SUMMARY STATEMENT

PROGRAM CONTACT: **(Privileged Communication)** ***Release Date:*** **07/01/2011**

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Application Number: **1 R21 CA164425-01**

Principal Investigators (Listed Alphabetically):
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Applicant Organization: UNIVERSITY OF TENNESSEE HEALTH SCI CTR

Review Group: **CONC**
Clinical Oncology Study Section

Meeting Date: **06/13/2011** ***RFA/PA:*** **PA08-267**
Council: **OCT 2011** ***PCC:*** **8SDR**
Requested Start: **10/01/2011**

Project Title: **In vivo and in vitro validation of a novel biomarker for melanoma**

SRG Action: **Impact/Priority Score: 33 Percentile: 27 +**
Human Subjects: **E4-Human subjects involved - Exemption #4 designated**
Animal Subjects: **30-Vertebrate animals involved - no SRG concerns noted**
Clinical Research - not NIH-defined Phase III Trial

Project Year	Direct Costs Requested	Estimated Total Cost
1	125,000	173,457
2	150,000	208,149
TOTAL	275,000	381,606

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

1R21CA164425-01 WALL, JONATHAN

RESUME AND SUMMARY OF DISCUSSION: This application proposes to test the efficacy of novel peptide-based reagents for binding to tumor specific hypersulfated proteoglycans for the identification and imaging of melanoma. Development of imaging tracers for the specific detection and identification of location of metastasis of melanoma is of high interest. Strengths include the innovative approach, use of the canine model of melanoma, and experience of the investigative team for imaging with peptides in small animal models. However, the potential lack of specificity of the peptides being studied may lower the significance of the research. Also, of concerns were reliance on imaging of euthanized animals and fixed tissues, lack of statistics and lack of details regarding the patient-derived samples to be studied. Following discussion, the study section recommended the project with moderately high level of enthusiasm.

DESCRIPTION (provided by applicant): Melanoma is the major cause of skin cancer mortality in the world. In contrast to many other malignancies the incidence and mortality trends have continued to increase over the last decade. The key to survival in patients with melanoma is early and accurate diagnosis and staging so that appropriate treatment regimes can be initiated. These important goals are currently achieved by measuring the physical and mitotic properties of the tumor and by determining the presence and distribution of metastases; however, it is likely that these techniques could be enhanced by additionally monitoring the expression of appropriate tumor biomarkers. One group of potential biomarkers is the sulfated proteoglycans expressed on the tumor cell surface or within the extra cellular matrix. While HSPGs are found in all tissue, their exact structure and amount can vary in tumor cells resulting in altered/aberrant biological functions. The importance of proteoglycans in tumorigenesis has been well established and their over-expression by melanoma cells likely contributes to the metastatic potential of the tumor and aids in tumor angiogenesis. We have recently identified a class of basic polypeptides that preferentially bind hypersulfated proteoglycans found in murine melanoma lung colonies as evidenced by single photon emission tomographic (SPECT) imaging using a radioiodinated peptide as a tracer. Furthermore, this class of peptides when appropriately labeled specifically stained pulmonary melanoma tumor colonies in formalin-fixed tissue sections. This advance provides a new and convenient method to evaluate the potential of specific HSPGs as biomarkers of melanoma that may have diagnostic or prognostic import. We now propose to validate and expand these studies by systematically evaluating a panel of related peptides to identify the most efficacious tumor-targeting reagent, in a mouse model of melanoma, by using quantitative imaging techniques. The expression of the proteoglycan biomarker in canine and human tumors will be assessed by using peptides to stain melanoma-containing tissue sections. Finally, we will perform imaging in dogs with metastatic melanoma using our most efficacious peptide and compare performance with the standard tumor targeting reagent 2- deoxy-2-(18F)fluoro-D-glucose (FDG) by using PET/CT imaging. The goal of this study is to demonstrate that melanoma tumors express a unique proteoglycan-related biomarker that can be identified in vivo and in tissue sections by using appropriately labeled peptide reagents. Preliminary evaluation of the temporal and spatial expression of this biomarker in melanoma tumors using human tissue sections and dogs with metastatic melanoma will provide impetus for a statistically rigorous study of the diagnostic and prognostic importance of its expression. These studies may also lead to novel peptide- based reagents for non-invasive diagnosis and prognostic imaging of patients with melanoma.

PUBLIC HEALTH RELEVANCE: The American Cancer Society predicted more than 68,000 new cases of melanoma and ~ 8,500 related deaths in the USA in 2010, rendering this the 5th most common cause of cancer related deaths. Despite intense research into the diagnostic and prognostic importance of tumor-biomarker expression, melanoma incidence and death rates continue to increase; however it is well recognized that the key to long term survival is early and accurate diagnosis and staging. We have identified, by using a peptide targeting reagent, a novel tumor biomarker present on melanoma tumors, whose expression may be of great diagnostic or prognostic importance, and whose detection may enhance early diagnosis, staging, and patient survival.

CRITIQUE 1:

Significance: 2
Investigator(s): 2
Innovation: 1
Approach: 4
Environment: 2

Overall Impact: The investigators plan to test the efficacy of novel peptide-based reagents for the identification and imaging of melanoma. These peptides bind to heparan sulfate proteoglycans expressed by melanoma cells and the investigators believe that this specific binding can be used to identify melanoma cells in tissue sections and can also be used with radio-labeling as an imaging modality. This is an interesting and potentially important concept and the investigators appear to have the necessary resources to pursue this line of investigation. Potential impact of this work is lessened by the use of a single murine melanoma model in the preliminary studies and lack of robust statistical analysis or discussion of statistical techniques to be employed.

1. Significance:

Strengths

- The concept of using peptides to bind tumor-specific HSPGs as a way to image tumor cells is novel and has the potential to change the way we diagnosis and stage melanoma and other cancer types.
- The ability to label peptides that bind to tumor-specific HSPGs is also a novel concept that has the potential to change the way we image and treat certain types of cancer.

Weaknesses

- The HSPG-binding peptides may not function in human or canine melanoma as they do in the single murine melanoma model presented in the preliminary studies section of the application
- Lack of a discussion of the statistical analysis that was used to design the proposed studies or how the data will be statistically analyzed

2. Investigator(s):

Strengths

- The Co-PIs bring different strengths to the application and they have a track-record of working together that increases the likelihood of a successful collaboration.
- The co-investigators include a Surgical Oncologist with experience in treating patients with melanoma and a radiologist experienced in large-animal imaging, both important collaborators on this project.

Weaknesses

- No Biostatistician is included in the list of personnel, possibly accounting for the general lack of statistical discussion found in this application.

3. Innovation:

Strengths

- The use of peptides that bind to tumor-specific HSPGs is a novel concept and is the main strength of this proposal.
- The use of a canine model of melanoma for pre-clinical imaging studies is moderately innovative.

Weaknesses

- None

4. Approach:

Strengths

- The investigators have designed a reasonable set of Specific Aims that move from peptide selection *in vivo*, to *in vitro* imaging using human and canine specimens, back to *in vivo* studies in a canine model of melanoma. This is a logical process that, in general, has a high likelihood of obtaining useful data.
- The investigators' documented ability to produce HSPG-binding peptides and to label these peptides for use in pre-clinical studies is impressive.
- The use of the canine model for pre-clinical imaging is a strength of the application, with the presumption that human studies would follow at the conclusion of these studies.

Weaknesses

- The investigators do not make clear why they think we need a new system for imaging melanoma. How will this system be superior to standard IHC staining? How will it be superior to PET/CT scanning? How will it potentially improve outcomes for patients with melanoma?
- One weakness of this application is that the investigators appear to have only tested their peptides in one murine melanoma model (B16F10).
- There is a general lack of statistical analysis of the preliminary data and a discussion of the statistical analysis that will be done to validate the investigators' hypothesis. This is especially important given the proposed use of dogs in these studies; it is important to justify the number of animals to be used in studies employing large animals to assure that usable data will be obtained.
- The investigators propose to study human melanoma samples in Specific Aim 2 but do not provide a justification for the number of samples to be tested or what variables will be used to compare standard IHC staining to peptide-based staining. How will the investigators prove that their method is better than what we have now?
- The investigators also do not take into consideration the diverse genotypes found in human melanoma in any of their studies. What impact does the presence of B-RAF, N-RAS, PTEN, or other common mutations have on peptide binding to HSPGs or in any other aspect of the proposed work? Melanoma is not a homogeneous disease and this must be taken into account when designing experiments like those proposed in this application.
- The use of dead, frozen mice in the imaging studies outlined in Specific Aim 1 might not be the best model to use when studying a new imaging system that you propose to use in living, breathing human patients.

5. Environment:

Strengths

- The environment at The University of Tennessee Health Science Center appears adequate for the proposed studies

Weaknesses

- Lack of statistical input might indicate a lack of Biostatistical support at this institution.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Data and Safety Monitoring Plan (Applicable for Clinical Trials Only):

Not Applicable (No Clinical Trials)

Inclusion of Women, Minorities and Children:

G4A - Gender Unknown, acceptable

M4A - Minority Representation Unknown, acceptable

C4A - Children Representation Unknown, acceptable

- No comments are made concerning the types of human tissues to be used or from whom these tissues will be obtained.

Vertebrate Animals:

Acceptable

Biohazards:

Not Applicable (No Biohazards)

Budget and Period of Support:

The budget was recommended as requested.

CRITIQUE 2:

Significance: 3
Investigator(s): 3
Innovation: 3
Approach: 4
Environment: 2

Overall Impact: The is an exploratory studies proposal aimed at testing the potential use as an imaging tool of a polypeptide that preferentially binds to heparin sulfate proteoglycans (HSPG) on B16 melanoma. A first peptide has been analyzed in limited preliminary studies leading to the proposal to expand this line of research leading to the *in vivo* imaging of melanoma in dogs. The applicant is new to the field of melanoma and veterinary medicine, so he has enlisted adequate collaborators to pursue the proposed studies. Caveats include the lack of biostatistical planning and the limited testing in a single murine melanoma cell line that may not be representative of human melanoma.

1. Significance:

Strengths

- Development of imaging tracers for the specific detection and distribution of melanoma is of high interest.

Weaknesses

- The potential lack of specificity of the peptides being studied may lower the significance of the research.

2. Investigator(s):

Strengths

- An investigator with significant experience in proteoglycans and radiolabeled probe molecular imaging who is collaborating with co-investigators with significant expertise to allow pursuing the goals of this research.

Weaknesses

- Biostatistician support is missing.

3. Innovation:

Strengths

- A new line of research based on the confluence of expertise in HSPG, molecular imaging and melanoma biology.

Weaknesses

- Other approaches to develop radiolabeled probes to image melanoma have been developed, most without significant advantages over the use of FDG for PET scans.

4. Approach:

Strengths

- A suitable project for exploratory studies in biomarkers for cancer based on an early lead arising from extensive prior expertise in a different field of research (peptides to recognize amyloid disease).
- Supportive preliminary data suggesting that all the expertise is in place for the proposed studies.
- Initial imaging in mice with lung metastasis from B16 melanoma with a low background and a high signal-to-noise ratio resulting in the visualization of lung and nodal metastasis.
- The proposal to screen an extended line of peptides based on the testing of the ability to bind to HSPG and functionality as imaging probes.
- The testing of the candidate HSPG-binding peptides in tissue blocks of human and canine melanomas, for which the feasibility of the approach is also provided.
- The translation of this research project to a relevant canine clinical setting of imaging metastatic melanoma in dogs.

Weaknesses

- The lack of understanding of the specific HSPG that the initial peptide binds to may be misleading for the ability to select new peptides with improved functions in Aim 1.
- Testing of the candidate probe in additional melanoma cell lines in vitro would strengthen the proposal.

- The proposal is missing biostatistical planning.

5. Environment:

Strengths

- The environment is outstanding for the proposed work.

Weaknesses

- No weaknesses.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Acceptable

Biohazards:

Not Applicable (No Biohazards)

Resource Sharing Plans:

Acceptable

Budget and Period of Support:

The budget was recommended as requested.

CRITIQUE 3:

Significance: 3
Investigator(s): 3
Innovation: 3
Approach: 5
Environment: 3

Overall Impact: This application proposes to examine whether peptides that bind to specific heparan sulfate proteoglycans expressed by melanoma can be used for tumor imaging. Strengths include the innovation, use of the canine model of melanoma, and experience of the investigative team for imaging and small animal models. Weaknesses include significant concerns regarding specificity; reliance on imaging of euthanized animals and fixed tissues; lack of statistics; and lack of detail regarding the patient-derived samples to be studied.

1. Significance:

Strengths

- Developing a specific and sensitive imaging approach to detect melanoma metastasis is highly significant.

Weaknesses

- Pet based imaging modalities are plagued with false-positive findings. While the proposed approach may be more sensitive in tumor detection, it is unclear whether this approach will be more specific.

2. Investigator(s):

Strengths

- The PI is a professor of medicine at University of Tennessee College of Medicine with significant experience in using peptides for imaging.
- The team is comprised of investigators with significant experience in imaging and animal models.

Weaknesses

- No biostatistician is included within the team.

3. Innovation:

Strengths

- The use of basic polypeptides that preferentially bind tumor-specific hypersulfated proteoglycans to detect melanoma metastasis is relatively novel.

Weaknesses

- The PI's experience is primarily in imaging of amyloid, so his experience in melanoma is limited.

4. Approach:

Strengths

- The PI and co-PI has significant experience in PET and SPECT imaging using peptides. They present preliminary data showing feasibility.

Weaknesses

- Hypersulfated proteoglycans are ubiquitous, raising concerns of specificity. The peptides identified by the PI were initially used for visualizing amyloid. Figure 2 shows avidity in pulmonary metastasis, but also shows significant activity below the diaphram (liver, stomach?). The rest of the imaged mouse is not shown.
- Only the B16F10 lung metastasis model will be used for peptide screening.
- The tissue staining (aim 2) will only be performed on fixed tissues.
- In vivo imaging (aim1) will be performed on euthanized mice. These results may therefore be different that in live animals.
- No justification for the sample sizes in any of the aims is provided.

5. Environment:

Strengths

- The environment is excellent. The PI has access to 2 microPET scanners.
- The animal facility is adequate for the proposed experiments.

Weaknesses

- Melanoma samples will be derived from a tissue repository at Moffitt Cancer Center. No letter from that center is provided, nor are there any details to these samples or collaborator from Moffitt.

Protections for Human Subjects:

Acceptable Risks and/or Adequate Protections

Data and Safety Monitoring Plan (Applicable for Clinical Trials Only):

Inclusion of Women, Minorities and Children:

G4A - Gender Unknown, Acceptable

M4A - Minority Representation Unknown, Acceptable

C4A - Children Representation Unknown, Acceptable

Vertebrate Animals:

Acceptable

Biohazards:

Acceptable

Budget and Period of Support:

The budget was recommended as requested.

CRITIQUE 4:

Statistical Evaluation:

Investigator(s):

Strengths

-

Weaknesses

- There is no statistician named on the project. Also, there is no individual responsible for data analysis.

Approach:

Strengths

- No statistical strengths identified.

Weaknesses

- There are no statistical or data analysis methods proposed.

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

PROTECTION OF HUMAN SUBJECTS (Resume): ACCEPTABLE
The applicant has addressed all of the issues regarding protection of human subjects and the committee has no concerns for the protection of human subjects in the proposed studies.

VERTEBRATE ANIMAL (Resume): ACCEPTABLE
The applicant has addressed all of the issues regarding protection of animal welfare and the committee has no concern for the vertebrate animals in the proposed studies.

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

+ Derived from the range of percentile values calculated for the study section that reviewed this application.

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-10-080 at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-10-080.html.
The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

MEETING ROSTER

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* Temporary Member. For grant applications, temporary members may participate in the entire meeting or may review only selected applications as needed.

Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.