereby reducing the risk of potentially life-threatening recurrence and minimizing the need for reconstructive therapies.

The current imaging modalities used for ameloblastoma include radiography, computed tomography (CT), and magnetic resonance imaging (MRI) [19, 20]. In some cases, contrast-enhanced MRI or cone beam CT (CBCT) may be used [21, 22]. Unfortunately, these approaches lack specificity for tumor tissue. In some cases, ^{18}F-fluorodexyglucose (FDG) positron emission tomography (PET) has been used of ameloblastic carcinoma, malignant ameloblastoma, or recurrent ameloblastoma [23-26]. However, Seno et al. reported that multicystic/solid ameloblastomas only demonstrate mild or moderate FDG uptake, likely due to the low proliferative state and glucose use of these tumors. The ability to specifically and visibly label tumor cells, regardless of their proliferative state, would be highly beneficial for these patients facing extensive surgeries.



	AB-1	AB-5	AB-6	AB-8	AB-10	AB-11	SCC-1	SCC-5	SCC-4	FaDu
EGFR	2.52	2.20	2.40	4.78	4.09	3.80	2.55	2.98	1.62	2.65

Figure 1. EGFR expression in ameloblastoma (AB) and squamous cell carcinoma (SCC) cells. Relative gene expression of EGFR was determined by qRT-PCR. The housekeeping gene GAPDH was used as normalizing reference. All the experiments were performed in duplicate and repeated two times (error bars denote SD).

Novel approaches employing FDA-approved anti-neoplastic antibodies for non-invasive imaging prior to or during surgical intervention have been gaining increasing attention [27]. Numerous approaches for targeted molecular imaging are currently under development. One approach is the use of antibodies targeting the epidermal growth factor receptor (EGFR), which is highly expressed in multiple human tumors. Two EGFR monoclonal antibodies are currently FDA approved; cetuximab for HNSCC and panitumumab for metastatic colorectal cancer. We have previously demonstrated that ameloblastoma cells highly express the EGFR *in vitro* and at similar levels as HNSCC tumor cell lines (**Fig. 1**). Clinical studies have confirmed that 100% of ameloblastomas express the EGFR protein [28-30]. We have also demonstrated that many cases of ameloblastoma express high levels of the EGFR protein (**Fig. 2**). These exciting discoveries led to the development of a new collaboration among the Amm, Morlandt, and Rosenthal laboratories. Our early studies examining the potential of using EGFR as a biomarker for the targeted imaging of ameloblastoma employed an antibody-based optical imaging approach, which allows targeting of known tumor-specific markers for the delivery of optical probes directly to the tumor. The optical dye, IRDye800 (LI-COR), had the advantages of being reliably conjugated to targeting-antibodies, being imageable *in vivo* using FDA-approved imaging equipment (SPY Imaging System), a lack of toxicity in rodent models, and no toxicity demonstrated in a human trial with bevacizumab-IRDye800 (www.clinicaltrials.gov) [31-35]. The Rosenthal and Warram laboratories have successfully used near-infrared-labeled anti-EGFR antibodies in many preclinical models to optically image human head and neck squamous cell carcinomas (HNSCC), melanomas, and breast cancers [31-34, 36]. In orthotopic xenograft models of HNSCC, they showed that panitumumab-IRDye800 and cetuximab-IRDye800, both anti-EGFR antibodies, were equivalent *in vivo* in terms of their specificity and sensitivity for labeling tumors [33]. The collaboration with the Amm lab has now shown that Cetuximab-IRDye800 can specifically label ameloblastoma tumor tissue and makes it possible to localize and visualize tumor tissue *in vivo* (see **Research Accomplishments**).

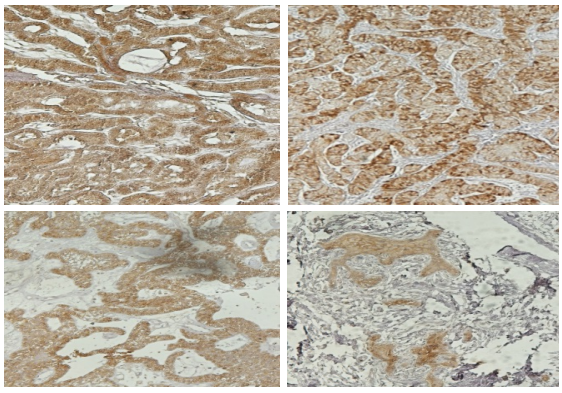


Figure 2. EGFR protein expression in ameloblastomas tumors. Protein expression was determined by immunohistochemisty using a specific polyclonal antibody (10X).

Although this fluorescent imaging demonstrated the specificity of the antibodies for tumor tissue *in vivo*, it was insufficient to detect intraosseous tumor cells within the mandible or the tibia. To overcome this limitation, we approached Dr. Suzanne Lapi, who was recently recruited to UAB as the Director of Advanced Imaging Research, the Cyclotron Facility, and the Radiochemistry Laboratory. These facilities have the ability to produce radiolabeled antibodies to cGMP manufacturing standards and have an approved Investigational New Drug application for the radiolabeled panitumumab. In contrast to the chimeric (mouse/human) cetuximab, panitumumab has the advantage of being a fully human antibody, which may make it less immunogenic in patients. Dr. Lapi's group has extensive experience with the production and study of zirconium-89 labeled (^{89}Zr) panitumumab [37].

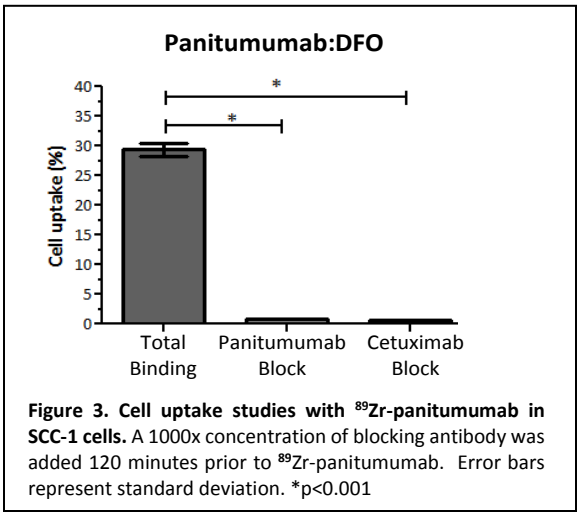


Figure 3. Cell uptake studies with ^{89}Zr-panitumumab in SCC-1 cells. A 1000x concentration of blocking antibody was added 120 minutes prior to ^{89}Zr-panitumumab. Error bars represent standard deviation. *p<0.001

Immuno-PET combines the specificity and sensitivity of antibody-mediated tumor targeting with the power and resolution of PET imaging. ^{89}Zr is a positron emitter with a longer half-life (3.27 days), which fits well with the 5-7 days required to obtain optimal tumor-to-nontumor ratios for antibody localization. Dr. Lapi's laboratory conjugates desferrioxamine-Bz-NCS (DFO) to panitumumab, then labels the product with ^{89}Zr [37]. They showed that this method produced ^{89}Zr-panitumumab with high radiochemical purity, high specific activity, and was stable in serum (up to 7 days). EGFR-positive and negative cancer cells were used to demonstrate that the ^{89}Zr-panitumumab uptake correlated with the EGFR expression on the cells. An HNSCC cell line with high EGFR expression, SCC-1, was used to confirm the uptake and blocking by unlabeled panitumumab and cetuximab (**Fig. 3**). The specific uptake *in vivo* is measured with background and decay correction and is presented as the percent of the injected dose per gram of tissue (%ID/g) normalized to the total activity injected. Xenograft tumor models also demonstrated uptake that correlated with the cellular EGFR expression. Tumors generated from high EGFR-expressing squamous cell carcinoma cells, A431, demonstrated uptake at 24 hours post-injection (17.3 %ID/g) that increased by 120 hours (32.9 %ID/g). In moderate EGFR-expressing HCT116 tumors, the ^{89}Zr-panitumumab uptake was 10.6 %ID/g at 24 hours and 19.8 %ID/g at 120 hours. Biodistribution studies showed that the blood uptake was highest at 24 hours, while the tumor demonstrated the highest uptake at 120 hours post-injection. In a small clinical study, ^{89}Zr-cetuximab was used for PET imaging in 10 patients with advanced colorectal cancer treated with cetuximab. In 6 of 10 patients, the tumors demonstrated ^{89}Zr-cetuximab uptake. Four of these patients had stable disease with cetuximab treatment, whereas only 1 of the 4 patients without detectable ^{89}Zr-cetuximab uptake responded to the antibody [38]. No additional toxicity beyond that of cetuximab alone was seen with ^{89}Zr-cetuximab in these 10 patients. In a dosimetry and first in human experiment, ^{89}Zr-panitumumab was given to three metastatic colorectal cancer patients at a dose of 37 Mbq [39]. The patients were imaged three times on day 1-7 post-injection. The whole body effective radiation dose was estimated to be 0.264-0.330 mSv/MBq, which was considered safe and within reasonable estimates for clinical imaging. In a study of colorectal cancer xenografts, ^{89}Zr-panitumumab was able to detect primary and metastatic tumors from an EGFR-expressing cell line [40]. Tumor uptake is measured as standardized uptake values (SUV = [(MBq/mL) x (animal wt (g)/injected dose (MBq)]) via microPET/CT imaging. *In vivo* detection showed that the uptake correlated with the level of EGFR expression in A431 tumors (high EGFR), which had a higher SUVmax compared to HCT116 tumors (lower EGFR), with the difference being greater at 120 hours post-injection. Following imaging, the tumors were removed, and the EGFR expression level was confirmed in each tumor xenograft.

The current options for imaging tumors within bone, whether jaw or long bones, remain limited to those previously described for imaging ameloblastomas (e.g. radiography, CT, MRI). In HNSCC patients with mandibular tumor involvement, CT is recommended because it can detect cortical erosion; however, MRI may better detect early marrow invasion and has better soft tissue contrast [41]. For patients with tumors that are difficult to evaluate clinically or that require extensive resection and reconstruction, FDG-PET imaging may be recommended. Kolk et al. compared CT, MRI, and single-photon emission computed tomography (SPECT/CT) with ^{99m}Tc-bisphosphate, which shows areas of bone metabolic activity [42]. In patients with histologically-proven bone infiltration, SPECT/CT showed cortical bone erosion that was not detected by prior MRI or CT. The sensitivity and specificity of SPECT/CT was shown to be greater than those of other modalities, and produced fewer false-negatives. PET imaging has an advantage over SPECT in providing better image resolution. Immuno-PET, such as that with ^{89}Zr-panitumumab, provides the added benefit of specificity for tumor tissue, which combined with the higher sensitivity of PET, allow for more precise imaging of the bone-tumor area and margins.

D. Hypothesis and Specific Aims:

Hypothesis: We hypothesize that the epidermal growth factor receptor (EGFR) expression in aggressive odontogenic neoplasms will make it possible to specifically identify tumor tissue *in vivo*. It has previously been demonstrated that intravenous administration of a radiolabeled anti-EGFR antibody successfully identified tumor tissue in multiple *in vivo* preclinical models via PET/CT imaging. Clinically, this approach may be valuable to guide the surgical removal of aggressive odontogenic neoplasms using preoperative, non-invasive imaging to define tumor margins, improving local tumor control while preserving healthy normal tissue. Two hypothesis-driven specific aims will be investigated as follows:

Specific Aim 1: Measure the tumor uptake of radiolabeled ^{89}Zr-panitumumab *in vivo* using patient-derived tumor models of ameloblastoma via PET/CT scanning.

Specific Aim 2: Develop *in vivo* bone models of ameloblastoma.
2a. Develop an intraosseous model via intratibial injection of primary ameloblastoma cells.
2b. Develop an orthotopic mandibular model via injection of primary ameloblastoma cells or implantation of patient-derived tumor into rat mandibles.
2c. Measure the tumor uptake of ^{89}Zr-panitumumab and the accuracy of using immuno-PET with this antibody for margin determination in an intraosseous model of ameloblastoma.

E. Experimental Design and Methods:
Specific Aim 1: Measure the tumor uptake of radiolabeled ^{89}Zr-panitumumab *in vivo* using patient-derived tumor models of ameloblastoma via PET scanning.

In Vivo Models of Ameloblastoma: We have previously developed *in vivo* patient-derived xenograft (PDX) models of ameloblastoma. Human neoplasms are heterogeneous, containing stromal tissue and neoplastic, endothelial, and immune cells. PDX models, where surgically-removed tissue is directly implanted into mice, retain this heterogeneity and more accurately reflect the tumor microenvironment. These models can be used to assess the *in vivo* specificity of ^{89}Zr-panitumumab for human tumors. Patients have been and will be recruited and consented by Dr. Amm (the applicant). The tumors will be surgically resected by Drs. Anthony Morlandt or Yedah Ying, and small pieces (2x2 mm) of primary tumors will be subcutaneously implanted into the flanks of female athymic nude mice by Dr. Amm (10 mice per group x 4 primary tumor samples = 40 mice). Once tumors are established (4-6 weeks post-implantation, based on our previous experience), animals will be transferred to Dr. Lapi at the UAB Advanced Imaging Facility, where they will receive ^{89}Zr-panitumumab and will be imaged via PET scan at 120 hours post-injection (similar design to Chang et al., 2013) [37]. Animals will be sacrificed post-imaging, and all tumors will be excised, weighed and stored for pathologic analysis.

^{89}Zr Production and Antibody Labeling: ^{89}Zr will be produced via the 89Y(p,n)89Zr nuclear reaction using the TR24 cyclotron and separated via ion exchange as described previously [37, 43, 44]. The radiochemical purity and specific activity (amount of radioactivity per mol antibody) will be determined by analytical size-exclusion chromatography. Antibodies with radiochemical purity $\geq$ 95% will be used for experiments. In Dr. Lapi's previous studies, ^{89}Zr-panitumumab was shown to be stable for 7 days in serum at 37°C with no degradation products visible [37].

Small Animal PET/CT experiments: All animal studies will be conducted in compliance with the Guidelines for the Care and Use of Research Animals and additional guidelines established by the UAB IACUC. Once ameloblastoma PDX tumors are established, mice will be randomly assigned to treatment and control groups. The mice (n=10) will be administered ^{89}Zr-labeled radiopharmaceuticals [3.7 MBq (100 μCi)/20-25 μg in 100 μL 0.9% sterile saline] via tail vein injection. One group of mice (n=5) will receive a blocking dose of 1 mg of unlabeled panitumumab (supplied by Dr. Jason Warram) 120 minutes prior to ^{89}Zr-panitumumab. At 7 days post-injection, the mice will be anesthetized with 1-2% isoflurane and imaged, with images collected. For all PET studies, PET and CT data will be co-registered using standard image display software. Regions of interest will be drawn and the mean and maximum standard uptake values (SUVs) for tumors will be determined using the formula: SUV = [(MBq/mL) x (animal wt. (g))/injected dose (MBq)]. Upon completion of immuno-PET scanning, all mice will be sacrificed, and their tumors and organs of interest will be assayed for radioactivity using a gamma counter.

Immunofluorescence Detection of EGFR: To confirm that the ^{89}Zr-panitumumab binding *in vivo* correlated with the EGFR expression in the tumors, immunofluorescence staining will be performed on resected specimens [37]. Tumors will be harvested after completion of microPET/CT imaging studies and immediately fixed in 10% formalin. After allowing for the radioactivity to decay to background levels, tumors will be embedded in paraffin. Five-micron sections will be prepared and blocked in PBST with 3% goat serum/1% BSA for 30 minutes at room temperature. Antigen retrieval will be performed in a citrate-based buffer using a pressure cooker. The sections will be incubated with rabbit polyclonal anti-EGFR primary antibody (1:200; Abcam) overnight at 4°C and visualized with Alexa Fluor 555-conjugated goat antirabbit IgG (1:200; Invitrogen). Finally, sections will be mounted with SlowFade Gold antifade reagent with 4,6-diamidino-2-phenylindole (DAPI) and coverslipped.

Figure 4. Intramandibular animal model development. Implantation of ameloblastoma tissue following creation of a defect in the angular process of the mandible. Small pieces of ameloblastoma tumor tissue (white arrow) were implanted directly adjacent to the mandible intramedullary space.

Specific Aim 2: Develop *in vivo* bone models of ameloblastoma.

Subcutaneous xenografts offer an easily accessible and stable environment, with little inconvenience to the animal, while providing proof-of-principle data. However, these models do not adequately represent the tumor environment. The intraosseous nature of ameloblastomas can by simulated using orthotopic models by injecting cells into the tibia (a model widely used, as described by Yang et al. in 2010), or via the injection of cells directly into mandible. This would be a completely novel animal model that has not been previously employed in cancer research.

Intra-tibial implantation of ameloblastoma: Tumor tissue will be collected after resection and will be dissected into 8x8 mm pieces. Half of each piece will be frozen sectioned and H&E stained to confirm the presence of tumor. The area(s) adjacent to the region(s) containing tumor will be used to form PDX tumors or digested with collagenase, strained through a cell strainer, and centrifuged. The resulting cells will be resuspended and loaded into a syringe (approximately $2x10^5$ cells in 10 µl of sterile PBS) and kept on ice (as described in Campbell et al., 2012) [45]. After sterile preparation, the needle will be inserted into the tibia under the patella through the patellar ligament. The cells will be injected and the needle extracted. Luciferase-positive tumor cells are available from our collaborator, Dr. Jason Warram, and will be implanted and imaged to confirm the uptake of tumor cells in a pilot study before the use of patient tissues. This surgery has previously been performed using a loading dye to see the injection site and successful injection. The surgery and protocol has been approved by the Institutional Animal Care and Use Committee.

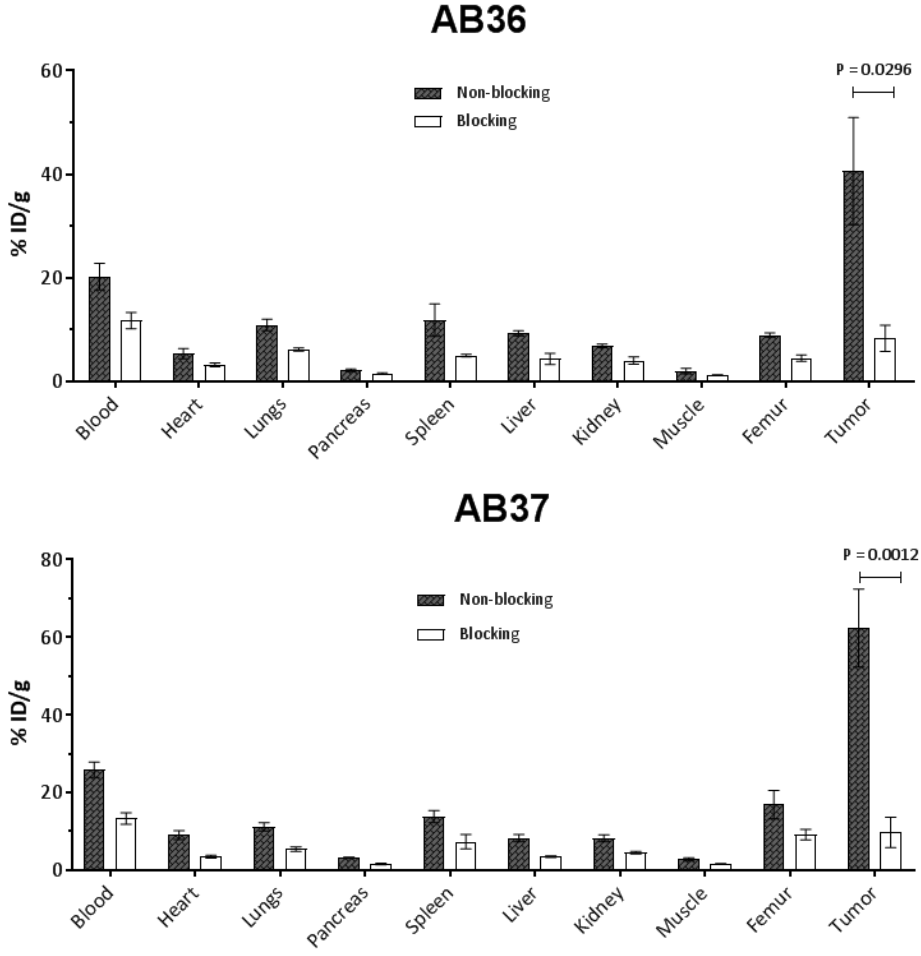


Figure 5. Biodistrubtion of ^{89}Zr-panitumumab in ameloblastoma PDX (AB-36 and AB-37) at 7 days post-injection. A 1 mg blocking (200x) dose of unlabeled panitumumab was given 120 minutes prior to ^{89}Zr-panitumumab. Data are presented as percent injected dose per gram ± standard deviation, n = 4.

Mandibular orthotopic model of ameloblastoma: For the mandibular model of ameloblastoma, athymic nude rats will be utilized instead of mice due to their larger mandible intramedullary space. PDX tissue pieces or a tumor cell suspension will be prepared as described as above. For the injection of cells, the mandibles of athymic nude rats will be palpated, and the needle will be inserted above the alveolar process into the medullary space of the mandible. For orthotopic models involving the implantation of a tumor piece into athymic nude rats, a transcervical incision will be made and deepened through subcutaneous tissues and the platysma muscle following the humane preparation for surgery. The pterygomasseteric sling will be incised, exposing the inferior border and lateral surface of the mandibular body and angle. Subperiosteal dissection then proceeds superiorly, and a defect in the angular process of the mandible will be created. Small pieces of ameloblastoma will be implanted directly adjacent to the mandibular intramedullary space (**Fig. 4**). Next, the periosteum, subcutaneous tissues, and skin will be closed with absorbable chromic gut sutures. Rats will be given access to a soft diet following

surgery until no difficulties eating solid food are observed. This surgery has been well tolerated in animals in previous studies, and is approved by the Institutional Animal Care and Use Committee.

Small animal PET/CT experiments: Four-to-six weeks post-implantation, the rats will be injected with ^{89}Zr-panitumumab and imaged on the same schedule and experimental plan as described previously (Specific Aim 1). These data will provide valuable information about the feasibility of imaging intraosseous odontogenic tumors, and potentially those that have expanded beyond the bone.

The UAB Center for Clinical and Translational Science and the UAB Biostatistics and Bioinformatics Shared Facility provide support for statistical analysis (study design, sample size and power calculations, and data analysis). The imaging equipment, antibodies, and other resources are all currently available at UAB laboratories and hospitals.

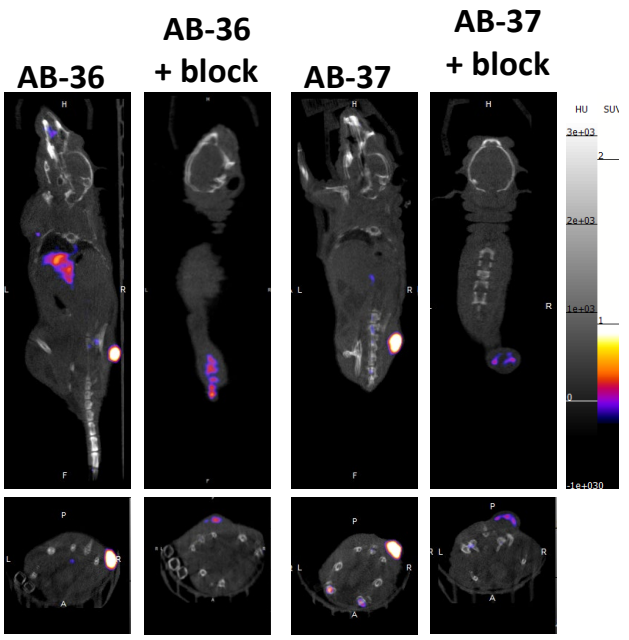


Figure 6. MicroPET/CT images obtained at 7 days post ^{89}Zr-panitumumab injection. Tumors were implanted in the right flank of each mouse. The coronal slice at the center of the tumor is shown in the upper panel and the axial slice in the bottom panel. The scale, shown as standardized uptake value (SUV) is shown on the far right. A 1 mg blocking (200x) dose of unlabeled panitumumab was given 120 minutes prior to ^{89}Zr-panitumumab.

F. Preliminary Studies

We have an active Oral and Maxillofacial Surgery Department at UAB. Within the last year, we have had several ameloblastoma patients, four of whom were eligible for our studies (the tumors were of large enough size and sufficient integrity). The patients were enrolled and provided written informed consent as approved by the UAB Institutional Review Board. Two of these samples were implanted as PDXs in the flanks of nude mice (AB-36 and AB-37). The biodistribution of ^{89}Zr-panitumumab was measured 120 hours post-injection and was reported as the injected dose per gram of tissue (%ID/g). The average tumor uptake was ~40 %ID/g for AB-36 and ~65 %ID/g for AB-37 (**Fig. 5**). The radiolabeled %ID/g was significantly greater than the uptake produced in mice receiving unlabeled panitumumab. MicroPET/CT imaging showed high uptake of ^{89}Zr-panitumumab in the ameloblastoma tumors compared to other areas of the mouse (**Fig. 6**). There is also low uptake in the bone (measured in the femur, **Fig. 5**), which makes ^{89}Zr-panitumumab an attractive target for imaging EGFR-expressing tumors within bone. With this technology, we believe we can more accurately assess neoplastic margins for the surgical removal of ameloblastomas, thus improving patient outcomes.

These aims and the planned research will generate necessary translational data required for the initiation of a Phase I clinical trial in patients with aggressive odontogenic tumors. Along with the described career development activities, including continuing my success as an Assistant Professor and increasing my research presence in the community, the proposed research plan would help me to achieve my scientific career goals to develop a clinical, multi-disciplinary research program as an independent investigator. This project demonstrates my ability to work in a collaborative team on a translational project with the goal of impacting patient care, and also shows my integration within my department. It also demonstrates my independence as I will develop new animal models of disease for a patient population under-represented in research. Participating in the Loan Repayment Program has, and would, provide stability during my career progression and would significantly enhance my future research success.

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Dr. Amm recently accepted an offer for a tenure-track Assistant Professor position from the UAB SOD Department of Oral and Maxillofacial Surgery, with which Dr. Amm has productive collaborations. The position includes financial support of salary and research funds, 75% protected research time, and support of a postdoctoral fellow. It also included laboratory and office space and all experimental resources and equipment required for the proposed research activities. This demonstrates the considerable institutional investment UAB has made and will continue to make for Dr. Amm's success.

The research environment at UAB is well-developed and provides every resource needed to facilitate Dr. Amm's progress as an independent investigator and achieve the outlined research goals. UAB has a long history of encouraging interdisciplinary collaborations as a strategy to maximize its research productivity, and has many interdisciplinary, interdepartmental research centers (University-wide Interdisciplinary Research Centers, UWIRCs) and core facilities that provide support and enhance research opportunities. All of the core facilities and enrichment programs at UAB are available for Dr. Amm's research and professional development.

UAB UWIRCs of particular benefit to this application are:

Center for Clinical and Translational Science (CCTS): This is a university-wide, interdisciplinary UAB Center, 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. Dr. Amm has regularly attended their events and successfully utilized their grant assistance and training programs. The CCTS also has an office for clinical research which will aid in the development of a Phase I clinical trial protocol by Dr. Amm.

Global Center for Craniofacial, Oral and Dental Disorders (GC-CODED): The GC-CODED is housed within the School of Dentistry (SOD) under the leadership of Dr. Rosa Serra. 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 odontogenic tumors. This center has been instrumental in supporting patient recruitment and the interdepartmental collaborations described in the proposed research.

Comprehensive Cancer Center (CCC): The UAB CCC is one of the nation's leading cancer research and treatment centers, and is expertly guided Dr. Michael Birrer. The UAB CCC is one of the original National Cancer Institute (NCI)-designated cancer centers, one of only 40 in the nation. 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 its members, the CCC hopes to foster an interactive and collaborative environment that will lead the way into the future of cancer research.

Advanced Imaging Facility: *Cyclotron Facility and Nuclear Pharmacy:* The UAB Cyclotron Facility is located within the Advanced Imaging Facility (AIF) at the CCC and houses state-of-the-art equipment for the preparation of radioactive compounds for preclinical and clinical use. The facility includes a TR24 cyclotron, hot cells and associated equipment in both GMP and non-GMP areas. Nearby is an animal imaging suite and animal vivarium. Animal elevator access from the 6th floor to the AIF allows access to the larger animals and the dedicated PET-CT and PET-MR scanners. Dr. Amm is collaborating with the Director of the Cyclotron Facility and Radiochemistry, Dr. Suzzane Lapi, for this project and will have access to her staff, facilities, and expertise.

The UAB SOD provides excellent support to and investment in junior faculty. As a postdoctoral scholar, Dr. Amm was supported by the SOD's NIDCR-funded T-90 Dental Academic Research Training (DART) Grant and the Institute of Oral Health Research prior to obtaining her own funding. Dr. Amm's current postdoctoral scholar, Dr. Burthia Booker, is also supported by the DART. The SOD provides many activities to enrich research and career development. As a postdoctoral scholar, Dr. Amm participated in the SOD's Annual Scholars Symposium, UAB Postdoctoral Research Day, a Laboratory Management course, and many other seminars regarding research and career development. As a faculty member, she has transitioned into leadership roles in these events and organizations. She is the co-chair for the Scholar's Day Judging Committee. She was elected as the SOD faculty representative to the Council for Postdoctoral Education. The SOD has fully supported Dr. Amm's career development and provided protected time to pursue research activities.