ACADEMIC RESEARCH ENHANCEMENT AWARDS **Issue Date:** 08/11/2014
Department of Health and Human Services
National Institutes of Health
NATIONAL CANCER INSTITUTE

Grant Number: 1R15CA182850-01A1
FAIN: R15CA182850

Principal Investigator(s):
Maria Cekanova, DSC

Project Title: Detection of COX-2-expressing bladder cancer by fluorocoxib A

Ms. Nichols, Cynthia L
Director of Sponsored Programs
2621 Morgan Circle Drive
103 Morgan Hall
Knoxville, TN 379964506

Award e-mailed to: nih@utk.edu

Budget Period: 08/11/2014 – 07/31/2017
Project Period: 08/11/2014 – 07/31/2017

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $422,444 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF TENNESSEE KNOXVILLE in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Cancer Institute of the National Institutes of Health under Award Number R15CA182850. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Nicholas Mitrano
Grants Management Officer
NATIONAL CANCER INSTITUTE

Additional information follows

SECTION I – AWARD DATA – 1R15CA182850-01A1

Award Calculation (U.S. Dollars)

Salaries and Wages	$121,920
Fringe Benefits	$37,738
Personnel Costs (Subtotal)	$159,658
Equipment	$10,000
Supplies	$71,000
Travel Costs	$4,500
Other Costs	$39,707
Consortium/Contractual Cost	$15,001
Federal Direct Costs	$299,866
Federal F&A Costs	$122,578
Approved Budget	$422,444
Federal Share	$422,444
TOTAL FEDERAL AWARD AMOUNT	$422,444
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$422,444

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$422,444	$422,444

Fiscal Information:

CFDA Number:	93.394
EIN:	1626001636A1
Document Number:	RCA182850A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2014

IC	CAN	2014
CA	8479566	$422,444

NIH Administrative Data:
PCC: 5FDI / **OC**: 414A / **Released**: MITRANON 08/05/2014
Award Processed: 05/08/2014 01:52:21 PM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R15CA182850-01A1

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 1R15CA182850-01A1

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is excluded from Streamlined Noncompeting Award Procedures (SNAP).
MULTI-YEAR FUNDED AWARD: This is a multi-year funded award. A progress report is due annually on or before the anniversary of the budget/project period start date of the award, in accord with the instructions posted at: http://grants.nih.gov/grants/policy/myf.htm.

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R15CA182850. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

This award represents the final year of the competitive segment for this grant. See the NIH Grants Policy Statement Section 8.6 Closeout for complete closeout requirements at: http://grants.nih.gov/grants/policy/policy.htm#gps .

A final Federal Financial Report (FFR) (SF 425) must be submitted through the eRA Commons (Commons) within 90 days of the expiration date; see the NIH Grants Policy Statement Section 8.6.1 Financial Reports, http://grants.nih.gov/grants/policy/policy.htm#gps, for additional information on this submission requirement. The final FFR must indicate the exact balance of unobligated funds and may not reflect any unliquidated obligations. There must be no discrepancies between the final FFR expenditure data and the Payment Management System's (PMS) cash transaction data.

A Final Invention Statement and Certification form (HHS 568), (not applicable to training, construction, conference or cancer education grants) must be submitted within 90 days of the expiration date. The HHS 568 form may be downloaded at: http://grants.nih.gov/grants/forms.htm.

Unless an application for competitive renewal is submitted, a final progress report must also be submitted within 90 days of the expiration date. Instructions for preparing a Final Progress Report are at: http://grants.nih.gov/grants/funding/finalprogressreport.pdf. Any other specific requirements set forth in the terms and conditions of the award must also be addressed in the final progress report. Institute/Centers may accept the progress report contained in competitive renewal (type 2) in lieu of a separate final progress report. Contact the awarding IC for IC-specific policy regarding acceptance of a progress report contained in a competitive renewal application in lieu of a separate final progress report.

NIH **strongly encourages** electronic submission of the final progress report and the final invention statement through the Closeout feature in the Commons, but will accept an email or hard copy submission as indicated below.

Email: The final progress report and final invention statement may be e-mailed as PDF attachments to the NIH Central Closeout Center at: NIHCloseoutCenter@mail.nih.gov.

Hard copy: Paper submissions of the final progress report and the final invention statement may be faxed to the NIH Division of Central Grants Processing at 301-480-2304, or mailed to:

NIH Division of Central Grants Processing, OER
6705 Rockledge Drive
Suite 5016, Room 5109
MSC 7986
Bethesda, MD 20892-7986 (for regular or U.S. Postal Service Express mail)
Bethesda, MD 20817 (for other courier/express mail delivery only)

NOTE: If this is the final year of a competitive segment due to the transfer of the grant to another institution, then a Final Progress Report is not required. However, a final FFR is required and should be submitted electronically as noted above. If not already submitted, the Final Invention Statement is required and should be sent directly to the assigned Grants Management Specialist.

Treatment of Program Income:
Additional Costs

SECTION IV – CA Special Terms and Conditions – 1R15CA182850-01A1

INFORMATION: Although the budget period start date for this award is 8/11/14, this award includes funds for thirty-six months of support. Allowable preaward costs may be charged to this award, in accordance with the conditions in the NIH Grants Policy Statement, (October 2013), and with institutional requirements for prior approval. The NIH GPS can be found at http://grants.nih.gov/grants/policy/nihgps_2013/.

INFORMATION: This award, including the budget and the budget period, has been discussed between Kelly Fritz of the National Cancer Institute and Cynthia Nichols on 8/4/14.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Kelly Fritz
Email: weaverke@mail.nih.gov **Phone**: (240) 276-6314 **Fax**: 301-451-5391

Program Official: Anne E Menkens
Email: menkensa@mail.nih.gov **Phone**: 240.276.5919

SPREADSHEET SUMMARY
GRANT NUMBER: 1R15CA182850-01A1

INSTITUTION: UNIVERSITY OF TENNESSEE KNOXVILLE

Budget	Year 1
Salaries and Wages	$121,920

Fringe Benefits	$37,738
Personnel Costs (Subtotal)	$159,658
Equipment	$10,000
Supplies	$71,000
Travel Costs	$4,500
Other Costs	$39,707
Consortium/Contractual Cost	$15,001
TOTAL FEDERAL DC	$299,866
TOTAL FEDERAL F&A	$122,578
TOTAL COST	$422,444

Facilities and Administrative Costs	Year 1
F&A Cost Rate 1	49%
F&A Cost Base 1	$250,159
F&A Costs 1	$122,578

ACADEMIC RESEARCH ENHANCEMENT AWARDS **Issue Date:** 09/19/2014

Department of Health and Human Services
National Institutes of Health
NATIONAL CANCER INSTITUTE

Grant Number: 1R15CA182850-01A1 REVISED
FAIN: R15CA182850

Principal Investigator(s):
Maria Cekanova, DSC

Project Title: Detection of COX-2-expressing bladder cancer by fluorocoxib A

Ms. Nichols, Cynthia **L**
Director of Sponsored Programs
2621 Morgan Circle Drive
103 Morgan Hall
Knoxville, TN 379964506

Award e-mailed to: nih@utk.edu

Budget Period: 08/11/2014 – 07/31/2017
Project Period: 08/11/2014 – 07/31/2017

Dear Business Official:

The National Institutes of Health hereby revises this award to reflect a decrease in the amount of $7,505 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF TENNESSEE KNOXVILLE in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Cancer Institute of the National Institutes of Health under Award Number R15CA182850. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Teresa Parker
Grants Management Officer
NATIONAL CANCER INSTITUTE

Additional information follows

SECTION I – AWARD DATA – 1R15CA182850-01A1 REVISED

Award Calculation (U.S. Dollars)

Salaries and Wages	$121,920
Fringe Benefits	$37,738
Personnel Costs (Subtotal)	$159,658
Equipment	$10,000
Supplies	$71,000
Travel Costs	$4,500
Other Costs	$39,707
Consortium/Contractual Cost	$15,001
Federal Direct Costs	$299,866
Federal F&A Costs	$115,073
Approved Budget	$414,939
Federal Share	$414,939
TOTAL FEDERAL AWARD AMOUNT	$414,939
AMOUNT OF THIS ACTION (FEDERAL SHARE)	($-7,505)

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$414,939	$414,939

Fiscal Information:

CFDA Number:	93.394
EIN:	1626001636A1
Document Number:	RCA182850A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2014

IC	CAN	2014
CA	8479566	$414,939

NIH Administrative Data:
PCC: 5FDI / **OC**: 414A / **Released**: PARKERT 09/17/2014
Award Processed: 05/08/2014 01:52:21 PM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R15CA182850-01A1 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 1R15CA182850-01A1 REVISED

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a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
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Suite 5016, Room 5109
MSC 7986
Bethesda, MD 20892-7986 (for regular or U.S. Postal Service Express mail)
Bethesda, MD 20817 (for other courier/express mail delivery only)

NOTE: If this is the final year of a competitive segment due to the transfer of the grant to another institution, then a Final Progress Report is not required. However, a final FFR is required and should be submitted electronically as noted above. If not already submitted, the Final Invention Statement is required and should be sent directly to the assigned Grants Management Specialist.

Treatment of Program Income:
Additional Costs

SECTION IV – CA Special Terms and Conditions – 1R15CA182850-01A1 REVISED

INFORMATION: This award corrects the F&A Rate as discussed in the correspondence between Kelly Fritz of the National Cancer Institute and Deborah Hampstead on 8/11/14. No action by the awardee is required.

THE FOLLOWING TERMS FROM THE PREVIOUS NOTICE OF AWARD ISSUED ON 9/16/2014 ALSO APPLY TO THIS AWARD:

INFORMATION: Although the budget period start date for this award is 8/11/14, this award includes funds for thirty-six months of support. Allowable preaward costs may be charged to this award, in accordance with the conditions in the NIH Grants Policy Statement, (October 2013), and with institutional requirements for prior approval. The NIH GPS can be found at http://grants.nih.gov/grants/policy/nihgps_2013/.

INFORMATION: This award, including the budget and the budget period, has been discussed between Kelly Fritz of the National Cancer Institute and Cynthia Nichols on 8/4/14.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Kelly Fritz
Email: weaverke@mail.nih.gov **Phone**: (240) 276-6314 **Fax**: 301-451-5391

Program Official: Anne E Menkens
Email: menkensa@mail.nih.gov **Phone**: 240.276.5919

SPREADSHEET SUMMARY

Budget	Year 1
Salaries and Wages	$121,920
Fringe Benefits	$37,738
Personnel Costs (Subtotal)	$159,658
Equipment	$10,000
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Consortium/Contractual Cost	$15,001
TOTAL FEDERAL DC	$299,866
TOTAL FEDERAL F&A	$115,073
TOTAL COST	$414,939

Facilities and Administrative Costs	Year 1
F&A Cost Rate 1	46%
F&A Cost Base 1	$250,159
F&A Costs 1	$115,073

PI: **Cekanova, Maria**	Title: Detection of COX-2-expressing bladder cancer by fluorocoxib A	
Received: 11/01/2013	FOA: PA13-313	Council: 05/2014
Competition ID: FORMS-C	FOA Title: Academic Research Enhancement Award (Parent R15)	
1 R15 CA182850-01A1	Dual: EB	Accession Number: 3634865
IPF: 578304	Organization: UNIVERSITY OF TENNESSEE KNOXVILLE	
Former Number:	Department: Small Animal Clinical Sciences	
IRG/SRG: ZRG1 SBIB-L (83)A	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 294,420	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Maria Cekanova	The University of Tennessee	PD/PI
Joseph Bartges	The University of Tennessee	Co-Investigator
Jashmin Uddin	Vanderbilt University Medical Center	Co-Investigator
Henry Manning	Vanderbilt University Medical School	Consultant

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

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

○ Pre-application ● Application ○ Changed/Corrected Application

4.a. Federal Identifier
CA182850

b. Agency Routing Number

2. DATE SUBMITTED	Application Identifier	c. Previous Grants.gov Tracking Number
2013-11-01	14-0208 Cekanova	

5. APPLICANT INFORMATION **Organizational DUNS*:** 003387891

Legal Name*: The University of Tennessee
Department:
Division:
Street1*: 2621 Morgan Circle Drive
Street2: 103 Morgan Hall
City*: Knoxville
County: Knox
State*: TN: Tennessee
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 37996-4506

Person to be contacted on matters involving this application
Prefix: Ms. First Name*: Cynthia Middle Name: L Last Name*: Nichols Suffix:
Position/Title: Director of Sponsored Programs
Street1*: 2621 Morgan Circle Drive
Street2: 103 Morgan Hall
City*: Knoxville
County: Knox
State*: TN: Tennessee
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 37996-4506
Phone Number*: 865-974-7357 Fax Number: 865-974-7451 Email: aggrant@utk.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 62-6001636

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

Other (Specify):
Small Business Organization Type ○ Women Owned ○ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*
○ New ● Resubmission
○ Renewal ○ Continuation ○ Revision

If Revision, mark appropriate box(es).
○ A. Increase Award ○ B. Decrease Award ○ C. Increase Duration
○ D. Decrease Duration ○ E. Other *(specify)* :

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

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Detection of COX-2expressing bladder cancer by fluorocoxib A

12. PROPOSED PROJECT
Start Date* 07/01/2014
Ending Date* 06/30/2017

13. CONGRESSIONAL DISTRICTS OF APPLICANT
TN-002

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: Maria Middle Name: Last Name*: Cekanova Suffix:

Position/Title: Research Assistant Professor
Organization Name*: The University of Tennessee
Department: Small Animal Clinical Sciences
Division:
Street1*: 2407 River Drive
Street2: A201 Veterinary Teaching Hospital
City*: Knoxville
County: Knox
State*: TN: Tennessee
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 37996-4506
Phone Number*: 865-389-5222 Fax Number: 865-974-5554 Email*: mcekanov@utk.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$414,938.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$414,938.00
d. Estimated Program Income*	$0.00

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

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

DATE:

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

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

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

● I agree*

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

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Ms. First Name*: Cynthia Middle Name: L Last Name*: Nichols Suffix:

Position/Title*: Director of Sponsored Programs
Organization Name*: The University of Tennessee
Department:
Division:
Street1*: 2621 Morgan Circle Drive
Street2: 103 Morgan Hall
City*: Knoxville
County: Knox
State*: TN: Tennessee
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 37996-4506
Phone Number*: 865-974-7357 Fax Number: 865-974-7451 Email*: aggrant@utk.edu

Signature of Authorized Representative*
Cynthia Nichols

Date Signed*
11/01/2013

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:1235CoverLetter.pdf

Funding Opportunity Number: PA-13-313 . Received Date: 2013-11-01T16:37:09.000-04:00

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

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

Project/Performance Site Location(s)

Project/Performance Site Primary Location

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

Organization Name:	The University of Tennessee
Duns Number:	0033878910000
Street1*:	2621 Morgan Circle Drive
Street2:	103 Morgan Hall
City*:	Knoxville
County:	Knox
State*:	TN: Tennessee
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	37996-4506
Project/Performance Site Congressional District*:	TN-002

Project/Performance Site Location 1

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

Organization Name:	The University of Tennessee
DUNS Number:	0033878910000
Street1*:	2407 River Drive
Street2:	A201 Veterinary Teaching Hospital
City*:	Knoxville
County:	Knox
State*:	TN: Tennessee
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	37996-4539
Project/Performance Site Congressional District*:	TN-002

Project/Performance Site Location 2

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

Organization Name:	Vanderbilt University Medical Center
DUNS Number:	0044134560000
Street1*:	1161 21st Avenue South
Street2:	MCN AA-1105
City*:	Nashville
County:	Davidson
State*:	TN: Tennessee
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	37232-2310
Project/Performance Site Congressional District*:	TN-005

File Name

Additional Location(s)

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

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**	
1.a. If YES to Human Subjects	
Is the Project Exempt from Federal regulations? ○ Yes ○ No	
If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6	
If NO, is the IRB review Pending? ○ **Yes** ○ **No**	
IRB Approval Date:	
Human Subject Assurance Number	
2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**	
2.a. If YES to Vertebrate Animals	
Is the IACUC review Pending? ○ Yes ● No	
IACUC Approval Date:	12-18-2012
Animal Welfare Assurance Number	A3668-01
3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**	
4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**	
4.b. If yes, please explain:	
4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No	
4.d. If yes, please explain:	
5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**	
5.a. If yes, please explain:	
6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**	
6.a. If yes, identify countries:	
6.b. Optional Explanation:	

	Filename
7. Project Summary/Abstract*	1236-ProjectSummary.pdf
8. Project Narrative*	1237-ProjectNarrative.pdf
9. Bibliography & References Cited	1238-BibliographyReferencesCited.pdf
10.Facilities & Other Resources	1239-FacilitiesOtherResources.pdf
11.Equipment	1240-Equipment.pdf

Funding Opportunity Number: PA-13-313. Received Date: 2013-11-01T16:37:09.000-04:00

Project Summary/Abstract

Despite the prevalence of white light for screening used in the clinic, it is widely assumed that the sensitivity and specificity of scoping (cystoscopy, endoscopy, and colonoscopy) can be significantly improved with the use of optical contrast agents that specifically accumulate in tumors. The early detection of urinary bladder dysplasia and tumor margins of bladder transitional cell carcinomas (TCC) will result in improved patient prognosis and long-term survival. The present situation with 50-70% recurrence rate of bladder cancer shows the inadequacy of white light cystoscopy for detection and resection of the bladder cancer lesions. In this study, we propose to explore the efficacy of a novel optical imaging agent, fluorocoxib A, to selectively target cyclooxygenase-2 (COX-2) enzyme expressed in bladder cancer. The COX-2 enzyme is expressed at high levels intracellularly in tumor cells, but not in most of normal cells, which enables molecular imaging in vivo with high signal-to-noise ratios. This property defines COX-2 as an attractive imaging target for carcinomas detection. Together with colleagues from Vanderbilt University, we have conducted proof-of-principle studies that have explored the use of a new class of imaging agents targeting COX-2 to image tumors in a variety of in vivo pre-clinical settings. Encouraged that fluorocoxib A could be used to image COX-2-expressing xenograft tumors originated from human cell lines, and from canine primary K9TCC, and in dogs with naturally-occurring carcinomas, we now propose an essential series of studies that will bridge the gap between proof-of-principle and clinical use. We hypothesize that fluorocoxib A will detect overexpressed COX-2 in bladder dysplasia, carcinoma in situ, as well as TCC. Our proposed study using fluorocoxib A in mouse and canine TCC models will assist in translating this optical imaging agent into clinical applications for early detection of bladder cancer, as well for monitoring responses to therapy, tumor margins, and recurrence of bladder cancer. To evaluate our hypotheses, we propose the following specific aims to: 1) Determine the ability of fluorocoxib A to detect the early bladder neoplasias using a mouse model of nitrosamine-induced bladder cancer and to 2) Use fluorocoxib A to detect K9TCC in a dog cancer model. This proposal will lay the framework for enhanced collaborations in translational imaging science between investigators from the University of Tennessee and Vanderbilt University and impart a major impact on the scientific atmosphere of the University of Tennessee. This project will create an appropriate scientific environment to attract high-quality students into UT graduate program to successfully accomplish the proposed research. Fluorescence imaging by fluorocoxib A will allow earlier detection of urinary bladder tumors, when interventions can be more successful, as well as improve detection of TCC margins for more complete resection. Improved biomarkers for detection of urinary bladder neoplasia, which can rapidly and cost effectively be deployed into clinical populations, are critically needed.

Project Narrative

Despite the prevalence of white light for cancer detection used in the clinic, it is widely assumed that the sensitivity and specificity of cystoscopy can be significantly improved by the optical contrast agent, fluorocoxib A that specifically accumulates in COX-2-expressing tumors. *Our proposed study using fluorocoxib A in mouse and dog bladder cancer models will assist in translating this optical imaging agent into clinical applications for early detection of bladder cancer, as well for monitoring responses to therapy, tumor margins, and recurrence of bladder cancer.* Improved bladder cancer detection and tumor surveillance can be rapidly and cost effectively deployed into the clinical populations, thereby improving patient's prognosis and long-termed survival.

Facilities & Other Resources

List of Facilities:

1) College of Veterinary Medicine, Veterinary Medical Center, University of Tennessee, Knoxville, Tennessee – Clinic

2) Laboratory and office of Dr. Maria Cekanova at the Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Veterinary Medical Center, University of Tennessee, Knoxville, Tennessee

3) Vanderbilt University in Nashville, Department of Biochemistry at the School of Medicine at Tennessee Laboratory and office of Dr. Jashim Uddin are a part of laboratory of Dr. Marnett and Vanderbilt University Institute of Imaging Sciences (VUIIS) by Dr. Charles Manning, Director of Molecular Imaging Research

1) **The University of Tennessee, College of Veterinary Medicine, Veterinary Medical Center** and the Agriculture/Veterinary Medicine Library are contained within a modern structure of 246,000 sq ft **for research, teaching and for clinic**.

The College of Veterinary Medicine (CVM) is a one of the 13 colleges and schools of the University of Tennessee (http://www.utk.edu/aboutut/) in Knoxville founded in 1794 as Blount College; became East Tennessee College in 1807, East Tennessee University in 1840, and the University of Tennessee in 1879. Institutions of the UT system are in Knoxville, Health Science Center in Memphis, in Chattanooga, in Martin, Space Institute in Tullahoma, Institute of Agriculture, and Institute for Public Service. The UT is a co-manager with Battelle of the nearby Oak Ridge National Laboratory and has also collaborations with Vanderbilt University in Nashville, TN. Faculty and students experience unparalleled research and learning opportunities at the Department of Energy's largest science and energy lab. The Clyde M. York Veterinary Medicine Building on the agricultural campus houses the Departments of Large Animal Clinical Sciences, Small Animal Clinical Sciences, and Biomedical and Diagnostics Sciences.

The CVM is an outstanding organization that is effective in all three of its mission areas: teaching, research and service. Our strategic plan outlined seven strategic goals that addressed organizational structure; revenues and budget, infrastructure, facilities, and personnel; homeland security and agroterrorism, teaching and curriculum; and assessment (http://www.vet.utk.edu/about/StrategicPlan.pdf). The mission of the CVM at the UT is to educate students in the art and science of veterinary medicine and related biomedical sciences, and to discover new knowledge and to disseminate it to veterinarians and others in order to advance human and animal well-being. The vision of the CVM at the UT is that we are an empowered and diverse organization with the resources to perform well in all mission areas, graduating highly trained veterinarians and biomedical scientists, while providing quality patient and client services, and advancing the knowledge of medical sciences. Our mission is to develop a research reputation for the CVM at national and international levels. We promote racial, cultural and gender diversity and equality to strengthen all programs and service areas of the college to ensure maximum integration with the community and world we serve. We honor our role in teaching, service and research by valuing the principles and spirit of veterinary medical ethics.

The Main Campus of the University of Tennessee in Knoxville (UTK) has a total of 28,512 students, and 20,286 of those are undergraduates. Of the undergraduate population, 48.9% are male, and 51.1% are female. Minorities make up approximately 15.2% of the current student body. Of those students who completed a professional degree program following receipt of a baccalaureate degree from UT, 25% were within the biological sciences/health-oriented programs. The University has actively engaged to increase those numbers by attracting high-quality students through its Top 25 Initiative that was implemented approximately three years ago. This program is designed to move UT into the Top 25 public research universities within the nation. To achieve this goal, the university has focused in on expanding facilities, improving research resources and actively recruiting the best students within the state and surrounding areas, all while centering around the university's three-part vision: value creation; original ideas; leadership.

Comparative & Experimental Medicine (CEM) graduate program (MS and PhD) is an interdisciplinary graduate program administered through the College of Veterinary Medicine that currently as of Spring of 2013 has enrolled three master degree and twenty-two PhD degree students. CEM is a jointly administered graduate

program intended to prepare students for teaching and/or research careers in the health sciences. This program emphasizes the comparative approach to the study of biomedical science. The program is open to approved graduate students seeking training in this area and is especially useful for individuals with professional degrees. Keeping with the program's interdisciplinary nature, other colleges and departments throughout the university participate in the program but retain their own administrative structure. For the student with an undergraduate biological science background, the CEM Program provides an unusual opportunity to study disease processes common in animals and humans from a multi-disciplinary perspective. The scope of this intercollegiate program, which pools faculty resources from both veterinary and human medicine, is broadened by faculty members representing wide-ranging interests in biomedical disciplines and areas of the life sciences. The interdisciplinary training environment includes such diverse support as facilities and personnel at the Veterinary Medical Center, University of Tennessee (UT) Graduate School of Medicine, life sciences departments, College of Agricultural Sciences and Natural Resources, College of Engineering, and the Department of Nutrition.

Dr. Cekanova developed multi-disciplinary collaboration with veterinary specialists at UT Veterinary Medical Center in Knoxville and Dr. Marnett's research group from Vanderbilt University and imaging group at VUIIS Vanderbilt University to evaluate novel imaging agents for detection of xenograft tumors in rodent models and of naturally-occurring cancers in dogs as a more advance translational model for human medicine. Dr. Cekanova has environment suitable for successful accomplishment of this project. The facility and equipment of Dr. Cekanova are mentioned in details below. The additional facilities available to Dr. Cekanova though Vanderbilt University are mentioned below also in Dr. Manning (VU) letter of support to provide access to the full resources of the Molecular Imaging Research program at VU which includes all the relevant imaging hardware housed within VUIIS and relevant imaging expertise. Projects supported by UTCVM and VU in last two years allowed Dr. Cekanova to accept five female undergraduate students to have their first hands on research experience and for three female DVM summer students to learn about the molecular and cellular cancer research. Dr. Cekanova served as a mentor for not only mentioned undergraduate students and DVM graduate students, but was assigned to become a major PhD mentor last year. Dr. Cekanova actively trained and co-mentored undergraduate researchers since 2009, one of whom have joined health related programs such as medicine at UTGSM in Memphis. In addition, Dr. Cekanova is a major mentor of post-doctoral research associate, Dr. Rathore who joined her laboratory last year. This proves that Dr. Cekanova has already experience, and available facilities to mentor additional graduate/undergraduate students. The new collaboration with Vanderbilt Imaging Center VUIIS in Nashville will allow to gain new experiences and student will have opportunity to interact with other investigators and learn new techniques.

The University of Tennessee is actively working to improve both the quality of undergraduate and graduate students as well as the quality of their education. The expansion of facilities and research resources and the active recruitment of the top undergraduate and graduate students are beginning to have a positive impact on the community. The additional support of an AREA grant will continue to assist the University pursue and achieve its goals. Specifically, this grant will provide opportunities for students at all levels to engage in biological research that will have direct effects on human health and disease. These experiences will help reinforce the lessons learned in the classroom, develop valuable critical thinking techniques, engage them both intellectually and creatively, and prepare them for their future careers in medicine, science and technology.

The Department of **Small Animal Clinic** is a 52,000 square foot facility located within the University of Tennessee Veterinary Medical Center. The facilities include: 15 examination rooms, 12 patient wards with a capacity of 190 small animals, 8 operating rooms with anesthesia and surgical preparation rooms, an intensive care unit, in-house diagnostic and treatment facilities with a complete radiology unit and all of the support laboratories necessary for diagnostic testing. Specific to the **oncology unit**, approximately 5,000 sq ft is dedicated to both radiation and medical oncology. The linear accelerator vault, which contain a Clinac iX Silhouette 120 MLC linear accelerator with multiple photon and electron energies, control room and staging areas comprise approximately 1,950 sq ft. Additionally there is 1,110 sq ft allocated for high and low dose radioisotope usage and isolation for radioactive animals. For medical oncology, 2,000 sq ft has been allocated for treatment and diagnostics, which includes a chemotherapy administration suite, separate canine and feline wards, exam rooms, office space, and rounds and consultation rooms. In addition to active clinical support services in clinical pathology, anatomic pathology, pharmacology, bacteriology and mycology, virology,

immunology, toxicology, endocrinology, and parasitology, some of our specialized facilities available for extensive case work-up and research application include.

The **caseload of the Small Animal Clinic** is approximately 14,000 dogs and cats per year. The department consists of 34 faculty representing board-certified specialists in 11 different specialties, including internal medicine, cardiology, oncology, neurology, nutrition, dermatology, ophthalmology, anesthesiology, radiology, radiation oncology, and surgery. Emergency and critical care services are provided on a 24-hour basis. Other personnel include 28 veterinary technicians, a physical rehabilitation team, and a support staff of receptionists and veterinary assistants. The **UTCVM clinical oncology caseload** is comprised primarily of dogs presenting with a varied array of different tumor types: non-Hodgkin's lymphoma, osteosarcoma, malignant melanoma, cutaneous mast cell disease, head and neck carcinomas, bladder carcinoma, thyroid carcinoma, soft tissue sarcoma, histiocytic malignancies, hemangiosarcoma, genitourinary carcinomas, and pulmonary cancers. The UTCVM-COP currently has minimal competition for referral oncology cases within a 150-mile radius. **Through the Center for Minimally Invasive Procedures at UTCVM, we perform approximately 150 cystoscopic procedures per year and evaluate approximately 20 patients with transitional cell carcinoma of the urinary bladder each year.** We have the capacity to recruit additional cases of targeted histology with the addition of technical support provided by the proposed grant.

The UTCVM is an AAALAC-accredited **facility for animal research activities**. We maintain a reputation for excellence in patient care and animal welfare with the UT-Knoxville Institutional Animal Care and Use Committee (IACUC) and Office of Laboratory Animal Care (OLAC). **The University of Tennessee, Knoxville laboratory animal facilities** are registered with the United States Department of Agriculture (63-R-0105) and have an assurance on file with the Office of Laboratory Animal Welfare (A3668-01). The animal care and use program at The University of Tennessee, Knoxville, is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC-I). The animal care and use program includes The University of Tennessee Medical Center, Knoxville; College of Education, Health, and Human Sciences; College of Arts and Sciences; College of Veterinary Medicine (CVM); College of Agricultural Sciences and Natural Resources; and Agricultural Research and Education Centers (Dairy, Greenville, Highland Rim, East TN, Middle TN, and Plateau).

Animals are cared for by veterinary technicians and American Association of Laboratory Animal Science (AALAS)-certified laboratory animal technicians that are supervised by an AALAS-certified laboratory animal technologist. These caretakers work under the supervision of veterinarians who are diplomats of the American College of Laboratory Animal Medicine. Veterinary care is provided by veterinarians licensed in the state of Tennessee. Laboratory animal facilities at the University of Tennessee meet and exceed NIH and U.S. Public Health Service standards for the humane care and use of laboratory animals. All animals housed in the dedicated animal facilities are obtained from approved vendors and are free from pathogens to which they are susceptible (viruses, bacteria, parasites, etc). A rodent sentinel health surveillance program is used to verify the absence of these infectious agents in rodents. Larger species are vaccinated appropriately and tested annually for infectious diseases to which they are susceptible.

2) Laboratory and office of Dr. Maria Cekanova at the Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Veterinary Medical Center, University of Tennessee, Knoxville, Tennessee

UT, CVM has designated approximately 40,000 sq ft for research activities. **Dr. Cekanova's laboratories** at the College of Veterinary Medicine in Knoxville have 900 sq ft located in two separate rooms with fully-equipped instruments at her disposal. Dr. Cekanova has also free access to different facilities to use the fluorescence-activated cell sorter (FACS), Laser Capture Microdissection System and to Histology laboratory to process and evaluate tissue samples.

Dr. Cekanova receives full institutional support as protected time for research with salary and benefits support since 2009. She is a junior faculty and she has strong support and guidance from senior faculty mentor, Dr. Legendre at SACS department. She has developed several collaborations with her colleagues from clinic to establish translational research at UTCVM. Recently, she becomes a leading mentor for PhD graduate student at CEM program at UTCVM. In addition, she mentored several veterinary students during their summer research program and currently she is a mentor of three undergraduate students.

Dr. Cekanova's office has 120 sq ft with office desk and computer to analyze the data. The computer available for Dr. Cekanova is DELL Optiplex980, Intel Core i5 Dual Core Processor 650 with VT (3.20GHz, 4M) with 64-bit operating system Genuine Windows 7 Professional with all software for data analysis, such as Microsoft Office 2007 package, Adobe Acrobat Professional 9.0, Adobe Photoshop CS5, Scion Image, End Note XIV, and Internet Explorer; printer, scanner, and fax. The computers in her office and laboratories are linked to the internet and available file servers. This networking enables file exchange within the research group. This office set up also provides personnel from the laboratory access to word processing, database management, graphics, literature searching, etc. In addition, the networking enables searching of Abstracts, MedLine, and Protein and Nucleic Acid Databanks. All major journals are available free of charge to all investigators through site licenses to the University of Tennessee Library System. She has Leica Leitz DMRB microscope with attached DP72 camera to evaluate the histology of the tissue sections and immunohistochemical staining data.

The six office staff is available to assist faculty and staff of that department that is equipped with copy machines, fax machine, and all necessary office supplies.

Other available facilities for Dr. Cekanova's research at UT:
Dr. Cekanova has also free access to **other laboratories at UTIA** equipped with additional equipment's for conducting her research, such as two PCR machines, cryostat, luminometer, spectrophotometers, microplate readers with luminescence and fluorescence, fluorescence microscopes, micro and macro-centrifuges, and others. Dr. Cekanova has also free access to different facilities at UTIA to use the fluorescence-activated cell sorter (FACS), Laser Capture Microdissection System, and to Histology laboratory to process and evaluate tissue samples.

Advanced Microscopy and Imaging Center at the University of Tennessee, Knoxville that is a multi-user, multi-disciplinary facility to provide microscopy and imaging services in support of teaching and research for students, faculty and staff at UTK. The Center was developed and is supported by the Division of Biology in the College of Arts and Sciences, the Department of Material Sciences in the College of Engineering and the Joint Institute of Applied Material. The Center provides microscopy services to academic units on the main UTK campus, the Institute of Agriculture, the College of Veterinary Medicine and the University of Tennessee Medical Research Center and Hospital, Knoxville.

The Center is a 5,500 sq ft. research laboratory located in the Science and Engineering Research Facility on the main campus of the University of Tennessee, Knoxville. It houses instrumentation for: transmission and scanning electron microscopy, optical microscopy including laser scanning confocal and total internal reflection (TIRF), and atomic force microscopy **and IVIS Lumina imaging system for in vivo mice imaging at UT Knoxville.**

Preclinical Molecular Imaging Graduate School of Medicine, The University of Tennessee in Knoxville.
Dr. Cekanova has developed a collaboration with Dr. Wall and Dr. Kabalka from UT, GSM in Knoxville, Tennessee, to initiate the long term goals to evaluate the novel PET imaging tracers using dogs and cats with naturally-occurring tumors as better translational models. In recent years SPECT, PET, and CT micro-imaging and corroborative studies have been used to study the efficacy of amyloid-binding biomolecules and provide a non-invasive method for quantifying the biodistribution of these agents *in vivo*. In September 2005 a new purpose-built micro-imaging laboratory, the Preclinical Imaging Laboratory (PIL), was constructed in the UT Medical Center immediately below a suite of radiochemistry laboratories, the cyclotron and a prototype, high-resolution, low-count clinical/preclincal PET scanner with a 52 cm axial field of view (Siemens). The new facility boasts 4 imaging suites: the first housing a microCAT II +SPECT hybrid scanner (Siemens Preclinical Solutions; described in detail below), and the second, a P4 microPET (Siemens Preclinical Solutions). The microCAT II +SPECT is a fully shielded high-resolution hybrid x-ray (CT) and single photon emission computed tomography (SPECT) *in vivo* imaging system. The x-ray source is a 40 W, tungsten anode, 35-80 kVp source mounted on a rotating gantry capable of step-and-shoot acquisition over the full 360° around the subject positioned on the animal bed. The x-ray detector is a 2048 × 3096 pixel CCD coupled to a high-resolution phosphor screen via a fiber optic taper. It boasts a 61 × 91 mm field of view and a resolution of 27 µm. The digitized images are 12 bit depth and the detector dynamic range when using 2 × 2 or 4 × 4 binning (standard in our mode of operation) is 72 dB. The CT data are reconstructed using the cone-beam Feldkamp algorithm, although iterative reconstruction methods are being developed and evaluated in our program. Real time reconstruction is performed using the Cobra software package (Exxim Computing Corporation, Pleasanton,

CA) running on a dual-processor Dell workstation with 8 GB of memory. A standard 512 × 512 × 768 (4 × 4 binned image) volumetric reconstruction onto 77 μm3 voxels usually completes within 2 minutes after the final projection of a 360-projection acquisition (1°azimuths). Offline reconstruction can alternatively be conducted using an in-house developed parallel implementation of the Feldkamp algorithm. This code is capable of providing volumetric images of the same dimensions as above in less than 5 min using a small group of PCs.

The SPECT detectors are mounted on stepper motor driven stages so that they can be readily retracted form the CT field of view. Detectors are available optimized for either high or low energy photon emitting nuclides and are housed within a 10 mm-thick casing of 6% antimonial lead. For low energy nuclides (< 170 KeV; such as 125I, 123I, and 99mTc) we employ two large-area detectors mounted facing each other on the rotating gantry and at 90° to the x-ray source and detector. The detector heads are 150 × 150 mm2 and composed of 5 mm-thick sodium iodide (NaI) crystals with a 1.5 × 1.5 mm or 1.25 × 1.45 mm crystal spacing. For high energy photon imaging (up to 300 KeV, e.g. 111In) similarly sized detectors (150 × 150 mm2) are available with 10 mm long NaI crystals and a crystal spacing of 2.2 × 2.2 mm.

For both sets of detectors parallel, pinhole, and multi-pinhole collimator configurations are available. Composed of 12 mm-thick 6% antimonial lead, the collimators can be fitted with pinholes made of 15 mm-thick tungsten containing either a 0.5, 1.0, 2.0, or 3.0 mm diameter aperture. For our standard mouse scans using ~ 100 μCi of tracer we use the 2.0 mm pinhole collimators on both detectors, which provides a modest degree of magnification and statistically good count rates (even when each detector is reconstructed independently). The SPECT data from each detector may be reconstructed either independently or following summation as a single ("multiple-detector") file. Reconstruction is performed post-acquisition using a dedicated ordered subset expectation maximization (EM) algorithm. A 52 × 52 × 68 image typically computes in approximately 2 minutes. Further upsampling of the image to smaller voxel sizes is normally performed post-reconstruction using the Lanczos filtering kernel in Amira.

We have recently added a clinical PET/CT to the floor above the small animal imaging facility. The state of the art mCT scanner is situated in a dedicated room and can be used for preclinical large animal imaging studies as well as research studies on human patients. The mCT boasts 64-slice CT, 78 cm bore that can accommodate patients up to 500 lbs in weight. The CT isotropic resolution is 0.24 mm and acquires at 0.3 sec per rotation. The PET system is composed of 52 detector rings comprising LSO crystals of 4 × 4 mm in size. The transaxial field of view is 70 cm with an axial view of 21.6 cm, which can be extended by 33% with TrueV™. The data can be reconstructed using both point spread function (HD/PET™) and time of flight (ultraHD/PET™) technologies to provide unprecedented resolution and image clarity (a 4× improvement in signal-to-noise).

The Immunohistochemisty Laboratory/Microscope room includes a Reichert cryostat and microtome; American Optical processor and Tissue-Tek embedding station; a 5-headed, Nikon Labophot microscope, an Olympus B-MAX Microscope and Leica DMR epifluorescent microscope with motorized Z-stage control. All scopes are equipped for polarizing light microscopy. In addition, the Leica is equipped with a Diagnostic Spot RT 2.2.0. 3-chip color CCD camera, directly linked to a Pentium PC, for image acquisition; the Olympus houses a Sony DXC-151A RGB CCD Video camera with direct analog feed into an overhead Sony 13" HR color monitor.

The Protein Biochemistry Laboratory includes a Millipore Dynacell culture system; two liquid nitrogen cryostorage units including an automated Taylor-Wharton system; NUAIRE hood; two CO2 water-jacketed, double-chambered, automated incubators; OMNI mixer; two water baths; and tabletop centrifuge. In addition, two BMG Labtech Thermostar microplate shakers and Perkin Elmer-Wallac time-resolved fluorescence and BioTek Synergy fluorescence microplate readers are available. For autoradiography we use a Packard Cyclone phosphorimager, New Brunswick Scientific shaking incubator, Fisher accuspin MicroR refrigerated microfuge, Fisher Isotemp floor incubator, and an Amersham Biosciences Ultrospec 3300 UV/visible spectrophotometer.

The Molecular Biology Laboratory is fully equipped for preparation of gene probes and DNA sequencing and includes an Applied Biosystems Model 391-04 hood, Beckman Model LS6000IC scintillation counter; radiological fume hood; three BRL Model S2 DNA sequencing apparatuses; BioRad GenePulser™ electroporation system; Millipore water purifying system (Milli-Q); various microcentrifuges; continuous flow spectrometer; Queue orbital shaker/incubator; Brinkmann polytron; microsonic disrupter; Stratagene UV Stratalinker 1800; transilluminator; Beckman 50pH meter; and other requisite equipment and a BioRad iQ5 Multicolor Realtime PCR and i cycler.

Four fully equipped **organic synthesis laboratories** outfitted with a complement of equipment including fume hoods, electronic balances, analytical HPLC units, and gas chromatographs. In addition, he has two fully equipped radiochemistry laboratories in the Molecular Imaging and Translational Research Program located at The University of Tennessee Medical Center. The laboratories are equipped with three vonGalen hot cells, a Capintec Radioiosotope Calibrator Model CRC-127R, a Bioscan, Autochanger 300 radio-TLC system, and a Waters HPLC fitted with UV, RI and radiation detectors. The Center houses an 11 MeV negative ion cyclotron dedicated to the routine production of PET radionuclides.

A full complement of MRI imagers (micro and macro) as well as radioisotope imaging systems (micro and macro) are available to the project (see equipment section.)

Analytical Equipment (Chemistry) - Available chemical analyses equipment include a variety of modern instruments including a high resolution double focusing mass spectrometer; a Nicolet R3m/V single crystal X-ray diffractometer; HP-5982 gc-mass spectrometer; Varian 600 MHz multinuclear NMR spectrometer; Bruker 400 MHz wide bore NMR Spectrometer multinuclear) equipped with a multinuclear micro-imaging MR system; Varian Mercury 300 MHz multinuclear NMR, Bruker 250 MHZ multinuclear analytical NMR; Nicolet 200 MHZ, magic angle multinuclear NMR spectrometer for solids; Digilab FTS Fourier Transform infrared spectrometer; a variety of UV-visible spectrometers; a Perkin-Elmer 137 and 257G infrared, Cary 14 and 171 UV-Vis spectrophotometer; and a Nuclear Data ND 660 multichannel analyzer. Also available are three vonGalen hot cells, a Capintec Radioiosotope Calibrator Model CRC-127R, a Bioscan, Autochanger 300 radio-TLC system, and a Waters HPLC fitted with UV, RI and radiation detectors. An 11 MeV negative ion cyclotron dedicated to the routine production of PET radionuclides.

Offices: There are sufficient offices (~300 sq ft each) in the Imaging Facility so that each investigator will have a private office.

3) Vanderbilt University in Nashville, Department of Biochemistry at the School of Medicine at Tennessee Laboratory and office of Dr. Jashim Uddin are a part of laboratory of Dr. Marnett and Vanderbilt University Institute of Imaging Sciences (VUIIS) by Dr. Charles Manning, Director of Molecular Imaging Research

The Marnett's Laboratory currently has 2,550 square feet of laboratory space in 850-860 Robinson Research Building, where Dr. Jashim Uddin is a Research Assistant Professor. The equipment description is described in more details in "Equipment" section
Office: Dr. Marnett has a 150 square foot office adjacent to the laboratory.

Computer: Drs. Marnett and Uddin utilize a MacBook Pro for routine computing. Each desk in the laboratory is equipped with MacIntosh IBooks or IMacs with expanded memory. All of the computers are linked to the internet and available file servers. This networking enables file exchange within the research group and provides individuals from the laboratory access to word processing, structure drawing, database management, graphics, literature searching, etc. In addition, the networking enables searching of Current Contents, Chemical Abstracts, MedLine, and Protein and Nucleic Acid Databanks. All major journals are available free of charge to all investigators through site licenses to the Vanderbilt University Library System.

X-ray Crystallography: A Bruker Microstar high-brilliance rotating anode X-ray generator is available, equipped with Montel multilayer confocal optics and two Proteum PT-135 CCD area detectors with kappa goniometers. This system is installed on the 5th floor of BIOSCI/MRB3. An Oxford XCalibur sealed tube generator with CCD detector is available at a site about 5 minutes from the laboratory. All detectors include low temperature devices. The facility maintains all software necessary for data reduction, phasing, model refinement and structure analysis. The cost of operations is supported by a flexible system of user fees. Vanderbilt owns a 1/2 share of the Life Sciences Collaborative Access Team (LS-CAT) beamline, which provides our institution with over 40 days of synchrotron experiments per year.

Biophysical: Fluorescence, CD and analytical ultracentrifuge instruments, and isothermal titration and differential scanning calorimeters are all within the shared BIOSCI/MRB3 space. These are operated by the Center for Structural Biology (CSB) on a fee for use basis. High performance and throughput computing are handled by three primary systems. The Advanced Computing Center for Research and Education (ACCRE) maintains a 1200-processor compute cluster with 1.5GB of RAM per processor. The CSB faculty typically

access ~15% of ACCRE. The facility is operated by ACCRE staff and supported through user fees. The Center for Structural Biology (CSB), of which Dr. Fesik is a member, maintains additional high performance computing resources: an SGI Altix system with 32 Intel Itanium2 CPUs and 64GB of RAM, and a 25-node cluster with 624GB of RAM and 200 Intel Nehalem processor cores interconnected with a 40 Gbit Infiniband fabric. These systems are all interconnected to a 20TB high-performance gigabit Ethernet network-attached storage system which facilitates data sharing between all the above computing resources and local workstations. These CSB facilities are operated by the CSB Computing Core, which is responsible for supporting all local computing resources. This group keeps the central computers and network updated, trouble-shoots, coordinates for outside repair and service contracts, does regular data backups and maintains shared software and databases, remote access into the network, color and black-and-white laser printers, and a data management system for short-term and archival file storage. The CSB Computing Core is supported through a system of user fees. Access to a comprehensive suite of continually updated and optimized structural biology software packages is provided through the SBGrid consortium, which charges an annual fee for this service.

Mass Spectrometry Core Facility – The Mass Spectrometry Core Facility directed by Dr. Richard Caprioli, is available to Drs. Marnett and Uddin, both of whom are a major user of the facility. It is located on the 9th floor of Medical Research Building IV, which is a short walk from Dr. Marnett's laboratory. There are 26 mass spectrometers in this facility capable of virtually all contemporary mass spectrometric experiments. These include LC/MS, GC/MS, electrospray, MALDI, etc. Dr. David Hachey oversees use of the facility for small molecule analysis.

Cell Biology Core Facility – Cell culture is available through the Center in Molecular Toxicology's Cell Biology Core Facility. This is a 1,085 square foot facility that is divided into one-half culture facility and one-half wet bench area. There are a total of four Laminar Flow Hoods (one 6-foot and three 4-foot units). Other equipment in this facility include CO2 regulated incubators (four double units and two single units); roller bottle apparatus; refrigerated incubator, Dewar for cryopreservation; two large capacity refrigerated media cabinets; a coulter counter; bioreactor; microtiter plate reader; electroporator; cytosine; low-speed centrifuges; and microscopes.

Proteomics Core Facility - The Proteomics Laboratory of the Mass Spectrometry Research Center provides state-of-the-art instrumentation and expertise in analytical proteomics, proteome profiling, and imaging to Vanderbilt researchers. The laboratory occupies approximately 3,500 sq. ft. of new laboratory space in the Mass Spectrometry Research Center on the 9th floor of Medical Research Building III. Instrumentation is available for protein and peptide separations by 2D-gel electrophoresis and multidimensional high performance liquid chromatography (LC). Proteins and peptides of interest are processed and subjected to mass spectral (MS) analysis, and protein identifications are made based on a comparison of MS data with sequence information from genomic, protein, and EST databases. Proteome profiling and imaging in frozen tissue sections are done by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) MS.

Proteomics Laboratory staff includes the Director, Daniel C. Liebler, Ph.D., three Associate Directors (David Friedman, Ph.D., Amy-Joan Ham, Ph.D. and Lisa Zimmerman, Ph.D.) and four research assistants. Proteomics Laboratory staff provides consultation on experimental design and sample preparation. Typically, digestion, protein and peptide separations, MS analyses and primary data workup are performed by Proteomics Laboratory personnel, although hands-on training in any aspect of proteomics analyses is provided to interested individuals. Both electrospray ionization (ESI) mass spectrometers for LC-MS-MS experiments and MALDI TOF and MALDI TOF-TOF instruments are available for MS analyses.The 2D-gel technology includes fluorescent dyes for multiplexing up to three samples on the same gel for direct quantitative comparisons, and automated workstations for protein excision and sample processing. Multidimensional LC is coupled directly with automated tandem mass spectrometry (MS-MS), followed by automated database searching with Sequent and Mascot software for protein identification. Sequent searches are done by parallel computing on a 50-node system in the Vanderbilt Scientific Computing Resource. Additional data analysis by SALSA enables identification of posttranslational modifications, adducts or sequence variants from MS-MS data. Tissue profiling and imaging by MALDI-TOF MS is done with the aid of specialized software developed at Vanderbilt. Dedicated file servers provide a secure, high-capacity resource for data access and archival.

VANDERBILT INSTITUTE OF IMAGING SCIENCE

Overview. The Vanderbilt University Institute of Imaging Science (VUIIS) is a University-wide interdisciplinary initiative that unites scientists whose interests span the spectrum of imaging research—from the underlying physics of imaging techniques to the application of imaging tools to address problems such as understanding

brain function. The VUIIS has a core program of research related to developing new imaging technology based on advances in physics, engineering, and computer science. In addition to high-field MRI and MR spectroscopy in human subjects, the VUIIS offers state-of-the-art options for small animal imaging in all modalities. Vanderbilt has just completed a four-floor, state-of-the-art facility adjacent to Medical Center North to house the VUIIS. The $28 million project ($21 million for construction) provides a 41,000-square-foot facility to integrate current activities in imaging research and provide research space for 24 faculty members and more than 60 graduate students and postdoctoral fellows in biomedical science, engineering, and physics. The following are housed in this new facility:

The **Center for Small Animal Imaging (CSAI)** is dedicated to research studies of small animals for a variety of applications. The CSAI is staffed with machine operators and animal technologists and is supported by instrumentation engineers and a computer systems manager. Gas ventilation devices, animal warming beds and physiological monitoring (blood gas, heart rate, respiration) are all present and run under the direction of Mr. Jarrod True. The wet laboratories contain fume hoods and allow preparation of animals, phantoms, chemical analysis, and contrast agent synthesis immediately adjacent to imaging facilities. Additionally, a 500 sq ft full time staffed (Mr. Ken Wilkens) fabrication/electronics shop is also adjacent to the wet lab and the animal imaging facility. The proximity of the electronic and fabrication shop allows efficient diagnostics of electronic components and other devices needed for experiments involving small animals.

Animal preparation and monitoring
This laboratory occupies 235 sq. ft. and consists of a fully-equipped operation bench with anesthesia equipment, surgical operating microscopes, temperature control water blankets, respiratory monitoring devices, blood gas analyzer (Bayer Rapidlab 800), Stereotaxic frames and biosafety flow hood for injection and brain perfusion. The laboratory is equipped with chemical resistance benches and safety cabinets for storing restricted chemicals and general supplies for animal work.

Chemistry Laboratories
Synthetic chemistry (460 sq. ft.). This lab is designed for synthesis and characterization of small organic molecules, peptidomimetics and other imaging probes. The lab is designed with modern instruments and acid, base-resistance bench tops. There are 4 Labconco safety hoods equipped with electronic sensors and detectors. In addition to major equipments such as high vacuum pumps, solvent purification system (custom design for 5 different solvents with 4 gallons each), temperature controlled centrifuge, the lab has fully equipped with small equipments such as glass wares, vortex, scales, pH meters, UV lamp, hot oven, dry box, Biofire safe solvent cabinet, vacuum line, Millipore Synergy water purification and contains over 2000 chemical reagents. Analytical chemistry and Molecular Biology (420 sq.ft.) This lab is designed with instruments for analyzing the synthesized probes or for screening biomolecules. The room is equipped with 2 Labconco biosafety hoods suitable for preparation of hazardous chemicals, buffer mixing, slides staining and immunohistochemistry. This laboratory is equipped with powerful HPLC system (Lachrome Elite, Hitachi), UV, spectrofluorometer, lyophilizer, Mettler Toledo balances, Mettler Toledo pH meter, lab-top shaker, Thermoelectron microcentrifuge, refrigerator/freezer sets, several stirrer/hotplates, Amicon stirred cells, ultrafiltration apparatus, Millipore Synergy water purification system with capacity to make 18Ω water

Molecular Biology
Tissue Culture Laboratory
Radiochemistry Laboratory
Hyperpolarization Laboratories

Electronic and machine shops (740 sq.ft.)
These are under the direction of Ken Wilkens, senior engineer. The electronic shop houses digital and analog oscilloscopes, an HP network analyzer and other instrumentation for the measurement of electrical signals. This shop is stocked with a wide array of circuit components for the construction and repair of the instrumentation used throughout VUIIS.

Center for Image Analysis
The CIA was established to develop, support and apply algorithms for the processing and analysis of images produced within the VUIIS. Common functions supported include the analysis of human structural and

functional MR data, the development of algorithms for rigid and non-rigid coregistration between images and between modalities, image segmentation, quantification of imaging data, correction of image distortions, and image restoration.

Computer and Data analysis laboratories (1200 sq.ft)
VUIIS maintains three computer labs dedicated to image analysis. These labs contains Sun and SGI workstations, a Linux cluster, and several PCs and Macs. Matlab, Mathematica, and IDL are available for data analysis and simulations. Specialized image analysis software includes Amira, AMIDE, AsiPro, Brain Voyager, SPM99, Analyze, and pMOD, as well as numerous other programs and utilities developed in house. The VUIIS file server provides approximately 20 terabytes of storage. The CIA is directed by a full time member of the faculty and is staffed with 2 programmers and 3 postdoctoral scientists as well as 3 image analysts.

Other Spaces
Faculty and administrative area
All faculty and administrative offices are located on the fourth floor. Facilities in this area include color laser printers, fax machines, color copy machines, and a faculty and staff lounge. Each faculty member is equipped with printer, computer and table, drawers, book shelves and a whiteboard. This floor also has a small conference room, complete with audio-visual presentation electronics, and a small kitchenette.
Offices
Shared offices are scattered throughout the second and fourth floors to house staff, graduate students, fellows And other trainees. All faculty have individual offices. Computer labs, wet labs, animal imaging facilities, the Electric & fabrication shops and personnel are located within the same building. Close proximity of these Components makes efficient use of space and time and allows a free flow of ideas with each of the Collaborators.

Other key Vanderbilt resources are:

1) **THE VANDERBILT MICROARRAY SHARED RESOURCE (VMSR)**
2) **DNA SEQUENCING SHARED RESOURCE**
3) **CELL IMAGING SHARED RESOURCE (CISR)**
4) **RESOURCES FOR ANIMAL CARE AND USE**
5) **RESOURCES FOR ANIMAL SYSTEMS**
   Transgenic Mouse/ES Cell Shared Resource. The objective of the Transgenic/ES Cell Shared Resource is to assist investigators in the generation, maintenance, and/or storage of genetically modified mice, thereby providing useful models for the study of cancer, diabetes, and neurobiological and other diseases. This core provides nine different services, including pronuclear DNA microinjection, ES cell microinjection into blastocysts, assisted reproduction to maintain transgenic and knockout mouse lines, gene targeting, and embryo cryopreservation. This facility has generated over 1755 transgenic founder mice from 482 different DNA constructs and 2566 chimeric mice from 203 different ES cell clones. The ES Cell Laboratory has assisted in the generation of at least 113 different gene-targeted mice. Other services include rederivation of established lines, assisted reproduction to maintain founder lines that are no longer breeding, embryo and sperm cryopreservation, and Cre and Flpe manipulation of conditional alleles. The facility also works closely with investigators to provide any needed advice and instruction on the screening strategies, maintenance, and analysis of the animals generated. Vanderbilt University researchers also have access to additional mouse models via the **Mouse Models of Human Cancers Consortium (MMHCC).** Dr. Robert J. Coffey is PI of Vanderbilt's MMHCC grant that brings more than twenty researchers at Vanderbilt together with researchers at other consortium member institutions. The overall aim of this NCI-sponsored program is to develop mouse models that parallel the mechanisms by which human cancer develop, progress and respond to therapy or preventive agents. In addition to these institutional sources, investigators at Vanderbilt also have the unique opportunity to obtain genetically altered mouse models of cancer from the recently established **Tennessee Mouse Genome Consortium** (TMGC, UO1 MH51971, PI David Goldowitz). This is a statewide consortium involving Oak Ridge National Laboratories, St. Jude's Children's Research Hospital at Memphis, the University of Tennessee at Knoxville and Memphis, the University of Memphis, Meharry Medical College and Vanderbilt.
6) **RESOURCES FOR COMPUTATION**

VUMC provides computation support to investigators through resources that include campus high-speed Ethernet backbone with connections to Internet and Internet-2. Various additional shared computing resources include a DNA sequence analysis core computer that runs the GCG suite of molecular biology programs and many other applications. Bioinformatics support service exists to provide training and support for mainframebased applications.The **Advanced Computing Center for Research and Education (ACCRE)** was built and is operated by Vanderbilt faculty.

7) **Proteomics core**
8) **Immunohistochemistry shared resource**
9) **Biostatistics shared resource**

EQUIPMENT

List of Equipment:

1) College of Veterinary Medicine, Veterinary Medical Center, University of Tennessee, Knoxville, Tennessee – Clinic

2) Laboratory and office of Dr. Maria Cekanova at the Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Veterinary Medical Center, University of Tennessee, Knoxville, Tennessee

3) Vanderbilt University in Nashville, Department of Biochemistry at the School of Medicine at Tennessee Laboratory and office of Dr. Jashim Uddin are a part of laboratory of Dr. Marnett and Vanderbilt University Institute of Imaging Sciences (VUIIS) by Dr. Charles Manning, Director of Molecular Imaging Research

1) College of Veterinary Medicine, Veterinary Medical Center, University of Tennessee, Knoxville, Tennessee – Clinic

In addition to active clinical support services in clinical pathology, anatomic pathology, pharmacology, bacteriology and mycology, virology, immunology, toxicology, endocrinology, and parasitology, some of our specialized facilities available for extensive case work-up and research application include:

- Color-flow Doppler ultrasonography and echocardiography
- Complete video-endoscopic and digital endoscopic equipment for gastrointestinal, respiratory, urinary and reproductive endoscopy
- Rigid endoscopy for laparoscopy, arthroscopy, videotoscopy
- Electrodiagnostic laboratory with testing capabilities in the areas of: electroencephalography, electromyography, electroneurography, evoked potentials including brainstem auditory evoked potentials, and electroretinography
- Cystometrography and urethral pressure profiles
- Nuclear medicine unit with 2 diagnostic gamma cameras, and Strontium-90 Beta probe as well as iridium implant capabilities and I131 facilities
- On-site CT-scan and MRI
- Endocrinology laboratory
- Clinical toxicology laboratory
- Ho:YAG, Diode, and CO2 laser units
- Force-plate unit for gait analysis
- Kinematic (motion) analysis equipment
- Abbot Nutrimix Multichannel TPN automated compounder
- Physical therapy service with underwater treadmill and wave pool
- SF2 Bioimpedance analyzer
- DEXA-scan for analysis of body composition; metabolic studies
- Intensive care unit with 24-hour nursing staffing
- Complete video-endoscopic and digital endoscopic equipment for gastrointestinal, respiratory, urinary and reproductive endoscopy
- Rigid endoscopy for laparoscopy, arthroscopy, videotoscopy

Endoscopy will be performed using a 1-mm rigid endoscope (Karl Storz Veterinary Endoscopy) attached to a Tricam SLII camera (Karl Storz Veterinary Endoscopy America), and a D-Light AF light source (Karl Storz Veterinary Endoscopy America). Images (still photographs and video) will be captured on an Aida DVD-M (Karl Storz Veterinary Endoscopy America) while projecting endoscopic images on a TV monitor. Captured images and video will be transferred to a computer for quantification of fluorescence and white light surface area.

IVIS LUMINA (Xenogen) imaging system is available for mice whole body imaging at UTK Knoxville, TN

2) Laboratory and office of Dr. Maria Cekanova at the Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Veterinary Medical Center, University of Tennessee, Knoxville, Tennessee

Dr. Cekanova laboratories include: three Nuaire biological safety cabinets class II, chemical fume hood, Queue Cell Culture CO_2 water-jacketed double-chambered automated incubator, Isotemp Fisher Scientific CO_2 water-jacketed double-chambered automated incubator; Fisher phase-contrast microscopes, GE Healthcare Life Sciences MegBACE sequencing system, BioRad C1000 Thermal Cycler, BioRad iCycler MyiQ Single color real-time PCR detection system, MJ Research PTC-200 Peltier Thermal cycler, ThermoSpectronic BioMate 3 UV spectrophotometer, LabLine Maxi-Rotators, Barnstead Nanopure Infinity water system, electrophoretic units, tabletop centrifuges, analytic balances, table shaker, liquid nitrogen cryostorage units, -80°C and -20°C freezers, four refrigerators, several water-baths, Western blotting apparatus, DELL OptiPlex computers. She has Leica Leitz DMRB microscope with attached DP72 camera.

Dr. Cekanova also has free access to different laboratories at UTIA equipped with additional equipment's for conducting her research, such as two PCR machines, cryostat, luminometer, spectrophotometers, microplate readers with luminescence and fluorescence, fluorescence microscopes, micro and macro-centrifuges, and others. Dr. Cekanova also has free access to different facilities at UT to use the fluorescence-activated cell sorter (FACS), Laser Capture Microdissection System, IVIS Lumina imaging system, and to Histology laboratory to process and evaluate tissue samples.

Other available facilities at UT:
Preclinical Molecular Imaging Laboratory of Dr. Jonathan Wall, Graduate School of Medicine, The University of Tennessee in Knoxville. The major equipment:

- MicroCAT II + SPECT scanner, ImTek/Siemens Preclinical, Knoxville, TN
- MicroPET P4 scanner, Concorde/Siemens, Knoxville, TN
- mCT clinical PET/CT, Siemens, Knoxville, TN
- 1480 Wallac Wizard 3 Automatic Gamma counter, Perkin Elmer
- Capintec CRC 15 PET digitally controlled dose calibrator
- Anesthesia apparatus for delivering isoflurane:oxygen anesthesia for small animals

BioVet apparatus for monitoring for small animal respiration rate, ECG, temperature and provided an output trigger for gated PET and CT image acquisition.

3) Vanderbilt University in Nashville, Department of Biochemistry at the School of Medicine at Tennessee Laboratory and office of Dr. Jashim Uddin are a part of laboratory of Dr. Marnett and Vanderbilt University Institute of Imaging Sciences (VUIIS) by Dr. Charles Manning, Director of Molecular Imaging Research

Laboratory of Dr. Marnett contains six fume hoods. Each researcher has a laboratory bench and an adjoining desk. Centrifuges are located in a central room located opposite the laboratory. A 150 square foot cold room is located opposite the laboratory. HPLC equipment includes a Waters 2695 Separations module capable of isocratic and gradient programs. Controlled by Millenium 32 3.20 software. Available detectors are Waters 2487 dual wavelength UV absorbance detector and SEDEX 7S evaporative light scattering detector. Waters 1525 Binary HPLC pump configured with a Waters 717 autosampler and 2996 photodiode array UV detector. This is also capable of automated isocratic or gradient analysis. Spectrophotometers include a Hewlett-Packard 8452A diode array spectrometer and a Shimadzu UV160U spectrometer. Centrifuge area contains a Sorval RC5C and RC80, Beckman Optima Tabletop Ultracentrifuge, Eppendorf Microcentrifuge Model 5415C, and a Savant SS11 basic component SpeedVac. Other items are a Biotage Flash Chromatography System with fraction collector and UV detector; Bioscan System 200 High performance Detector for thin layer chromatography with a Dell computer; various manual DNA sequencing gels; a Perkin-Elmer Cetus DNA Thermal Cycler; Cruachem DNA synthesizer; and an assortment of routine organic and biochemical preparative equipment; gel electrophoresis equipment, shaking water baths, etc.

Others: Small Molecule NMR Facility- The Small Molecule NMR Facility is currently comprised of four Bruker NMR spectrometers operating at 600, 500, 400 and 300 MHz.
The 300, 400, 500, and 600 MHz instruments

Major instrumentation in the Proteomics Laboratory includes: *ETTAN DIGE 2D gel electrophoresis system* (Amersham Biosciences). Includes equipment for first dimension isoelectric focusing (Multiphor II and IPGphor) and second dimension SDS-PAGE (Ettan DALT II) for large format (25 x 20 cm) gels. Protein samples are individually labeled with sensitive (sub picogram) cyanine fluorescent dyes (Cy2, Cy3 or Cy5) that exhibit a linear dynamic range of approximately four orders of magnitude. Additional instrumentation includes Amersham Ettan Spot Picker, Digester and MALDI plate spotter, which permit high throughput, automated analyses of protein features selected from 2D gels.

Applied Biosystems Voyager 4700 MALDI-TOF-TOF system. This new instrument combines a MALDI source with a TOF-TOF tandem mass analyzer to acquire both peptide mass fingerprint data and MS-MS spectra of peptide ions via high energy collisionally-induced dissociation. The instrument is equipped with Explorer 4700 instrument control software and GPS Explorer 2.0 software suite for proteomics data analyses. Unique automation capabilities of the ABI 4700 provide for high-throughput analyses and enable LC-MALDI-MS-MS analyses via off-line collection of LC-fractionated peptide mixtures. This approach complements electrospray LC-MS-MS for analysis of complex protein and peptide mixtures.

Applied Biosystems Voyager-DE STR. This instrument has an upgraded high current detector with greater sensitivity for high mass ions (*m/z* >200,000) relative to other MALDI-TOF instruments. The improved electronics, ion detector, and tilted ion reflector design result in a 4-fold improvement in resolution (*m/z* >20,000 for insulin) and wider dynamic range for detection of trace components in biological matrices. This is the principal instrument for both tissue proteome profiling and tissue protein imaging

Other Equipment – Other facilities available through the Vanderbilt University include a Bioanalytical NMR Facility and a Biomolecular NMR Facility with the following NMR spectrometers (Bruker 200, 300, 400, 500, and 600 MHz for 1H; broad band probes and other probes for 13C, 15N, and other nuclei are available, plus Silicon Graphics workstations), an 800 MHz NMR instrument, CD spectrometers (JASCO 720), facilities for carrying out molecular genetics studies include oligonucleotide synthesis (two Applied Biosystems 291 systems, with preparative cartridges), an Applied Photophysics 05-109 stopped flow/rapid scan spectrometer and a Kintek rapid quench apparatus, two capillary gel electrophoresis apparatuses. The Vanderbilt-Ingram Comprehensive Cancer Center maintains automated fluorescent-based nucleotide sequence analysis (ABI-3700), a Molecular Dynamics phosphorimager and a Packard Instant-Imager. All of these facilities are available for use at nominal cost. Other facilities available in the University include EPR spectrometry, cytofluorography, X-ray diffraction, and a BiaCore surface plasmon resonance instrument, and a high capacity insert cell culture facility for baculovirus expression of large amounts of protein.

VANDERBILT INSTITUTE OF IMAGING SCIENCE
CSAI Equipment. The CSAI facilities offer a wide array of options for MRI/MRS studies, including field strength, bore size in addition to multinuclear and decoupling capabilities. State-of-the-art equipment for ultrasound, optical, x-ray, and nuclear imaging of small animals is also available. The core instrumentation available is as follows:

- 0.5T (2.5cm bore) Maran Benchtop Relaxometer; Proton relaxation, diffusion and imaging of solutions and phantoms.
- 4.7T (31 cm bore) Varian Animal Imaging system: Dual channel, multinuclear capabilities, imaging and spectroscopy (rats, guinea pigs and rabbits)
- 7.0T (16 cm bore) Varian Animal Imaging system: Single channel, imaging and spectroscopy (rat and mouse imaging only)
- 9.4T (21 cm bore) Varian Animal Imaging system: Dual channel, multinuclear capabilities, imaging and spectroscopy (rat and mouse imaging only)
- 4.7T (60 cm bore) vertical Varian Inova system for imaging non-human primates
- An Imtek (now Siemens) MicroCAT II X-ray micro-CT system: microfocal x-ray tube (40W, 80kVp) with a cooled 4096x4096 CCD array (rodent and specimen imaging)

- A Concorde Microsystems (now Siemens) MicroPET Focus 220: positron emission tomography system suitable for small-animal (mice,rats) and primate imaging, including dynamic and quantitative studies;fluoro-deoxyglucose (FDG) is routinely available from the PETNet facility located in VUMC
- A Bioscan NanoSPECT combined SPECT and CT scanner for nuclear imaging of small animals
- A Xenogen IVIS 200 bioluminescent and fluorescent imaging system
- Two CRI Maestro: optical imaging systems designed for in vivo fluorescence imaging that uses spectral un-mixing techniques to reduce autofluorescence background and to resolve multiple fluorescentprobes
- A Visen FMT system for quantitative optical tomography in small animals
- A Li-Cor Pearl imager for small animal optical imaging
- A VisualSonics Vevo 770 high-resolution ultrasound system: B-mode (2D and 3D), M-mode, power Doppler (2D and 3D), and pulsed Doppler imaging capabilities operated at 20-55 MHz
- A Faxitron digital X-ray imaging system
- A Scanco microCT 40 for high resolution 3D CT imaging of bone specimens
- A Vibratome whole body small animal cryomacrotome equipped with optical imaging recording of animal sections

Molecular Biology

The molecular biology and connected laboratories are well equipped with modern instruments for work including isolation, purification and determination of DNA, cloning, protein purification. -Major equipment include a Sorvall ST 40R refrigerated centrifuge with multiple rotors and swinging-bucket adapters, a Sorvall RC-5B refrigerated ultracentrifuge with multiple fixed rotors, a IEC Centra MP4 bench-top centrifuge, an Eppendorf 5415R refrigerated microcentrifuge, a Skil 9", 2 speed bench-top bandsaw, two Brandel M-24TI harvester systems, a Brandel M-48TI harvester system, a BioTek Synergy 4 multi-channel microplate reader equipped with MicroFlo-Select automated dispenser system, two BioRad C1000 thermal cycler (PCR) equipped with CFX-96 real-time fluorescence detector system, a Reichert-Jung Cryocut 1800 cryomicrotome, a Vibratome 8800 whole animal cryomicrotome, a Zeiss Axio Observer A.1 inverted fluorescence microscope equipped with multiple wet and dry objectives, a CRI Nuance GNIR-Flex multispectral fluorescence camera, a Fisher Isotemp Plus multi-door, powered refrigerator, two Fisher Isotemp Plus -80 freezers, a Brinkmann Polytron PT 3000 tissue homogenizer, a Millipore Direct-Q 5 water purification system, a Retriever 2100 high-temperature/pressure antigen retrieval system and numerous electrophoresis set ups for immunoblotting and other ancillary small equipment.

Tissue Culture Laboratory

The tissue culture facility occupies 140 sq. ft. on the second floor of the VUIIS building. Access is tightly controlled to prevent unnecessary contamination.

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

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Maria Middle Name Last Name*: Cekanova Suffix:

Position/Title*:	Research Assistant Professor
Organization Name*:	The University of Tennessee
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County:	Knox
State*:	TN: Tennessee
Province:	
Country*:	USA: UNITED STATES
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Phone Number*: 865-389-5222 Fax Number: 865-974-5554 E-Mail*: mcekanov@utk.edu

Credential, e.g., agency login: eRA Commons User Name(s)

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

Degree Type: MS, RNDr, PhD Degree Year: 1999, 2002, 2005

	File Name
Attach Biographical Sketch*:	1245-Biosketch_Cekanova.pdf
Attach Current & Pending Support:	

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Joseph Middle Name W. Last Name*: Bartges Suffix:

Position/Title*:	Professor
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Division:	
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County:	Knox
State*:	TN: Tennessee
Province:	
Country*:	USA: UNITED STATES
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Credential, e.g., agency login: eRA Commons User Name(s)

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: DVM, PhD Degree Year: 1987, 1993

	File Name
Attach Biographical Sketch*:	1246-Biosketch_Bartges.pdf
Attach Current & Pending Support:	

Funding Opportunity Number: PA-13-313 . Received Date: 2013-11-01T16:37:09.000-04:00

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Jashmin Middle Name Last Name*: Uddin Suffix:

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Division:
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Street2:
City*: Nashville
County:
State*: TN: Tennessee
Province:
Country*: USA: UNITED STATES
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Phone Number*: 615-343-7326 Fax Number: E-Mail*: jashmin.uddin@vanderbilt.edu

Credential, e.g., agency login: eRA Commons User Name(s)

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: Ph.D. Degree Year: 2001

File Name

Attach Biographical Sketch*: 1247-Biosketch_Uddin.pdf

Attach Current & Pending Support:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Henry Middle Name Charles Last Name*: Manning Suffix:

Position/Title*: Associate Professor
Organization Name*: Vanderbilt University Medical School
Department: Institute of Imaging Sciences
Division:
Street1*: 1161 21st Avenue South
Street2: MCN AA-1105
City*: Nashville
County: Davidson
State*: TN: Tennessee
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 37232-2310

Phone Number*: 615-322-3793 Fax Number: E-Mail*: henry.c.manning@vanderbilt.edu

Credential, e.g., agency login: eRA Commons User Name(s)

Project Role*: Consultant Other Project Role Category:

Degree Type: PhD Degree Year: 2004

File Name

Attach Biographical Sketch*: 1248-Biosketch_Manning.pdf

Attach Current & Pending Support:

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Maria Cekanova	**Research Assistant Professor**
eRA COMMONS USER NAME (credential, e.g., agency login) eRA Commons User Name(s)	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Pavol Jozef Safarik in Kosice, Slovakia	M.S.	06/1999	Biology and Chemistry
University of Pavol Jozef Safarik in Kosice, Slovakia	RNDr.	08/2002	Molecular Biology
University of Pavol Jozef Safarik in Kosice, Slovakia and The University of Tennessee, Knoxville, TN	Ph.D.	01/2005	Genetics
The University of Tennessee, Knoxville, TN	Post-Doctoral training	01/2005-04/2009	Molecular and Cellular Biology

A. Personal statement: My role as Principal Investigator for this grant proposal is based on my research experiences in detection and characterization of cancer at molecular/cellular levels for over 12 years. I have accomplished my PhD thesis and my post-doctoral training in the cancer research field of investigating the role of interactions between connective tissue and adenocarcinoma cells at molecular and cellular levels. In addition, I was involved in PET imaging using novel radiolabeled NSAIDs tracer in carcinogen-induced pancreatic and lung tumors in hamsters. In the summer of 2010, I was selected by the Cancer Imaging program at NCI in partnership with the AACR to participate in the Cancer Imaging program at Washington University, St. Louis, Missouri. Since I have joint Department of Small Animal Clinical Sciences, I have focused on the translational research to evaluate novel optical and PET imaging agents for detection and treatment of canine and feline naturally-occurring adenocarcinomas as model for the human cancers. I successfully administrated the projects, collaborated with other researchers, and produced several peer-reviewed publications from each project. As a result of these previous experiences, I am aware of the importance of frequent communication among project members, creating a realistic research plan, timeline, and budget. The current application logically builds on my prior work and I have chosen co-investigator, Dr. Bartges a certified veterinary internal medicine specialist who will assist with cystoscopy and Dr. Uddin (from Dr. Marnett's laboratory at Vanderbilt University) who will synthesize fluorocoxib A for optical imaging and Dr. Charles Manning who will assist as consultant with the imaging experiments and analyzing data for this project. This highly enthusiastic team involving multidisciplinary expertise in tracer chemistry development, veterinary care oncology, optical imaging, and basic molecular biology have the knowledge to achieve the goals of this proposed study in order to improve detection of bladder cancer. In summary, I have the expertise, leadership and motivation necessary to successfully carry out the proposed work. This project will make appropriate scientific environment to attract high-quality student through our graduate program to successfully accomplished proposed research. I have already experiences and available facilities to mentor additional graduate/undergraduate student.

B. Positions and Honors:

1999-2005 Research and Teaching Assistant, Faculty of Science, The Institute of Biology and Ecology, UPJS in Kosice, Slovakia
2000-2003 Senior Research Assistant, Dept of OB/GYN, Graduate School of Medicine, UT, Knoxville
2003-2005 Research Assistant, Dept of Pathobiology, College of Vet. Med., UT, Knoxville
2005-2006 Post-Doctoral Research Associate, Dept of Pathobiology, CVM, UT, Knoxville

2006-2009 Research Associate I, Dept of Pathobiology, CVM, UT, Knoxville
2009-present Research Assistant Professor, Department of Small Animal Sciences, College of Veterinary Medicine, The University of Tennessee, Knoxville
2010-present member of College Research Committee at UTCVM, Knoxville

1999 Award for excellent study results, Dean of Faculty of Science, UPJS in Kosice, Slovakia
2008 Award from AACR Pathobiology of Cancer: The Edward A. Smuckler Memorial Workshop, Snowmass Village, Colorado
2010 Award from NCI/AACR Cancer Research Imaging Camp, St. Louis, Missouri to attend this workshop
2012 Award of Excellence, University of Tennessee Research Symposium of Comparative and Experimental Medicine in Knoxville, Tennessee for the first place in category of Research Assistant Professor

Professional Memberships:
2006 - present Associate member of American Society for Cell Biology
2006 - present Associate member of American Association for Cancer Research
2010 - present Honorary member of the Society of Phi Zeta
2012 - present Professional member of Veterinary Cancer Society

C. Selected Peer-reviewed Publications (Selected from 25 peer-reviewed publications)
Most relevant to proposed research in area of molecular cancer and imaging:

1. Cavarga, I., Brezani, P., **Cekanova-Figurova, M**., Solar, P., Fedorocko, P. & Miskovsky, P. (2001). Photodynamic therapy of murine fibrosarcoma with topical and systemic administration of hypericin. Phytomedicine, 8(5), 325-330.
2. Solar, P., Cavarga, I., Hofmanova, J., **Cekanova-Figurova, M**., Miskovsky, P., Brezani, P., Hrckova, G., Kozubik, A. & Fedorocko, P. (2002). Effect of acetazolamide on hypericin photocytotoxicity. Planta Medica, 68(7), 658-660.
3. Schuller, H.M. & **Cekanova, M.** (2005). NNK-induced hamster lung adenocarcinoma over-express β2-adrenergic and EGFR signaling pathways. Lung Cancer, 49(1), 35-45.
4. Schuller, H.M., Kabalka, G., Smith, G., Mereddy, A., Akula, M. & **Cekanova, M. (2006).** Detection of overexpressed COX-2 in precancerous lesions of hamster pancreas and lungs by molecular imaging: implications for early diagnosis and prevention. ChemMedChem, 1(6), 603-610.
5. **Cekanova, M.,** Majidi, M., Masi, T., Al-Wadei, H.A.N. & Schuller, H.M. (2007). Over-expressed Raf-1 and p-CREB are early markers for lung adenocarcinoma. Cancer, 109(6), 1164-1173.
6. **Cekanova, M**., Masi, J.T., Plummer, III H.K., Majidi, M., Fedorocko, P. & Schuller, H.M. (2006). Pulmonary fibroblasts stimulate the proliferation of cell lines from human lung adenocarcinomas. Anti-Cancer Drugs, 17(7), 771-781.
7. Laag, E., Majidi, M., **Cekanova, M.,** Masi, T., Takahashi, T. & Schuller, H.M. (2006). NNK activates ERK1/2 and CREB/ATF-1 via beta-1-AR and EGFR signaling in human lung adenocarcinoma and small airway epithelial cells. International Journal of Cancer, 119(7), 1547-1552
8. Fernando R, Foster J, Bible A, Strom A, Pestell R, Rao M, Saxton A, Baek SJ, Yamaguchi K, Donnell R, **Cekanova M**, and Wimalasena J: "Breast cancer cells proliferation is inhibited by BAD: regulation of cyclin D1" J. Biol. Chem., 2007; 282(39):28864-73
9. **Cekanova, M.,** Yuan, J.S., Li, X., Kim, K. and Baek, S.J. (2008). Gene alterations by peroxisome proliferator-activated receptor γ agonists in human colorectal cancer cells. International Journal of Oncology, 32(4), 809-819. PMCID: PMC2504864
10. **Cekanova, M.,** Lee, S.H., Donnell, R.L., Sukhthankar, M., Eling, T.E., Fischer, S.M. & Baek, S.J. (2009). Non-steroidal anti-inflammatory drug activated gene-1 (NAG-1) expression inhibits urethane-induced pulmonary tumorigenesis in transgenic mice. Cancer Prevention Research, 2(5), 450-458. PMCID: PMC2697576
11. **Cekanova M**, Lee SH, McEntee MF, and Baek SJ: "MCC-555-induced NAG-1 expression is mediated in part by KLF4." Eur J Pharmacol. 2010 Jul 10; 637(1-3):30-7. Epub 2010 Apr 10
12. **Cekanova M,** Uddin JM, Legendre A, Galyon G, Bartges J, Callens A, and Marnett L: "Preclinical single-dose safety and pharmacokinetic evaluation of fluorocoxib A, a novel COX-2-targeted optical imaging agent" J Biomed Opt., 2012, November 1, 17(11),):116002. doi: 10.1117/1.JBO.17.11.116002
13. Anderson CJ, Piwnica-Worms D. AACR/SNMMI State-of-the-Art Molecular Imaging in Cancer Biology and Therapy: Abstracts. Abstract #5 **Cekanova M**, Bilheux HZ, RathoreK, Bilheux JC, Walker L, Donnell R, and

Legendre AM:"Neutron Radiography with combine computed tomography: a novel tool for cancer diagnosis and imaging." J Nuclear Med., 2013, Feb 1;54(Supplement 1), doi: 10.2967/jnm542abs. *This publication involved students under my supervision*

14. Siriwardhana N, Kalupahana NS, **Cekanova M**, LeMieux M, Greer B, Moustaid-Moussa N. Modulation of adipose tissue inflammation by bioactive food compounds. J Nutr Biochem. 2013 Apr;24(4):613-23. doi: 10.1016/j.jnutbio.2012.12.013. Review. PMID: 23498665
15. **Cekanova M**, Uddin MJ, Bartges JW, Rathore K, Callens A, Legendre AM, Wright L, Carters A, and Marnett LJ: "COX-2 expressing transitional cell carcinomas detected by fluorocoxib A: NSAIDs as novel class of imaging agent." Cancer Prev Res (Phila). 2013 May;6(5):466-76. doi: 10.1158/1940-6207.CAPR-12-0358. Epub 2013 Mar 26. PMID: 23531445. *This publication involved students under my supervision*

C. Research Support:

Active:

Private Support

1. [redacted] (PI: Bartges)
   Role: Co-Investigator 04/01/2006 – 03/31/2016
   Title: "Research in oncology and metabolism in dogs and cats"
2. Private Support
   Role: PI 06/01/2013 – 05/31/2014
   Title: *"Monitoring the responses of carcinomas to radiation therapy using COX-2-targeted imaging probes"*
3. Private Support
   Role: Co-PI 06/01/2009 – 05/31/2014
   Title: *"Isolation and characterization of the Mesenchymal Stem Cells in Horses and Dogs"*
4. Private Support
   Role: PI 8/31/2012 – 01/31/2014
   Title: *"Evaluation of neutron radiography and computed tomography for detection of breast cancer"*

There is no overlap with scope of proposed R15 grant application

Completed Grants:

1. Private Support 4 completed studies
2. Private Support
   Role: PI 04/01/2010-12/31/2012
   Title: "*New staging techniques and evaluation of therapies for oral SCCs in cats."*
3. Private Support
   Role: Co-Investigator 10/01/2010 – 09/30/2012
   Title: "Neutron imaging for the determination of tumor margins"
4. Private Support
   Role: Co-PI 09/01/2010 - 12/31/2012
   Title: *"Safety and efficacy of a novel anthracycline, AD198 in dogs with refractory lymphoma."*
5. Private Support
   Role: Principal Investigator 09/01/2010 - 12/31/2012
   Title: "Role of estrogen receptor beta (ERβ) in breast cancer"
6. Private Support
   Role: Co-Investigator October 2010-2012
   Title: "Investigation of a novel approach to forensic analysis using neutron imaging techniques." The goal of this study is to investigate the novel approach to determine the time of death using neutron imaging as possible new technique for forensic analysis.
7. Vanderbilt In Vivo Cellular and Molecular Imaging Center (VICMIC) Vanderbilt University/NIH
   Role: UT-Principal Investigator 10/30/2011-9/30/2013
   Title: *"The synthesis and evaluation of Fluorocoxib A in domestic animals with naturally-occurring tumors"*

D. Undergraduate, graduate students, residents, interns and post-doctoral associates trained by Dr. Cekanova:

1. **Olya Smrkovski, DVM,** Residency in Oncology (2008-2010), Diplomate ACVIM Oncology 2011 (partial contribution to training)
2. **Rebecca Ward, DVM,** Residency in Oncology (2009-2011), Diplomate ACVIM Oncology 2011 (partial contribution to training)
3. **Shannon Flood, DVM, ACVIM,** Residency in Oncology (2010-2011), research co-advising with the project
4. **Meredith Westling, DVM,** Research Intern in Orthopedics (Stem cells aspect of the study), (2011-2012) research co-advising with the project
5. **Kristina Andrews**, the 1st year Vet Student; COE Summer Student Research Program; research training; The UT, June 2010- August 2010; 40h/week; 10 weeks, total of 400h
6. **Kim Lucy**, the 1st year Vet Student; COE Summer Student Research Program; research training; The UT, June 2010- August 2010; 40h/week; 10 weeks, total of 400h
7. **Sherri Townley,** the 1st year Vet Student; COE Summer Student Research Program; research training; The UT, June 2011- August 2011; 40h/week; 10 weeks, total of 400h
8. **Casey Grier Smith**, undergraduate student BCMB, UT; Fall 2010; 2 credits, 8h/week; 15 weeks
9. **Ann Carr,** undergraduate students Biomedical Engineering, UT; Summer 2011; 16h/week
10. **Kimberley Sanders**, undergraduate students BCMB, UT, Fall 2011; 2 credit, 8h/week; 15weeks
11. **Mandy Carter**, undergraduate students BCMB, UT, Fall 2011 and Spring 2012; 2 credit, 8h/week; 15weeks – 2012 successfully accepted for Medical School
12. **Laura Wright**, undergraduate students BCMB, UT, Fall 2011 and Spring 2012; 3 credit, 12h/week; 15 weeks
13. **Valeria Tanco, DVM, MSc, DACT,** I served as major advisor for PhD study, September 2011-November 2012
14. **Kusum Rathore, PhD**, I serve as major advisor for post-doctoral research associate, 2012-present
15. **Mary Mc-Alexander,** undergraduate 3rd year student of Maryville College, Summer 2013, 40h/10 weeks

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Bartges, Joseph William	Professor of Medicine and Nutrition
eRA COMMONS USER NAME	The Acree Endowed Chair of Small Animal
eRA Commons User Name(s)	Research

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Marshall University	B.S.	May 1983	Zoology
University of Georgia	D.V.M.	June 1987	Veterinary Medicine
University of Minnesota	Internship	1987-1988	Small Animal Medicine and Surgery
University of Minnesota	Residency	1988-1993	Veterinary Internal Medicine
University of Minnesota	Residency	1988-1993	Veterinary Nutrition
University of Minnesota	Ph.D.	1988-1993	Veterinary Medicine

A. Personal Statement

The goal of the proposed research is to evaluate a newly synthesized optical imaging tracer, fluorocoxib A, for detection of urinary bladder tumors expressing cyclooxygenase-2 (COX-2) enzyme in mice and canine cancer models. I am a board-certified veterinary internal medicine specialist and my role will be a Co-Investigator. I have a brad backround in veterinary internal medicine and nutrition, with specific expertise in urinary animal dissorders. I will perform cystoscopic optical imaging and biopsy acquisition of bladder cancers with standard veterinary care. I have successfully aministered the projects, collaborated with other researchers, and produced several peer-reviewed publications from each project. The current aplication builds logically on my prior work. In summary, I have demostrated record of succesfull and productive research projects in an area of the urrinary diseases in dogs, and my the expertise and experience have prepared me to be a part of this proposed study.

B. Positions and Honors

Positions and Employment

1994 - 1997	Assistant professor, Departments of Small Animal Medicine, and Physiology and Pharmacology, Small Animal Clinical Nutritionist, Veterinary Teaching Hospital, College of Veterinary Medicine, University of Georgia.
1997 - 2002	Associate Professor, Department of Small Animal Clinical Sciences, Internist and Clinical Nutritionist, Veterinary Teaching Hospital, College of Veterinary Medicine, The University of Tennessee.
2002 - present	Professor, Department of Small Animal Clinical Sciences, Internist and Clinical Nutritionist, Veterinary Teaching Hospital, College of Veterinary Medicine, The University of Tennessee.
2002 - present	Acree Endowed Chair of Small Animal Research, Department of Small Animal Clinical Sciences, Internist and Clinical Nutritionist, Veterinary Teaching Hospital, College of Veterinary Medicine, The University of Tennessee.

Other Experience and Professional Memberships

1995 - present	Diplomate, American College of Veterinary Internal Medicine (Small Animal)
1995 - present	Diplomate, American College of Veterinary Nutrition

Honors (40)

2007 The University of Tennessee National Alumni Association Excellence in Teaching Award
2008 Pfizer Carl J. Norden Distinguished Teacher Award, College of Veterinary Medicine, The University of Tennessee
2009 Coughlin Distinguished Professor, The University of Tennessee, College of Veterinary Medicine Annual Conference
2009 B. Ray Thompson, Sr. Outstanding Faculty Performance Award, The University of Tennessee, Institute of Agriculture
2010 Bark Magazine. Top 100 Best and Brightest in Canine Medicine

C. Selected Peer-reviewed Publications (Selected from 101 peer-reviewed publications)

1. Hezel, A., **Bartges, J.W.,** Kirk, C.A., Cox, S., Geyer, N., Moyers, T. & Hayes, J. (2007). Influence of hydrochlorothiazide on urinary calcium oxalate relative supersaturation in healthy, young, adult, female, domestic short-haired cats. Vet Therapeutics, 8(4), 247-254.
2. Geyer, N., **Bartges, J.W.,** Kirk, C.A., Cox, S., Hezel, A., Moyers, T. & Hayes, J. (2007). Influence of prednisolone on urinary calcium oxalate and struvite relative supersaturation in healthy, young, adult, female, domestic short-haired cats. Vet Therapeutics, 8(4), 239-246.
3. **Bartges, J.W.** (2008). Bad bugs and bladder drugs: treating UTI's in dogs and cats. Compendium on Continuing Education for the Practicing Veterinarian, 30(3B), 4-10.
4. Lusby, A.L., Kirk, C.A. & **Bartges, J.W.** (2009). The role of key adipokines in obesity and insulin resistance in cats. Journal of the American Veterinary Medical Association, 235(5), 518-522.
5. Stenske, K.A., Bemis, D.A., Gillespie, B.E., DiSouza, D.H., Oliver, S.P., Draughon, F.A., Matteson, K.J. & **Bartges, J.W.** (2009). Comparison of clonal relatedness and antimicrobial susceptibility of fecal Escherichia coli from healthy dogs and their owners. American Journal of Veterinary Research, 70(9), 1108-1116.
6. Stenske, K.A., **Bartges, J.W.,** Draughon, F.A. & Bemis, D.A. (2009). Prevalence of urovirulence genes cnf, hlyD, sfa/foc, and papGIII in fecal Escherichia coli from healthy dogs and their owners. American Journal of Veterinary Research, 70(11),1401-1406.
7. Baldwin, K., **Bartges, J.,** Buffington, T., Freeman, L.M., Grabow, M., Legred, J. & Oswald, Jr. D. (2010). AAHA nutritional assessment guidelines for dogs and cats. Journal of the American Animal Hospital Association, 46(4), 285-296.
8. Roshwalb, S., Gorman, S., Hurst, S., **Bartges, J.W.,** Agarwal, S., Sommardahl, C. & Dhar, M. mRNA expression of canine ATP10C, a P4-type ATPase, is positively associated with body condition score. American Journal of Veterinary Research, Epub 5 Oct 2011. PMID: 20932785
9. Murphy, M., Lusby, A.L., **Bartges, J.W.** & Kirk, C.A. (2011). Size of food bowl and scoop affects amount of food owners feed their dogs. Journal of Animal Physiology and Animal Nutrition, Epub 19 Apr 2011. PMID: 21501249.
10. **Bartges, J.W.** (2011). Chronic renal failure-up to date. Journal of Veterinary Medicine, 64, 743-747.
11. **Bartges JW,** Boynton, B, Vogt AH, Krauter E, Lambrecht K, Svec R, Thompson S. AAHA canine life stage guidelines. *J Am Anim Hosp Assoc* 2012;48(1):1-11
12. Stafford JR, **Bartges JW.** A clinical review of pathophysiology, diagnosis, and treatment of uroabdomen in the dog and cat. *J Vet Emerg Crit Care.* 2013 Mar-Apr;23(2):216-29.Epub 2013 Mar 7.
13. Cekanova M, Uddin MJ, Legendre AM, Galyon G, **Bartges JW,** Callens A, Martin-Jimenez T, Marnett LJ. (2012). Single-dose safety and pharmacokinetic evaluation of fluorocoxib A: pilot study of novel cyclooxygenase-2-targeted optical imaging agent in a canine model. *J Biomed Opt.* Nov 1;17(11):116002.
14. Cekanova M, Uddin MJ, **Bartges JW,** Callens A, Rathore K, Legendre AM, Wright L, Carters A, and Marnett LJ: "Molecular imaging of COX-2 in K9TCC in vitro and in vivo." Cancer Prev Res (Phila). 2013 May; 6(5):466-76 [Epub 2013 March 26] diu,: 10.1158/1940-6207. CAPR-12-0358. PMID: 23531445
15. **Bartges JW,** Kirk CA, Cox S, Moyers T. Influence of Alkalinization and Acidification on Urine Saturation with Calcium Oxalate and Struvite and Bone Mineral Density in Healthy Cats. *Am J Vet Res* 2013 Oct;74(10):1347-52. doi: 10.2460/ajvr.74.10.1347

Additional Publications:

Research abstracts: 83 total; 51 primary or mentoring author and 32 other author

Review articles: 44 total; 29 primary or mentoring author and 15 other author
Textbook chapters: 83 total; 78 primary or mentoring author and 5 other author
Editor: 6 total; 4 primary and 2 other editor; including 1 textbook (released January 2011)

D. Research Support (98 grants total)

Ongoing Research Support

1) Bartges JW, Callens A. Evaluation of Polydimethylsiloxane urethral bulking agent for dogs with urinary incontinence. Private Support 2010. *Principle investigator*
2) Bartges JW. Funding for summer veterinary student. Private Support Private Support 2011 *Principle investigator*
3) Bartges JW, Murphy M. Evaluation of a nutraceutical for weight loss in dogs. 2011. *Co-investigator, wrote proposal for graduate student project.*
4) Olin S, Bartges JW. Evaluation of a point-of-care urine culture test. Private Support Private Support 2011. *Co-investigator, co-wrote proposal*
5) Cekanova M, Uddin J, Bartges JW. Fluorocoxib: An evaluation in domestic animals. NIH/Vanderbilt University. 2012. *Co-investigator.*
6) Cekanova M, Uddin MJ, Bartges JW, Legendre AM, Callens A. Evaluation of a novel optical imaging tracer Fluorocoxib in canine cancer. Private Support Private Support 2012. *Co-investigator, co-wrote proposal, perform clinical evaluations.*
7) Bartges JW. Evaluation of a diet for managing uroliths in dogs. Private Support 2012. *Principle investigator*
8) Cline ME, Witzel A, Bartges JW, Kirk CA. Evaluation of a circulating water bowl on water intake, urine volume, and urinary saturation in cats. Private Support 2012. *Assisted with grant preparation and statistics*

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Md. Jashim Uddin	Research Assistant Professor
eRA COMMONS USER NAME (credential, e.g., agency login) eRA Commons User Name(s)	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Dhaka University, Bangladesh	B.S.	1991	Chemistry
Dhaka University, Bangladesh	M.S.	1993	Organic Chemistry
Shinshu University, Japan	Ph.D.	2001	Organic Chemistry
University of Alberta, Edmonton Canada	Postdoc	2001-2004	MedChem Drug Design
Vanderbilt University, Nashville TN	Postdoc	2004-2005	Medchem Drug Design

A. Personal Statement

I am a Research Assistant Professor of Biochemistry at Vanderbilt University School of Medicine. I was invited to Vanderbilt because of my training with Dr. Yamamoto and Dr. Knaus that gave me hand-on-skills and outstanding knowledge in Organic and Medicinal Chemistry Drug Discovery. I acquired the expertise and understanding on the development of new synthetic strategies to control complicated organic transformations and new drug design concepts. In the last 10 years, I have made significant contributions to the field of drug design and discovery and developed quite a few new drug design concepts for cyclooxygenase-2 (COX-2) inhibition and invented the next generation COX-2 inhibitors that are useful for molecular imaging of inflammation and cancer. My training with Dr. Marnett gave me an extensive knowledge to develop Fluorocoxib A and Fluorocoxib B, the two of hundreds of fluorescently labeled or radiolabeled cyclooxygenase-2 (COX-2) inhibitors, which is being used for imaging of tissues that contain high levels of COX-2, like inflammation, esophageal cancer or skin cancer, colon cancer in pre-clinical settings. These compounds are being commercialized by U.S. Biotech Companies. In addition, I have published numerous scientific publications in the world's leading organic, bioorganic, medicinal chemistry and cancer research journals.

B. Positions and Honors

06/94-07/96 Production Officer (Chemist), Basic Chemicals Plant, Beximco Pharmaceuticals Ltd., Bangladesh
08/96-06/97 Lecturer, Department of chemistry, Cox's Bazar College, Bangladesh National University, Bangladesh
07/97-09/97 Research Chemist, ciba Specialty chemicals Group, Research and Development laboratory, Beximco Pharmaceuticals ltd., Bangladesh
10/97-03/98 Research Scholar, Yamamoto Research lab, Department of Functional Polymer Sciences Graduate School of Science and Technology, Shinshu University, Japan
2006-Present Research Assistant Professor, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville TN

Honors

10/97-03/01 Japanese Ministry of Education, Sports and Culture Scholarship, Japan
01/02-12/04 The Alberta Heritage Foundation for Medical Research Postdoctoral Fellowship Award

C. Selected Peer-Reviewed Publications or Manuscripts In Press

1. **M.J. Uddin**, P.N.P. Rao, and E.E. Knaus. "Design and Synthesis of Acyclic Triaryl (Z)-Olefins: A Novel Class of Cyclooxygenase-2 (COX-2) Inhibitors." *Bioorg. Med. Chem.* **12**:5929 (2004).
2. **M.J. Uddin,** P.N.P. Rao, and E.E. Knaus. "Design and synthesis of (Z)-1,2-Diphenyl-1-(4-Methanesulfonamidophenyl)-2-alk-1-enes and (Z)-1-(4-Azidophenyl)-1,2-Diphenyl-2-alk-enes: Novel Inhibitors of Cyclooxygenase-2 (COX-2) with Antiinflammatory and Analgesic Activity." *Bioorg. Med. Chem.* **15**:417 (2005).

3. **M.J. Uddin**, P.N.P. Rao, R. McDonald, and E.E. Knaus. "Design and Synthesis of (E)-1,1,2-Triarylethenes: Novel Inhibitors of the Cyclooxygenase-2 (COX-2) Isozyme." *Bioorg. Med. Chem. Letts.* ***15****:439 (2005).*
4. P.B. Anning, B. Coles, J. Morton, H. Wang, **J. Uddin**, J.D. Morrow, S.K. Dey, **L.J. Marnett**, and V.B. O'Donnell. "Nitric Oxide Deficiency Promotes Vascular Side Effects of Cyclooxygenase Inhibitors." *Blood* **108**:4059-4062 (2006) [PMCID: PMC1895442].
5. **M.J. Uddin** and L.J. Marnett. "Synthesis of 5- and 6-Carboxy-X-Rhodamines." *Org. Lett.* **10**:4799-4801 (2008) [PMCID: PMC2646678].
6. M.E. Konkle, t.Y. Hargrove, Y.Y. Kleshchenko, J.P. von Kries, W. Ridenour, **M.J. Uddin**, R.M. Caprioli, L.J. Marnett, W.D. Nes, F. Villalta, M.R. Waterman, and G.I. Lepesheva. "Indomethacin Amides as a Novel Molecular Scaffold for Targeting Trypanosoma cruzi Sterol 14α-Demethylase." *J. Med. Chem.* **52**:2846-2853 (2009) [PMCID: PMC2744100].
7. **M.J. Uddin**, D.C. Smithson, K.M. Brown, B.C. Crews, M. Connelly, F. Zhu, L.J. Marnett, and R.K. Guy. "Podophyllotoxin analogues Active versus Trypanosoma brucei." *Bioorg. Med. Chem. Lett.* **20**:1787-1791 (2010) [PMCID: PMC2826502].
8. **M.J. Uddin**, B.C. Crews, A.L. Blobaum, P.J. Kingsley, D.L. Gorden, J.O. McIntyre, L.M. Matrisian, K. Subbaramaiah, A.J. Dannenberg, D.W. Piston, and **L.J.** Marnett. "Selective Visualization of Cyclooxygenase-2 in Inflammation and Cancer by Targeted Fluorescent Imaging Agents." *Cancer Res.* **70**:3618-3627 (2010) [PMCID: PMC2864539].
9. **M.J. Uddin**, M.I. Schulte, **L.** Maddukuri, J. Harp, and L.J. Marnett. "Semisynthesis of 6-Chloropurine-2'-Deoxyriboside 5'-Dimethoxytrityl 3'-(2-Cyanoethyl-N,N-Diisopropylamino) Phosphoramidite and Its Use in the Synthesis of Fluorescently Labeled Oligonucleotides." *Nucleosides Nucleotides Nucleic Acids* **29**:831-840 (2010) [PMCID: PMC3019237].
10. **M.J. Uddin**, B.C. Crews, K. Ghebreselasie, M.N. Tantawy, and **L.J.** Marnett. [I]-Celecoxib Analogues as SPECT Tracers of Cyclooxygenase-2 in Inflammation." *ACS Med. Chem. Lett.* **2**:160-164 (2011) [PMCID: PMC3037034].
11. **M.J. Uddin**, B.C. Crews, K. Ghebreselasie, I. Huda, P.U. Kingsley, M.S. Ansari, M.N. Tantawy, J. Reese, and **L.J.** Marnett. "Fluorinated COX-2 Inhibitors as Agents in PET Imaging of Inflammation and Cancer." *Cancer Prev. Res.* **4**:1536-1545 (2011) [PMCID: PMC3214660].
12. M. Cekanova, **M.J. Uddin,** A.M. Legendre, G. Galyon, J.W. Bartges, A. Callens, T. Martin-Jimenez, and L.J. Marnett. "Single-Dose Safety and Pharmacokinetic Evaluation of Fluorocoxib A: Pilot Study of Novel Cyclooxygenase-2-Targeted Optical Imaging Agent in a Canine Model." *J. Biomed. Opt.* **17**:116002 (2012).
13. **M.J. Uddin,** B.C. Crews, K. Ghebreselasie, and **L.J.** Marnett. "Design, Synthesis, and Structure-Activity Relationship Studies of Fluorescent Inhibitors of Cyclooxygenase-2 as Targeted Optical Imaging Agents." *Bioconjug. Chem.* **24**:712-723 (2013) [PMCID: PMC3630741].
14. M. Cekanova, **M.J. Uddin**, J.W. Bartges, A. Callens, A.M. Legendre, K. Rathore, L. Wright, A. Carter, and L.J. Marnett. "Molecular Imaging of Cyclooxygenase-2 in Canine Transitional Cell Carcinomas In Vitro and In Vivo." *Cancer Pres. Res.* **6**:466-476 (2013) [PMCID: PMC3671760].
15. A.L. Blobaum, **M.J. Uddin,** A.S. Felts, B.C. Crews, C.A. Rouzer, and **L.J.** Marnett. "The 2-'Trifluoromethyl Analogue of Indomethacin is a Potent and Selective COX-2 Inhibitor." *ACS Med. Chem. Lett.* **4**:486-490 (2013) [PMCID: PMC3654564].

D. Research Support

ACTIVE

R37 CA087819 (L.J. Marnett, PI) 6/01/05 - 5/31/15
NCI/NIH
"Chemistry and Biology of Malondialdehyde-DNA Adducts"
The specific aims of this grant are (1) Determine the structural basis for frameshift mutagenesis by M_1dG by comparing the ability of a series of ring-closed and ring-opened analogs to induce frameshift mutations in $(CpG)_4$ sequences replicated in *E. coli.* (2) (a) Define the mutagenicity of OPdA, εdA, and H-εdA in reiterated poly-dA sequences carried on site-specifically modified vectors replicated in mammalian cells; and (b) compare the ability of these adducts to induce frameshift mutations in reiterated dA_n sequences in wild-type and mismatch repair-deficient cells. (3) (a) Determine the products and kinetics of the reaction of M_1dG, OPdG, OPdA and N^2-oxopropyl-dG with amino acids and peptides and (b) define the chemistry and functional consequences of oxopropyl-dG with amino acids and peptides and (b) define the chemistry and functional consequences of modification of DNA binding proteins by N^2-oxopropyl-dG, M_1dG, OPdG and OPdA.
Role: Salary Support Only

COMPLETED RESEARCH

P50 CA128323 (Gore, John C., PI) 9/22/08 - 8/31/13
NIH/NCI
"In Vivo Cellular and Molecular Imaging Centers (ICMICs)"
The proposal aims to establish a new In Vivo Cellular and Molecular Imaging Center at Vanderbilt University which will be dedicated to providing the scientific and technical resources to develop, support and integrate highly innovative molecular imaging studies of cancer biology of direct relevance and translational potential to clinical cancer care.
Role: Salary Support Only

U54 CA136465 (Contag, Christopher H., PI) 9/22/08 – 8/31/13
NIH/NCI
"Multimodality Imaging of GI Cancers for Diagnosis and Directed Therapy"
This is a subcontract from Stanford that represents the Phase II portion of 5U54 CA105296-05 that ends 3/31/09. Dr. Marnett is PI of the subcontract and will be the lead investigator on one task-specific project for optimizing cyclooxygenase-2 targeting in vivo in order to synthesize optical imaging agents to visualize premalignancy and malignancy in humans. A principal goal of this research is to optimize the imaging agents using animal models of cancer that can be translated into the clinic.
Role: Salary Support Only

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
H. Charles Manning, Ph.D.	Associate Professor
eRA COMMONS USER NAME (credential, e.g., agency login) eRA Commons User Name(s)	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Tarleton State University, Stephenville, TX	B.S.	2000	Chemistry
Texas Tech University, Lubbock, TX	Ph.D.	2004	Chemistry
Vanderbilt University, Nashville, TN	Post-Doc	2004-2006	Imaging Science

A. Research and Professional Experience

I am an Associate Professor of Radiology, Biomedical Engineering, Neurosurgery, and Chemical and Physical Biology and serve as the Vanderbilt University Institute of Imaging Science (VUIIS) faculty director of Molecular Imaging Research. In addition to these activities, I am the scientific director of the VUMC Research Radiochemistry Core Facility. The primary focus of my research program is the discovery and biological validation of novel molecular imaging probes and cancer imaging biomarkers. My laboratory team is highly multidisciplinary, featuring an equal mixture of synthetic organic chemists and molecular biologists. I am a recipient of multiple awards from the National Cancer Institute (NCI) to pursue discovery and biological validation of novel molecular imaging compounds, preclinical biological validation of PET/MR imaging metrics, clinical validation of PET imaging in patients with advanced colorectal cancer. I am a member of the Vanderbilt Ingram Cancer Center, co-direct Project 1 of Vanderbilt's GI SPORE Program, lead imaging cores in Vanderbilt's Digestive Disease Research Center (DDRC), Breast SPORE, and GI SPORE, and serve on the VUMC PET Center Executive Steering Committee. As a trained chemist and imaging scientist, I have been involved in the development and validation of molecular imaging metrics for more than a decade. It is my privilege as part of this R15 application to consult with Dr. Cekanova on all aspects of non-invasive molecular imaging in preclinical models. I have sufficient time to allocate to this endeavor and am happy to do so. I am very familiar with fluorocoxib-A, which was developed by my colleague, Dr. Marnett, and I am very enthusiastic about the propose studies.

B. Positions and Honors

Positions and Employment

1996-2000	Undergraduate Research Assistant, Dept. of Physical and Environmental Sciences, Tarleton State University, Stephenville,TX
2000-2004	Graduate Student and Research Associate, Dept. of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX (Mentor: Dr. Darryl J. Bornhop)
2004-2006	Post-Doctoral Research Fellow, Institute of Imaging Science and Dept. of Radiology, Vanderbilt University, Nashville, TN (Mentor: Dr. John C. Gore)
2006-2008	Research Assistant Professor of Radiology and Radiological Sciences, Vanderbilt University, Nashville, TN
2006-2008	Research Assistant Professor of Neurosurgery, Vanderbilt University, Nashville, TN
2006-	Faculty Director of Molecular Imaging Research, Vanderbilt University Institute of Imaging Sciences (VUIIS), Vanderbilt University, Nashville, TN
2008	Assistant Professor of Radiology, Biomedical Engineering, and Neurosurgery, Vanderbilt University, Nashville, TN
2008	Faculty Member, Program in Physical and Chemical Biology, Vanderbilt University, Nash, TN
2012	Associate Professor of Radiology, Biomedical Engineering, and Neurosurgery, Vanderbilt University, Nashville, TN

Other Experience and Professional Memberships

2004-Present	American Chemical Society (ACS)
2007-Present	Society of Nuclear Medicine
2007-Present	Vanderbilt Ingram Cancer Center
2007-Present	VICC Experimental Therapeutics Program

Honors

1997	Outstanding Freshman Chemistry Award, Tarleton State University
1998	Outstanding Student in Organic Chemistry, Tarleton State University
1997-2000	Phi Eta Sigma
1997-2000	Alpha Chi
2000	Top Graduate, Dept. of Physical and Environmental Sciences, Tarleton State University
2000-2002	Southwestern Bell Chancellor's Fellowship Scholar, Texas Tech University
2004-2006	NIH-T32 Post-Doctoral Fellowship
2007	Society of Nuclear Medicine Molecular Imaging Center of Excellence (MI CoE) Editorial Board

C. Selected Peer-reviewed Publications (Most relevant selected from 45)

1. **Manning HC**, Merchant NB, Foutch AC, Virostko JM, Wyatt SK, Shah C, McKinley ET, Xie J, Mutic NJ, Washington MK, Lafleur B, Tantawy MN, Peterson TE, Ansari MS, Baldwin RM, Rothenberg ML, Bornhop DJ, Gore JC, Coffey RJ. Molecular imaging of therapeutic response to epidermal growth factor receptor blockade in colorectal cancer. Clin Cancer Res 2008;14:7413-22. PMCID: PMC2657180
2. **Manning HC**, Lander A, McKinley E, Mutic NJ. Accelerating the development of novel molecular imaging probes: a role for high-throughput screening. J Nucl Med 2008;49:1401-4. PMCID: PMC2576283
3. Shah C, Miller TW, Wyatt SK, McKinley ET, Olivares MG, Sanchez V, Nolting DD, Buck JR, Zhao P, Ansari MS, Baldwin RM, Gore JC, Schiff R, Arteaga CL, **Manning HC**. Imaging biomarkers predict response to anti-HER2 (ErbB2) therapy in preclinical models of breast cancer. Clin Cance Res 2009;15(14):4712-21. PMCID: PMC2819132 [Available on 2010/7/15]
4. Miller TW, Forbes JT, Shah C, Wyatt SK, **Manning HC**, Olivares MG, Sanchez V, Dugger TC, de Matos Granja N, Narasanna A, Cook RS, Kennedy JP, Lindsley CW, Arteaga CL. Inhibition of mammalian target of rapamycin is required for optimal antitumor effect of HER2 inhibitors against HER2-overexpressing cancer cells. Clin Cancer Res 2009; 15(23):7266-76. PMCID: PMC2787848 [Available on 2010/12/1]
5. Virostko JM, Xie J, Hallahan DE, Arteaga CL, Gore JC, **Manning HC**. A molecular imaging paradigm to rapidly profile response to angiogenesis-directed therapy in small animals. Mol Imaging Biol 2009;11(3): 204-12. PMCID: PMC2677126
6. Ayers GD, McKinley ET, Zhao P, Fritz JM, Metry RE, Deal BC, Alderz KM, Coffey RJ, **Manning HC**. Volume of preclinical xenograft tumors is more accurately assessed by ultrasound imaging than manual caliper measurements. J Ultrasound Med 2010;29(6):891-901. PMCID: PMC2925269
7. R. Adam Smith, Saffet Guleryuz, **H. Charles Manning**. "Molecular imaging metrics to evaluate response to preclinical therapeutic regimens." *Front Biosci.* 2011 Jan 1;16:393-410. PMCID: PMC3023459
8. Eliot McKinley, Joseph E. Bugaj, Ping Zhao, Saffet Guleryuz, Christine Mantis, Prafulla C. Gokhale, Robert Wild and **H. Charles Manning**. "^{18}FDG-PET predicts pharmacodynamic response to OSI-906, a dual IGF-1R/IR inhibitor, in preclinical mouse models of lung cancer." Clin Cancer Res. (2011) 17; 3332. PMCID: PMC3122480
9. Matthew R. Hight, Donald D. Nolting, Eliot T. McKinley, Adam D. Lander, Shelby K.Wyatt, Mark Gonyea, Ping Zhao, **H. Charles Manning**. "Multispectral Fluorescence Imaging to Assess pH in Biological Specimens." J Biomed Opt. 2011 Jan-Feb;16(1):016007. PMCID: PMC3041815
10. Garrett JT, Olivares MG, Rinehart C, Granja-Ingram NM, Sánchez V, Chakrabarty A, Davé B, Cook RS, Pao W, McKinely ET, **Manning HC**, Chang JC, Arteaga CL. "Transcriptional and post-translational upregulation of HER3 (ErbB3) compensates for inhibition of the HER2 tyrosine kinase". Proc Natl Acad Sci USA. (2011) 108: 5021-5026. PMCID: PMC3064360
11. Jason R. Buck, Eliot T. McKinley, Matthew R. Hight, Allie Fu, Dewei Tang, R. Adam Smith, Mohammed Noor Tantawy, Todd E. Peterson, Daniel Colvin, M. Sib Ansari, Ronald M. Baldwin, Ping Zhao, Saffet Guleryuz, and **H. Charles Manning**. "Quantitative, preclinical PET imaging of TSPO expression in glioma using [^{18}F]PBR06." J Nucl Med. 2011; 52:107-114. PMCID: PMC3027353
12. Emily M. Fox, Todd W. Miller, Justin M. Balko, Maria G. Kuba, Violeta Sánchez, R. Adam Smith, Shuying Liu, Ana María González-Angulo, Gordon B. Mills, Fei Ye, Yu Shyr, **H. Charles Manning**, Elizabeth Buck, and Carlos L. Arteaga. "A kinome-wide screen identifies the insulin/IGF-I receptor pathway as a mechanism of escape from hormone dependence in breast cancer." Cancer Res. 2011 Nov 1;71(21):6773-84. PMCID: PMC3206206
13. Dewei Tang, Matthew R. Hight, Eliot T. McKinley, Allie Fu, Jason R. Buck, R. Adam Smith, Mohammed Noor Tantawy, Todd E. Peterson, Daniel Colvin, M. Sib Ansari, Mike Nickels, and **H. Charles Manning**.

"Quantitative preclinical imaging of TSPO expression in glioma using *N,N*-diethyl-2-(2-(4-(2-(^{18}F)-fluoroethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl)acetamide." J Nucl Med 2012; 53 (2): 287-294. PMCID: PMC3391587

14. Jason R. Buck, Sam Saleh, Md. Imam Uddin, **H. Charles Manning**. Rapid, microwave-assisted organic synthesis of selective ^{V600E}BRAF inhibitors for preclinical cancer research. Tetrahedron Letters 2012, Volume 53, Issue 32, pages 4161--4165. Publ.ID: TETL41348; Available 06/13.
15. Eliot T. McKinley, R. Adam Smith, Jarred P. Tanksley, Mary Kay Washington, Ronald Walker, Robert J. Coffey, **H. Charles Manning.** "[^{18}F]FLT PET to predict pharmacodynamic and clinical response to cetuximab therapy in Ménétrier's disease. Ann Nucl Med. 2012 Jul 22; Epub ahead of print.

D. Research Support

Ongoing Research Support

5P50 CA098131-10 Arteaga (PI) 09/11/08-05/31/13

NIH/NCI SPORE in Breast Cancer

Specific Aim 1: To determine the antitumor effect of the HER signaling inhibitor OSI-774 in patients with untreated operable breast cancer and determine a molecular profile associated with evidence of response in situ. Specific Aim 2: To determine if OSI-774 inhibits EGFR and HER2 signaling in situ and whether drug-induced changes in phospho-HER3 and of phosphorylated p21 and p27 are surrogate markers of drug action. Specific Aim 3: To determine by imaging mass spectrometry the protein profiles in pre- and post-OSI-774 tumor specimens that predict for evidence of response in situ. Specific Aim 4: To determine if a threshold level of p27 is required for the antitumor action of HER signaling inhibitors like OSI-774. Role: Co-Investigator

5P50 CA128323-05 Gore (PI) 09/22/08-08/31/13

NIH/NCI Vanderbilt In Vivo Cellular and Molecular Imaging Center

This proposal aims to establish a new In Vivo Cellular and Molecular Imaging Center at Vanderbilt University which will be dedicated to providing the scientific and technical resources to develop, support and integrate highly innovative molecular imaging studies of cancer biology of direct relevance and translational potential to clinical cancer care. Role: Leader, Project 3; Leader, Core Resource C: Radiochemistry

5R01 CA140628-04 (Manning) 07/17/09-06/30/14

NIH/NCI

Biological Basis of Imaging Biomarkers in Colorectal Cancer

The goal of this project is to evaluate and validate the biological basis of emerging non-invasive imaging biomarkers for use in evaluation of treatment response in colorectal cancer (CRC). Role: Principal Investigator

2P30DK058404-11 (Peek) 06/01/12-05/31/17

NIH/NIDDK

Molecular and Cellular Basis for Digestive Diseases

The goals to this proposal are: 1) to promote digestive disease-related research in an integrative, collaborative and multidisciplinary manner; 2) to develop and implement programs for attracting, training, and retaining young investigators in digestive disease-related research; 3) to enhance the basic, translational, and clinical research capabilities of VDDRC members; 4) to facilitate the transfer of basic research discoveries to improvements in prevention and/or clinical care; and 5) to attract investigators not involved in digestive disease-related research to pursue these lines of investigation. Role: Co-Leader

1R01CA163806-01A1 (Manning) 09/01/12-06/30/17

NIH/NCI

TSPO Ligands for Cancer Imaging

This proposal seeks to develop and validate a novel class of cancer imaging biomarkers, TSPO positron emission tomography (PET) ligands, suitable for rapid translation to patients. Role: Principal Investigator

2P50 CA095103-11 Coffey (PI) 09/07/12-04/30/17

NIH/NCI

SPORE in GI Cancer

In this proposal, we apply the translational research strengths of the VICCC and its affiliated institutions towards reducing the incidence, morbidity and mortality of colorectal cancer.

Role: Project 1 Co-Leader; Translation Pathology and Imaging Core Co-Leader

2R01CA109106-06A1 Gore (PI) 01/01/12-12/31/16

NIH/NCI

Private Support

MRI Diffusion in Tumors Using Oscillating Gradients
To develop new magnetic resonance imaging methods that measures the apparent diffusion coefficient (ADC) of water in tissues as a function of temporal frequency, and to evaluate whether so-called diffusion spectra are useful for the detection of tumors and for monitoring their response to treatment. Role: Co-Investigator
Manning (PI) 01/18/12-01/17/14
Pharmaceutical
Establishment of Tumor Model with FDG and FLT
To evaluate ^{18}FDG-PET as a non-invasive, pharmacodynamics (PD) biomarker of response following the administration of a Lilly PI3K/mTOR inhibitor, LY3023414. Secondarily evaluate ^{18}FLT-PET as a non-invasive, pharmacodynamics (PD) biomarker of response. Role: Principal Investigator
1R01 CA163499-01A1 Guelcher (PI) 09/01/12-06/30/17
NIH/NCI
The Role of Mechanotransduction in Progression of Tumor-Induced Bone Disease
This proposal studies will allow us to evaluate whether mechanotransduction inhibition (early inhibition) and/or Gli2 inhibition (more established tumors) will be promising targets for the development
of clinical treatment strategies for treating patients with tumor-induced bone disease. Role: Co-Investigator
1R01 CA173593-01 Gore (PI) 09/01/12-08/31/17
NIH/NCI
Comprehensive Evaluation of OGSE DWI for Assessing Tumor Treatment Response
There are three specific aims to this proposal. [1] to apply OGSE methods to measure changes that occur in OGSE spectra with the growth of tumors over time, and in response to three specific, different classes of targeted treatments, in mouse models *in vivo*. [2] to establish how early OGSE methods can detect the response of tumors to specific targeted treatments, how well these changes predict later outcomes, and which OGSE parameters correlate with changes in cellularity, apoptosis and proliferation. [3] to further validate and assist the interpretation of diffusion measurements by performing elaborate computer simulations of water in compartmental systems of appropriate complexity. Role: Co-Investigator
Private Support **Manning, (PI)** 01/01/13-12/31/14
Novel TSPO PET Ligands to Image Pancreatic Cancer
Our primary goal is to develop a novel, early biomarker of pancreatic neoplasia. Role: Principal Investigator
1R01 HL116358-01 Blackwell (PI) 09/25/12-06/30/15
NIH/NHLBI
Imaging Activated Macrophages in the Lungs
In this proposal, we hypothesize that developing molecular imaging techniques to identify functional subsets of activated macrophages will advance understanding of inflammatory lung diseases and could lead to novel, macrophage-targeted therapies. Role: Co-Investigator

Completed Research Support

Private Support **Manning (PI)** 04/10/08-04/10/11
Private Support
PET Imaging Metrics of OSI-IGF-1R Inhibitor
The specific aim of this research is to assess PET molecular imaging metrics as biomarkers of response to therapy. Role: Principal Investigator
1RC1 CA145138-01 (Manning) 09/30/09-08/31/12
NIH/NCI
Molecular Determinants Affecting FLT-PET in Colorectal Cancer
This proposal has two Specific Aims: Aim 1 - To identify molecular determinants that affect [^{18}F]-FLT PET imaging in preclinical mouse models of human CRC. Aim 2 - To explore the utility of [^{18}F]-FLT PET to assess clinical and biological effects of inhibiting EGF receptor and combined EGFR/SRC in neoadjuvant trials of patients with advanced CRC. Role: Principal Investigator
5K25 CA127349-05 Manning (PI) 04/01/08-03/31/13
NIH/NCI
Synergizing Quantitative HTS Technologies to Guide MIA Discovery and Development
To develop quantitative, high-throughput screening (HTS) technology to accelerate the discovery and development of small molecule imaging agents(MIAs). Role: Principal Investigator

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

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

ORGANIZATIONAL DUNS*: 0033878910000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The University of Tennessee

Start Date*: 07-01-2014 **End Date*:** 06-30-2017 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Maria		Cekanova		PD/PI	Inst. Base Salary	Calendar Months			67,920.00	29,206.00	97,126.00
2.	Dr.	Joseph		Bartges		Co-Investigator					0.00	0.00	0.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **97,126.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
1	Graduate Students	12.00			54,000.00	8,532.00	62,532.00
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	**62,532.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**159,658.00**

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

Funding Opportunity Number: PA-13-313 . Received Date: 2013-11-01T16:37:09.000-04:00

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

ORGANIZATIONAL DUNS*: 0033878910000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The University of Tennessee

Start Date*: 07-01-2014 **End Date*:** 06-30-2017 **Budget Period:** 1

C. Equipment Description

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

Equipment Item	Funds Requested ($)*
1 . Multifunction microplate reader	10,000.00
Total funds requested for all equipment listed in the attached file	
Total Equipment	**10,000.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,500.00
2. Foreign Travel Costs	
Total Travel Cost	**4,500.00**

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

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

Tracking Number: GRANT11516368
Funding Opportunity Number: PA-13-313 . Received Date: 2013-11-01T16:37:09.000-04:00

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

ORGANIZATIONAL DUNS*: 0033878910000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The University of Tennessee

Start Date*: 07-01-2014 **End Date*:** 06-30-2017 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	71,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	15,000.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Tuition	39,707.00
Total Other Direct Costs	**125,707.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**299,865.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified Total Direct Costs	46.00	250,185.00	115,073.00
		Total Indirect Costs	**115,073.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 202-401-2808

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**414,938.00**

J. Fee	Funds Requested ($)*

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

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

Tracking Number: GRANT11516368
Funding Opportunity Number: PA-13-313 . Received Date: 2013-11-01T16:37:09.000-04:00

Budget Justification

A. Senior and Key Personnel Justification – total $97,126

Maria Cekanova – Principal Investigator (effort= [ ] calendar months in year one and [ ] months in years two and three) Dr. Cekanova will be the responsible for overall coordination of the research team. She will carry out the design of all experiments related to optical imaging method, evaluating the data from optical imaging and coordinate all aspects of the research project involving fluorocoxib A. She will coordinate the in vivo experiments and supervise the graduate student. She will coordinate analysis obtained samples using variety molecular techniques, such as WB, IHC, IF assays and tissue culture work with isolated K9TCC cells in vitro. She will coordinate all meetings and closely interact will her graduate student and Drs. Bartges from UTVMC, Jashim Uddin and Charles Manning from Vanderbilt University via e-mail, phone and video-conferences, and personal visits. She will analyze and interpret the all data and coordinate the writing of manuscript and progress reports. She will designate [ ] calendar months/3years [ ] calendar months in year one, and [ ] months in years two and three) of her time effort to this study. PI benefits are calculated at the actual rate of 43%.

Joseph Bartges – Co-Investigator – (effort = [ ] calendar months/ 3 year) a board-certified veterinary internal medicine specialist will perform all endoscopic optical imaging and biopsy acquisition in dogs with bladder cancers with standard veterinary care. He will devote [ ] month/calendar year for 3 years of his time effort with no salary support from this project.

B. Other Personnel – total $62,532

Graduate MS/PhD Student – (effort = 12 calendar months in year one and two and 8.4 calendar months in year three) Salary is requested at $20,000 in year one and two and $14,000 in year three for a PhD/MS student who will designate 8.4 calendar months/year of her/his time effort to this project and her/his salary. GRA benefits are calculated at 9% plus student health insurance of $117/month. The graduate student will perform in vivo experiments and analyze data from experiments under PI guidelines using variety molecular techniques, such as WB, IHC, IF assays using tissue specimens and K9TCC cells.

C. Equipment Description - $10,000

Funding is requested to purchase a Microplate reader multifunction fluorescence, luminescence, absorbance instrument for measuring samples.

D. Travel – total $4,500

Domestic travel $1,000 in year one; $1,500 in year two and $2,000 in year three is requested to cover travel, lodging cost and conference fees of international research meetings, such as AACR, or WMIC imaging meetings to present data. This will also include any travel fees connected with travels to Nashville, Vanderbilt University for student to interact and learn about optical imaging data analysis by consultant Dr. Charles Manning.

E. Participant/Trainee Support Costs – N/A

F. Other Direct Costs – total $125,707

1. Materials and supplies – total $71,000

- **Mice -** optical imaging of mice (30 mice B6D2F1 mice $25/mouse plus shipping and housing for 6 months with total of $4,000 in year one
- **Dogs -** Cystoscopy and optical imaging of dogs - ($1,600/dog) including anesthesia, cystoscopy, histology evaluation; N=15 dog x $1,600/dog with total of $24,000
- **Reagents and supplies for *in vitro* experiments including Western blotting, immunohistochemistry, immunofluorescence, tissue culture work, HPLC assay** – ($40,000/3 years) – Antibodies, reagents for assays, histology, tissue culture disposables (tips, plates, tubes, flasks, pipettes), glassware, etc.

2. **Subawards/consortium/contractual costs – total $15,000**
   Jashim Uddin – Co-Investigator – (effort CAL. MONTHS per year) His laboratory is a part of Dr. Marnett's laboratory who is actively involved in cancer prevention research. They have focused on the enzyme cyclooxygenase-2 as a target for this research since it is expressed early in tumorigenesis and helps drive tumor progression. Its involvement as a contributing factor in carcinogenesis has been demonstrated in multiple solid tumor sites. Their laboratory has defined the molecular mechanisms of binding of a variety of non-steroidal anti-inflammatory drugs to COX-2 and used that information to design novel COX-2 inhibitors and COX-2 targeting agents. The latter provide new strategies for imaging and delivery of therapeutics to tumors that express COX-2. Dr. Uddin will conduct the design and execution of all experiments related to organic synthesis, characterization of fluorocoxib A. He will interact closely with personnel with regard to enzyme inhibition assay and cell culture experiments. The expenses covered with supplies and reagents to synthesize fluorocoxib A will be $15,000 for total amount for 3 years including F&A. He will assist with interpretation of data, writing the manuscripts and progress reports. He will interact regularly with Dr. Cekanova via telephone and video-conference to review research progress and identify high priority research goals. There are no salary and benefits support for Dr. Uddin provided by this grant.

   Charles Manning – Consultant – Dr. Manning research is focused on the development, validation, and translation of non-invasive molecular imaging metrics to assess disease progression and response to therapy. Projects within his program emphasize molecular imaging of cancer, and notably imaging-based assessment of response to anti-cancer pharmaceuticals in colorectal and breast cancers. He will contribute to the design and advice on the imaging experiments performed at UTCVM, participate in the interpretation of results and writing of manuscripts as mentioned in details in his LOS (see attached file). There are no consultations fees for Dr. Manning.

3. **Graduate Student Tuition – total $39,707** – tuition fee for graduate student is requested for fall, spring, and summer semesters for three years. We estimate $12,113/year with 9% increase/year.

G. Direct Costs – total $299,865

H. Indirect Costs – Calculated at our federally negotiated rate of 46% of modified total direct costs, total $115,073.

I. Total Direct and Indirect Costs - $414,938

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		**97,126.00**
Section B, Other Personnel		**62,532.00**
Total Number Other Personnel	1	
Total Salary, Wages and Fringe Benefits (A+B)		**159,658.00**
Section C, Equipment		**10,000.00**
Section D, Travel		**4,500.00**
1. Domestic	4,500.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		**125,707.00**
1. Materials and Supplies	71,000.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs	15,000.00	
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	39,707.00	
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		**299,865.00**
Section H, Indirect Costs		**115,073.00**
Section I, Total Direct and Indirect Costs (G + H)		**414,938.00**
Section J, Fee		

Funding Opportunity Number: PA-13-313 . Received Date: 2013-11-01T16:37:09.000-04:00

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

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

ORGANIZATIONAL DUNS*: 0044134560000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 07-01-2014 **End Date*:** 06-30-2017 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Md	Jashim	Uddin		PI	Inst. Base Salary	Calendar Months			0.00	0.00	0.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

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

Total Number Other Personnel **Total Other Personnel**

Total Salary, Wages and Fringe Benefits (A+B) **0.00**

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

Funding Opportunity Number: PA-13-313 . Received Date: 2013-11-01T16:37:09.000-04:00

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

ORGANIZATIONAL DUNS*: 0044134560000
Budget Type*: ○ Project ● Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 07-01-2014 **End Date*:** 06-30-2017 **Budget Period:** 1

C. Equipment Description

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

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

Additional Equipment: File Name:

D. Travel **Funds Requested ($)***

1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)
2. Foreign Travel Costs

Total Travel Cost

E. Participant/Trainee Support Costs **Funds Requested ($)***

1. Tuition/Fees/Health Insurance
2. Stipends
3. Travel
4. Subsistence
5. Other:

Number of Participants/Trainees **Total Participant Trainee Support Costs**

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

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

ORGANIZATIONAL DUNS*: 0044134560000
Budget Type*: ○ Project ● Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 07-01-2014 **End Date*:** 06-30-2017 **Budget Period:** 1

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	9,555.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**9,555.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**9,555.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Costs	57.00	9,555.00	5,445.00
		Total Indirect Costs	**5,445.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS: Steven Zuraf (301) 492-4855, agreement dated: 04/09/2013

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**15,000.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: 1241-Justification1.pdf
(Only attach one file.)

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

Tracking Number: GRANT11516368
Funding Opportunity Number: PA-13-313 . Received Date: 2013-11-01T16:37:09.000-04:00

BUDGET JUSTIFICATION

Personnel:

Dr. Md. Jashim Uddin will be the PI of the subcontract. He will synthesize fluorocoxib A and ship it to Dr. Cekanova's laboratory at the University of Tennessee-Knoxville for her use in imaging studies. Dr. Uddin will devote [ ] calendar months to the project without salary.

Calendar Months

Supplies:

Funds are requested for the chemicals required for the synthesis of fluorocoxib A.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		**0.00**
Section B, Other Personnel		
Total Number Other Personnel		
Total Salary, Wages and Fringe Benefits (A+B)		**0.00**
Section C, Equipment		
Section D, Travel		
1. Domestic		
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		**9,555.00**
1. Materials and Supplies	9,555.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1		
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		**9,555.00**
Section H, Indirect Costs		**5,445.00**
Section I, Total Direct and Indirect Costs (G + H)		**15,000.00**
Section J, Fee		

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OMB Number: 0925-0001

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

Prefix: Dr.
First Name*: Maria
Middle Name:
Last Name*: Cekanova
Suffix:

2. Human Subjects

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

3. Permission Statement*

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

● Yes ○ No

4. Program Income*

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

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

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

Tracking Number: GRANT11516368
Funding Opportunity Number: PA-13-313. Received Date: 2013-11-01T16:37:09.000-04:00

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5. Human Embryonic Stem Cells

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

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

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

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ○ Yes ● No

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

Previously Reported*: ○ Yes ○ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	1242-Introduction.pdf
2. Specific Aims	1243-SpecificAims.pdf
3. Research Strategy*	1244-ResearchStrategy.pdf
4. Progress Report Publication List	
Human Subjects Sections	
5. Protection of Human Subjects	
6. Inclusion of Women and Minorities	
7. Inclusion of Children	
Other Research Plan Sections	
8. Vertebrate Animals	1249-VertebrateAnimals.pdf
9. Select Agent Research	
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	1250-UddinLOI.pdf
12. Letters of Support	1251-LettersofSupport.pdf
13. Resource Sharing Plan(s)	1252-DataSharingPlan.pdf
Appendix (if applicable)	
14. Appendix	

Introduction to Resubmission Application

Response to Critique

SPECIFIC AIMS

This Academic Research Enhancement Award application (R15) *resubmitted* to the National Cancer Institute will explore the efficacy of an optical molecular imaging probe, fluorocoxib A, to selectively target cyclooxygenase-2 (COX-2) enzyme in *urinary bladder neoplastic tissues*. Our overall hypothesis is that fluorescence imaging of COX-2 expression will allow earlier detection of *urinary bladder* tumors, when interventions can be more successful, as well as improved detection of disease margins that may allow more complete resection. Improved biomarkers for *detection of urinary bladder neoplasia*, which can rapidly and cost effectively be deployed into clinical populations, are critically needed. The early detection of *urinary bladder dysplasia and tumor margins of bladder transitional cell carcinomas (TCC)* result in improved patient prognosis and long-term survival. The present situation with 50-70% recurrence rate shows the inadequacy of white light cystoscopy for detection and resection of the tumor lesions. The sensitivity and specificity cystoscopy can be significantly improved with the use of optical contrast agents that specifically accumulate in urinary *bladder cancer*.

Development of new and cancer-specific biomarkers is necessary in order to improve the detection of cancer (1). Despite the listing of approximately 1,350 contrast agents in the Molecular Imaging and Contrast Agents Database (as of January 2013) for *in vitro* and *in vivo* studies (2, 3), no selective tumor-targeted optical imaging agents have been successfully translated into clinical use to date. *We propose to utilize a canine model with naturally-occurring bladder cancer to assist with more rapid and effective translation of novel imaging contrast agents to clinical applications.* The COX-2 enzyme is expressed at high levels intracellularly in tumor cells, but not in most of normal cells, which enables molecular imaging in vivo with high signal-to-noise ratios (4-6). This property defines COX-2 as an attractive imaging target for *carcinomas* detection. Together with colleagues from Vanderbilt University, we have conducted proof-of-principle studies that have explored the use of a new class of imaging agents targeting COX-2 to image tumors in a variety of *in vivo* pre-clinical settings (4-6). Encouraged that fluorocoxib A could image COX-2 expressing xenograft tumors originated from human cell lines (5), *and from canine primary K9TCC (7), and in dogs with naturally-occurring colorectal and TCC carcinomas (6, 7),* we now propose an essential series of translational studies that will bridge the gap between proof-of-principle and translation. We hypothesize that fluorocoxib A will detect *overexpressed COX-2 in bladder dysplasia, carcinoma in situ (CIS), as well as TCC. We propose to use a carcinogen-induced urinary bladder tumorigenesis mouse model to investigate the uptake of fluorocoxib A during early bladder tumorigenesis (8-11). The canine model will allow us to study late stages of bladder cancer. Our proposed study using fluorocoxib A in mouse and canine bladder cancer models will assist in translating this optical imaging agent into clinical applications for early detection, as well for monitoring responses to therapy, tumor margins, and recurrence of bladder cancer.* To evaluate our hypotheses, we propose the following specific aims:

***Specific Aim 1:* Determine the ability of fluorocoxib A to detect the early bladder neoplasias using a mouse model of nitrosamine-induced bladder cancer.**

i. *Detect fluorocoxib A uptake in carcinogen-induced bladder pre-neoplastic urothelium in mice at 12 and 18 weeks after N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) administration.*
ii. *Compare fluorocoxib A uptake and Cox-2 expression between normal and pre-neoplastic tissues of the bladder.*

***Specific Aim 2:* Use fluorocoxib A to detect K9TCC in a dog cancer model.**

i. *Detect fluorocoxib A uptake during cystoscopy by COX-2-expressing heterogeneous urinary bladder carcinomas that occur naturally in dogs. Compare fluorocoxib A uptake and COX-2 expressions between normal and bladder carcinoma tissues.*
ii. *Determine the ability of fluorocoxib A to predict responses to tyrosine kinase inhibitors and novel COX-2 inhibitors therapies using primary K9TCC in vitro and using COX-2 expressing primary K9TCC xenograft mouse models in vivo.*

This proposal will lay the framework for enhanced collaborations in translational imaging science between investigators from the University of Tennessee and Vanderbilt University and impart a major impact on the scientific atmosphere of the University of Tennessee. This project will create an appropriate scientific environment to attract high-quality students into our graduate program to successfully accomplish the proposed research.

RESEARCH STRATEGY

A. SIGNIFICANCE

A.1. Bladder Cancer: *Bladder cancer is the fourth most common cancer in men and the eighth most common malignancy in women in according to the ACS in USA. An estimated 105,000 new cases of bladder cancers are expected to occur in 2013 in the USA. An estimated 11,000 bladder cancer-related deaths will occur in 2013 (www.cancer.org).* Precise early detection of tumors and accurate monitoring of tumor response to treatment are keys for survival of patients. With bladder cancer, there is currently no screening or early detection method recommended for individuals at average risk. Bladder cancer is diagnosed by microscopic examination of cells from urine, or by direct visualization of bladder wall during cystoscopy. However, conventional cystoscopy is not able to identify bladder cancer in early stages, such as dysplasia and carcinoma in situ (CIS), but only at more advanced stages (12). CIS is strongly related to recurrence, progression, and disease specific mortality. Up to 70% of patients with non-muscle-invasive bladder cancer will develop a local recurrence after transurethral resection (TUR) of the bladder tumor (13, 14). ***Therefore, improved detection of urothelial neoplasias that are difficult to visualize under white light examination during cystoscopy and reduced the rate of unidentified residual tumors after TUR is highly desirable for patients' survival. Sensitivity and specificity of conventional cystoscopy of urinary bladder cancer can be significantly improved by the tumor-specific fluorescence agents, such as fluorocoxib A.*** *It has been shown that the fluorescence produced by 5-aminolevulinic acid (5-ALA) improved white light cystoscopy detection of mouse bladder CIS by 13-fold (15).*

A.2. Cyclooxygenase 2 (COX-2) enzyme as target for bladder cancer: Thus, development of tumor-specific molecular imaging probes is required to improve detection of TCC at earlier stages as well as during surgical removal of TCC from bladder. Cancer is usually detected when structural changes in a tissue or organ have occurred. Molecular imaging may allow, in ways not previously possible, the dynamic underlying causes of disease, while providing better treatment monitoring. Because COX-2 enzyme expresses at high levels in cancers, but not in surrounding normal tissues, that's why COX-2 is an attractive target for selective detection of cancers. COX enzymes catalyze the synthesis of prostaglandins from arachidonic acid (16) and there are two isoforms of COX: COX-1 and COX-2 (17). COX-1 is expressed constitutively in normal tissues (18). In contrast to COX-1, COX-2 is not expressed in normal tissue, but rather is induced by many growth factors and cytokines in neoplastic and inflamed tissues. Many types of carcinomas that overexpressed COX-2 have also shown overexpression of EGFR signaling pathways (19). Growth factors, oncogenes, cytokines, and tumor promoters stimulate COX-2 expression via protein kinase C (PKC), and Ras-mediated signaling (20-22). Tumor suppressor genes, such as wild-type p53, inhibit the expression of COX-2 (23, 24). COX-2 increases the production of prostanoids that leads to promoting cell proliferation, angiogenesis, motility, and metastasis (25-30). COX-2 increases in inflammatory and premalignant lesions (31-33) and is expressed at even higher levels in carcinomas (20, 29, 34) including those of bladder (35, 36), head and neck (37), lung (38, 39), esophagus (40), stomach (41), pancreas (42), breast (43), ovary (44), colon (45, 46), skin (47, 48), and prostate. Numerous tumors in dogs, including TCC, (49-52) similarly to human bladder tumors show strong overexpression of COX-2 in the perinuclear area of the tumor cells and in surrounding stromal cells in the area of the tumors. These studies have shown that COX-2 is highly expressed in carcinomas and in precursor lesions that lead to the malignancy and provided direct evidence that COX-2 plays a key role in tumorigenesis of urothelium of bladder. The carcinomas that overexpress COX-2 are aggressive malignancies associated with high morbidity and mortality rates (37). COX-2 expression significantly increases with bladder tumor stage (35, 53). COX-2 is undetectable in normal bladder samples 0/10, but COX-2 is expressed in 29/29 (100%) of bladder squamous cell carcinomas, and in 12/35 (34%) of transitional cell carcinomas (35, 54). *We have also confirmed that canine normal urothelium doesn't express COX-2 and canine urinary bladder inflammation is COX-2 positive only in present macrophages (7). We have shown that COX-2 is strongly expressed by K9TCC cells at the periphery of bladder lesions, and COX-2 negative TCC cells are mostly present at the center of the K9TCC lesions (7). This observation suggests that COX-2 is increased in more aggressive growing regions of bladder neoplastic lesions (7).* Several studies, including animal model and epidemiological studies, have provided evidence that inhibition of COX-2 pathways may have significant benefits for cancer treatment and prevention (19, 20, 49, 55-58) particularly in the bladder cancer (36, 56, 59, 60). Numerous genetic studies using transgenic mice overexpressing COX-2 confirmed the increased development of metastatic tumors (61, 62). The non-steroidal anti-inflammatory drugs (NSAIDs) that inhibit COX enzyme (e.g. aspirin, diclofenac, piroxicam, sulindac, indomethacin, ibuprofen, and naproxen) are among the most widely used prescription and non-prescription drugs in the world (18, 63). Identification of the second isoform of COX enzyme, COX-2, led to

discoveries of a new class of COX-2 selective inhibitors (COXIB, e.g. rofecoxib, celecoxib, valdecoxib, etoricoxib, and lumiracoxib), which exhibit anti-inflammatory and anti-neoplastic activities (18, 64).The COX-2 inhibitor, etodolac, suppresses an epithelial-mesenchymal transition (EMT that promotes carcinoma progression) by reducing SNAIL and vimentin expression and by inducing E-cadherin expressions (65). Interestingly it has been shown that the patients with COX-2 negative, non-small-cell lung cancer (NSCLC) fared worse if treated with celecoxib as adjuvant treatment to carboplatin and gemcitabine than patients given chemotherapy alone (66). None of the currently used imaging agent in clinic has the ability to detect the expression of COX-2 in tumors and to predict the beneficial role of COXIBs for patients' therapies. Our proposed study is focused on evaluation of fluorocoxib A to detect COX-2 expressing early stages of bladder cancer such as dysplasia, CIS and high grades, such as TCC using mouse and dog models, respectively.

***A.3.* Fluorocoxib A - optical imaging agent for bladder cancer:** Development of new and cancer-specific biomarkers is necessary in order to improve the detection of cancer (1). In the 1980's, detection procedures for the visualization of urothelial neoplasias were developed using systemically applied synthetic porphyrin compounds (67). The acceptance of this procedure as part of the clinical routine was hindered by fluorescence excitation interfering with auto-fluorescence of normal tissue, but predominantly by phototoxic skin reactions caused by porphyrin compounds. Promising optical imaging agents are molecular-targeted near-infrared (NIR) and red-shifted fluorescent dyes. Those imaging agents abrogate the tissue auto-fluorescence and can propagate efficiently through centimeters of living tissues (68-70). The use of the COXIBs as a new class of imaging agents is based on the selective uptake by COX-2-expressing neoplastic lesions (19 , 71). Syntheses of radiolabeled COX-2 inhibitors have been reported (72, 73). During my post-doctoral studies in laboratory of Dr. Schuller, we characterized a newly synthesized derivative of celecoxib as SPECT radiotracers in a hamster model and showed promising results in identifying carcinogen-induced COX-2 expressing lung and pancreatic lesions (73). Unfortunately, the s.c. administration routes of the tracers, small body size of hamsters, and low resolution equipment were major limitations for successful translation to clinical use (73). New PET and SPECT imaging NSAIDs tracers were synthesized and evaluated by Dr. Marnett's laboratory using rodent models; however stability of new tracers were major limitation for successful translation to clinical trials (74, 75). Dr. Lawrence Marnett with Dr. Jashim Uddin at Vanderbilt University synthesized optical imaging agents that have shown promising properties *in vitro* and *in vivo* using rodent cancer models (4, 5). Fluorocoxib A is a rhodamine-labeled derivative of indomethacin (λ_{ex} = 580 nm, λ_{emit} = 602 nm) and selective COX-2 inhibitor with an IC_{50} value of 700 nmol/L for COX-2 in contrast to IC_{50} value of 25 µmol/L for COX-1 (35-fold) as assessed by purified enzyme assay. Once the fluorocoxib A is associated to COX-2 enzyme, it binds tightly with a dissociation rate of almost zero. *Fluorocoxib's potential for in vivo imaging was evaluated by Dr. Marnett's group using carrageenan-induced acute inflammation in the mouse footpad, human tumor xenografts in nude mice, and in APC^{Min} mice with spontaneous tumors (5).*

A.4. Carcinogen-induced bladder carcinogenesis mouse model: *For detection of early pre-neoplastic lesions in the bladder by fluorocoxib A, we will utilize a well-established carcinogen-induced bladder carcinogenesis mouse model. Bladder dysplasia and CIS develop in B6D2F1 mice after 12 weeks of the carcinogenic nitrosamine (N-butyl-N-(4-hydroxybutyl)nitrosamine; BBN) administration in drinking water (8-11). If carcinogenic treatment is prolonged to 18 weeks of BBN exposure, mice tend to develop high grade, nonpapillary, and invasive bladder carcinomas. The TCC and pre-neoplastic lesions overexpress Cox-2 (76) and are responsive for the cancer-preventive effects of COX-2 inhibitors (celecoxib) (60).*

A.5. Dog model of naturally occurring bladder cancer: Despite the listing of approximately 1,350 contrast agents in the Molecular Imaging and Contrast Agents Database (as of January 2013) for *in vitro* and *in vivo* studies, there are only 113 contrast agents, including four optical agents, that are currently approved by the United States Food and Drug Administration for clinical use in human medicine (as of October 2011) (2, 3). However, no selective tumor-targeted optical imaging agents have been approved by FDA and translated into clinical use to date. The development of *in vivo* animal models that recapitulate the natural history of human cancers and their clinical response to therapy constitute a major prerequisite for rapid bench-to-bedside translation of investigational anticancer therapies. As previously published, several optical and PET imaging agents detecting COX-2-expressing tumors were synthesized and characterized using human cell lines *in vitro* as well *in vivo* using xenograft mice models (5, 73), but their translation to clinical use failed. *To fill this gap to more efficiently translate novel imaging agents into clinic, we propose to evaluate efficacy of fluorocoxib A to detect and monitor the therapeutic responses of naturally-occurring TCC in dogs.* Companion animals with spontaneous neoplasms are a formidable and underexploited tool to make rapid advances in human cancer therapy by testing new compounds and delivery systems that have shown promise *in vitro*. Spontaneous

cancers in companion dogs offer a unique model for human cancer biology and translational cancer therapeutics (57). The relatively high incidence of cancers, similar biological behavior and response to therapy, comparable responses to cytotoxic agents, shared environment with their owners, and a shorter overall lifespan are contributing factors to the advantages of a companion animal model (51, 57, 77). The average age of the affected dog with spontaneous cancers is 8.4 years, which corresponds to an average age of 50 years for humans, suggesting that as in humans, spontaneous carcinomas are influenced by age and environment.

The histologic and biologic characteristics of bladder COX-2-overexpressing cancers in dogs are similar to bladder cancers in humans (34, 36). Several studies, including animal model and epidemiological studies, have provided evidence that inhibition of COX-2 pathways may have significant benefits for treatment and prevention of bladder cancer (36, 56, 59, 60). Compared to human bladder cancer, K9TCC are low grade with superficial papillary appearance or high grade invasive tumors that spreads through the bladder wall to lymph nodes and to other organs, such as liver and lung predominantly (36, 56, 78). The exact cause of TCC in dogs is still not know, however a genetic predisposition, pesticides, insecticides, and second hand smoke are considered major factors (36, 56, 78). Exploitation of dog bladder cancer model provides an opportunity to rapidly translate fluorocoxib A to clinical use for patients to detect and monitor the bladder cancer. Initially, we propose to evaluate the efficacy of fluorocoxib's A to detect the bladder dysplasia and CIS using carcinogen-induced rodent bladder carcinogenesis model. Followed by experiments to determine fluorocoxib A uptake during cystoscopy by COX-2 expressing urinary K9TCC bladder cancer to assist with more efficient translation of fluorocoxib A into clinical applications. The fact that Caliper Life Sciences has recently begun distribution of fluorocoxib A for pre-clinical imaging studies is evidence of the intense research interest in the imaging of COX-2-expressing tumors non-invasively. ***Dogs are big enough to assess optical imaging using cystoscopy to diagnose and monitor bladder cancer. The canine model of spontaneously occurring heterogeneous bladder cancer will provide critical information of fluorocoxib A efficacy for detection of bladder cancer in human clinical population.***

B. INNOVATION

The COX-2 enzyme is expressed at high levels intracellularly in cancer cells, but not in most of normal cells, which enables molecular imaging *in vivo* with high signal-to-noise ratios (4-6). The use of the COX-2 selective inhibitors as a new class of imaging agents is based on the selective uptake by COX-2-expressing neoplastic lesions. Together with colleagues from Vanderbilt University, we have conducted proof-of-principle studies that have explored that use of a new class of imaging agents targeting COX-2 to image tumors in a variety of *in vivo* preclinical settings (4-6). The research conducted by Dr. Marnett's lab at VU identified several of the most promising optical imaging agent derived from NSAIDs, such as fluorocoxib A, out of few hundreds of newly synthesized compounds. *They evaluated specificity of fluorocoxib A uptake in rodent xenograft models; however limitation of subcutaneous inoculation of homogeneous xenograft tumor using human stable cancer cell lines doesn't reflect the heterogeneity of primary human cancer. We got encouraged results that fluorocoxib A could be used to image COX-2 expressing xenograft tumors originated not only from human (5) but also primary K9TCC (7)* cell lines, *as well in naturally-occurring colorectal carcinomas and TCC during scoping of dogs (6, 7). Our clinic at the University of Tennessee, College of Veterinary Medicine (UTCVM) provides unique access to dogs diagnosed with naturally occurring bladder carcinomas. A dog cancer model is valuable pre-clinical model and important link between the rodent model and humans. In laboratory of Dr. Cekanova, we have successfully isolated and established 5 primary K9TCC cell lines (7) and evaluate imaging using fluorocoxib A (see preliminary data). We initiated the pilot study to evaluate fluorocoxib A uptake by COX-2-expressing canine carcinomas during scoping using Karl Storz Imaging photodynamic detection system (PDD) system. Recently, we have received new custom-made filter system to improve detection of fluorocoxib A uptake using in vivo modified PDD system at UTCVM. Our proposed study using fluorocoxib A in rodent and dog cancer models will assist translation of fluorocoxib A into clinical applications for detection of early bladder neoplasias, more precise detection of tumor margins of TCC during surgery, as well for monitoring the responses of TCC to therapy. Because dogs are diagnosed with bladder cancers mostly with TCC, we propose to use the mouse bladder carcinogenesis model to detect the early bladder pre-neoplastic lesions. Rodent model with the early stages of bladder tumors will be used as a proof-of principle model to support our hypothesis that fluorocoxib A can detect early bladder dysplasia and CIS lesions. Dog model with TCC is a valuable model that mimics the behavior of heterogeneous and spontaneous human bladder cancers. Primary cell lines from K9TCC are more biologically relevant controls than stable cell lines cultured for decades that may have accumulated numerous mutations over time.* ***In conclusion, the innovations of this proposal are: 1) detection of Cox-2 expressing bladder dysplasia and CIS as early stages of bladder cancer***

development by optical imaging agent, fluorocoxib A, using a mouse model of nitrosamine-induced bladder cancer and 2) detection of COX-2 expressing heterogeneous naturally occurring canine bladder carcinomas by optical imaging during cystoscopy in dogs.

C. APPROACH

We have established a unique multi-institutional collaboration of experts in the field to apply their knowledge from basic molecular (Dr. Cekanova), organic chemistry (Drs. Uddin and Marnett), optical imaging (Drs. Cekanova, Uddin, Bartges, and Manning), and veterinary medicine research (Dr. Bartges). This combination of expertise will optimize the likelihood of developing clinically useful probes for detecting and monitoring of the responses to treatments using non-invasive imaging tools. In our study, we propose to use rodent and dog animal models to evaluate efficacy of fluorocoxib A to detect COX-2 expressing bladder neoplasia. The rodent carcinogen-induced bladder cancer model *(8-11)* will allow us to evaluate efficacy of fluorocoxib A to detect bladder early dysplasia and CIS. The dog model with naturally occurring TCC will allow us to evaluate the efficacy of fluorocoxib A to detect and monitor responses of high grade invasive transitional cell carcinomas to therapy.

C.1. Preliminary Data

C.1.1.Clinical oncology case load at the University of Tennessee at the Veterinary Medical Center in Knoxville (UTVMC) – UTVMC is a state-of-the-art referral center providing veterinary care for the southeast region seeing approximately 15,000 patients per year. The UTVMC clinical oncology caseload is composed primarily of dogs with range of tumor types: non-Hodgkin's lymphoma, osteosarcoma, malignant melanoma, cutaneous mast cell disease, bladder carcinomas, colorectal carcinomas, pulmonary cancers, and others. *Through our Center for Minimally Invasive Procedures, our veterinary clinicians perform approximately 150 cystoscopic and endoscopic procedures per year in dogs and evaluate approximately 20 dogs with TCC of the urinary bladder each year, which provides sufficient number of cases for enrolment to our study.* The UTVMC is an AAALAC-accredited facility for animal research activities. We maintain a reputation for excellence in patient care and animal welfare with the UT-Knoxville Institutional Animal Care and Use Committee (IACUC) and Office of Laboratory Animal Care (OLAC).

***C.1.2.* COX-2 expressions in canine urinary bladder cancers** – The degree of COX-2 expression shows a significantly proportional increase ($p < 0.05$) with advance in pathological stage of the bladder carcinomas (35, 53, 79). Canine bladder carcinomas show strong expression of COX-2 in the cytoplasm with perinuclear localization in the tumor cells similarly to humans (36, 52) as shown in **Figure 1**. We confirmed, that normal urothelium has no expression of COX-2 **(Figure 1 inset)**, while canine TCC cells show strong expression of COX-2 enzyme **(Figure 1,** brown staining**)** as assessed by immunohistochemistry (IHC). The COX-2 positive cells in TCC are predominantly localized around the edges of tumor lesions supporting the evidence that COX-2 is involved in tumor invasion and metastasis (79-81).

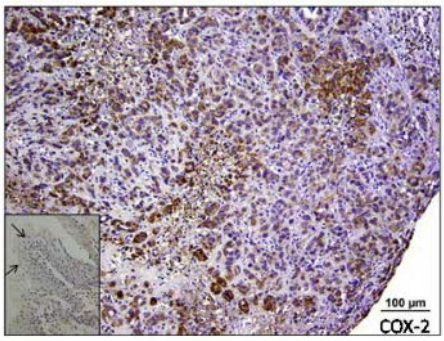


Figure 1: COX-2-expressing K9TCC by IHC. COX-2 is localized intracellularly in K9TCC cells (brown color). Nuclei were counterstained with hematoxylin (blue color). Insert picture shows negative expression of COX-2 in normal urothelium (arrows). Objective 20x, scale bar 100 µm.

The epidemiological studies indicate that the development of the bladder cancer is closely associated with chronic inflammation of the urinary tract, but the underlying mechanisms are still unknown (54, 82). COX-2 is markedly expressed in the bladder carcinomas, suggesting that chronic inflammation stimulates the production of the COX-2 and in turn induces the development of bladder cancer. We evaluated the expression of COX-2 in the biopsy samples obtained during cystoscopy in dogs (n=8) diagnosed with acute and chronic inflammations *(7). No detectible COX-2 expression was observed in neutrophilic and lymphocytic inflammation of bladder, only in the infiltrating perivascular macrophages in the submucosa layer as we published in Cancer Prevention Research in 2013 (7).*

C.1.3. Fluorocoxib A uptake by COX-2-expressing K9TCC *- We confirmed specific fluorocoxib A* ***(Figure 2A)*** *uptake by primary K9TCC in vitro, in K9TCC xenograft tumors in athymic mice in vivo, and in 5 dogs diagnosed with TCC during cystoscopy in vivo (7). Canine TCC cells were isolated from tumor with confirmed COX-2, E-cadherin and cytokeratin expressions. Specific uptake of fluorocoxib A by canine COX-2 in K9TCC cells* ***(Figure 2B)*** *was confirmed and published in Cancer Prevention Research by Cekanova et al. (7). We also confirmed tumorigenic behavior of isolated K9TCC cells after subcutaneous implantation to the athymic mice. We evaluated the ability of fluorocoxib A to target COX-2 in K9TCC xenograft tumors 24 hours after i.v. administration of fluorocoxib A (1 mg/kg) using whole body imaging by the IVIS Lumina system with DsRed Filters excitation 500-550nm and emission 575-650nm with background 460-490nm. Fluorocoxib A specifically binds to COX-2 expressing K9TCC#2Dakota xenograft tumors (intensive red/yellow signal in ROI green circle) as shown in* ***Figure 2C*** *after up to 24 h post-administration of fluorocoxib A. Specific and selective uptake of fluorocoxib A by K9TCC#2Dakota was statistically significantly (**p<0.01) blocked by pre-administration of celecoxib (10 mg/kg, i.v., 4 hours before fluorocoxib A) as shown in* ***Figure 2C and 2D*** *(7). Furthermore, no uptake of fluorocoxib A was detected in human UMUC-3 xenograft tumors that have no expression of COX-2 as shown in* ***Figure 2C and 2D****. We confirmed the expression of COX-2 in xenograft tumors by IHC* ***(Figure 2E)*** *and by WB* ***(Figure 2F)*** *in TCC cells (7). Confirmed epithelial-cell origin of K9TCC#2Dakota xenograft tumors was shown by IHC with positive E-cadherin expressions as shown in* ***Figure 2E*** *(7).*

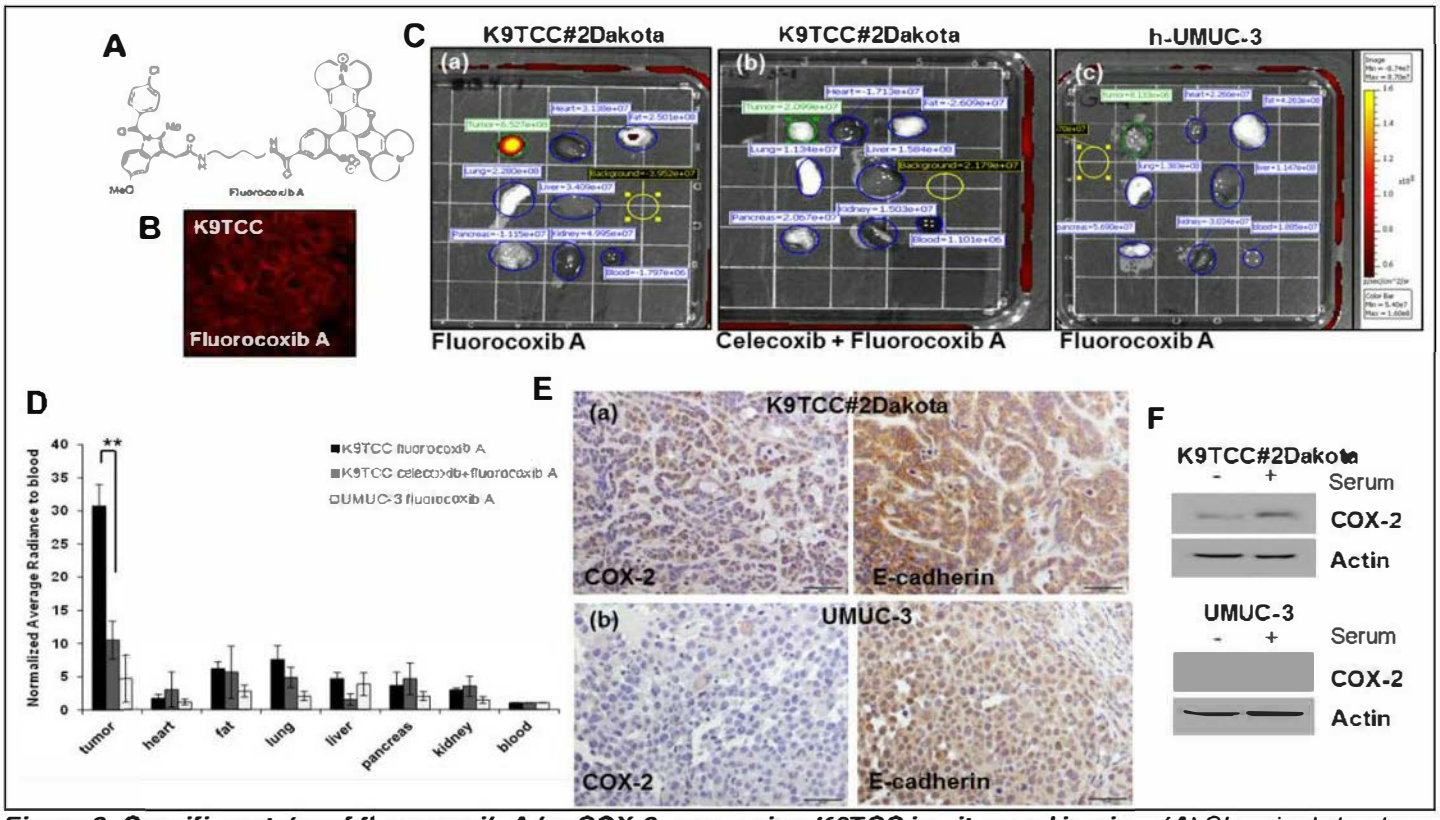
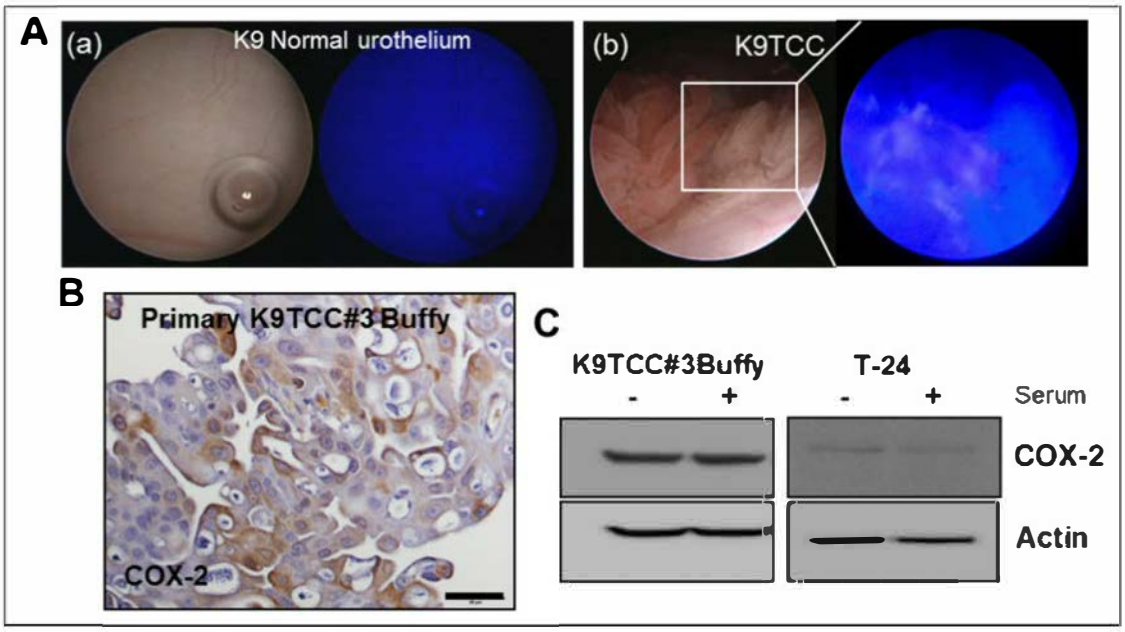


Figure 2. Specific uptake of fluorocoxib A by COX-2-expressing K9TCC in vitro and in vivo. ***(A)*** *Chemical structure of fluorocoxib A* ***(B)*** *Fluorocoxib A uptake by K9TCC in vitro.* ***(C)*** *Specific uptake of fluorocoxib A by COX-2 expressing K9TCC#2Dakota in tumor (green circle) as compared to normal organs from top left column: tumor, lung, pancreas, middle column: heart, liver, kidney, right column: fat, background, blood. Mice inoculated with (a) K9TCC#2Dakota after administration with fluorocoxib A, (b) K9TCC#2Dakota after administration with celecoxib 4 h before administration of fluorocoxib A, and (c) h-UMUC-3 cells after administration with fluorocoxib A.* ***(D)*** *Quantitative evaluation of averaged normalized radiance to blood from imaged tissue specimens (p<0.01**).* ***(E)*** *COX-2 and E-cadherin expressions in dissected K9TCC and UMUC-3 xenograft tumors by IHC. Objective 20x with scale bars 100μm. (F) COX-2 expressions in K9TCC#2Dakota and UMUC-3 cells treated with/without serum for 24 hours by WB. Actin was used as loading control.*

To evaluate the feasibility of our plan to evaluate novel optical imaging agents in dogs diagnosed with naturally occurring tumors during cystoscopy in vivo, we first performed pharmacokinetic and safety study of single dose of i.v. administration of fluorocoxib A in normal Beagle dogs (6, 7). We confirmed specific uptake of fluorocoxib A by COX-2 expressing colorectal carcinoma with no uptake by normal mucosa of colon during in vivo colonoscopy in dog, as we published in 2012 in Biomedical Optics (6, 7). No uptake of fluorocoxib A was observed by normal mucosa of bladder urothelium ***(Figure 3Aa)****, and specific fluorocoxib A uptake by K9TCC*



*during cystoscopy in five K9TCC cases **(Figure 3Ab)** in our published pilot study in Cancer Prevention Research in 2013 by Cekanova et al. (7). Specific COX-2 expression in K9TCC#3Buffy was confirmed by IHC **(Figure 3B)** and WB **(Figure 3C)** analysis. Human bladder T24 carcinoma cells were used as control for COX-2 expression by WB analysis **(Figure 3C)**.*

***Figure 3. Specific fluorocoxib A uptake during cystoscopy by COX-2 expressing bladder cancer in dogs. (A)** No detection of fluorocoxib A was observed by normal canine urothelium in contrast to (B) specific fluorocoxib A uptake during cystoscopy by COX-2-expressing bladder cancer 24 h after administration (1mg/kg, i.v.). (B) Confirmed COX-2 expression in K9TCC#3Buffy xenograft tumor by IHC and (C) in cells by WB analysis. Human T-24 TCC cells were used as control for COX-2 expression. Actin was used as loading control.*

C.2. Research Plan and Design:

Precise and early detection of tumors is important for improving the survival of patients with bladder cancer. There is currently no diagnostic method recommended for detection of early bladder cancer in individuals at average risk. Bladder cancer is diagnosed by microscopic examination of cells from urine, or bladder wall during cystoscopy. However, conventional cystoscopy is not able to identify bladder cancer at the early stages, but only at advanced stages (12). Together with colleagues from Vanderbilt University, we have conducted proof-of-principle studies exploring the usage of a new class of imaging agents targeting COX-2 to detect tumors in *vitro* and *in vivo* in a preclinical settings using rodent models (4-6). *Encouraged that fluorocoxib A could be used to image COX-2 expression in xenograft tumor originated from human (5) and canine cell lines (7), and in dogs with naturally-occurring colorectal carcinomas (6) and TCC (7), we now propose an essential series of translational studies that will bridge the gap between proof-of-principle and clinical application. We hypothesize that fluorocoxib A might be able to detect COX-2 expressing bladder dysplasia, CIS, and TCC. In this study, we hypothesize that fluorocoxib A can detect changes in COX-2 expression by different treatment in primary K9TCC cells in vitro as well in the athymic nude mice model in vivo. Our proposed study will help to efficiently translate optical imaging agent into clinical applications for early detection of bladder cancer. Fluorocoxib A-enhanced imaging can also be used to monitor the responses of bladder cancer to therapy. To evaluate our hypotheses, we propose the following specific aims:*

***C.2.1.SPECIFIC AIM 1:* Determine the ability of fluorocoxib A to detect the early bladder neoplasias using a mouse model of nitrosamine-induced bladder cancer.**

i. *Detect fluorocoxib A uptake in carcinogen-induced bladder pre-neoplastic urothelium in mice at 12 and 18 weeks after N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) administration.*
ii. *Compare fluorocoxib A uptake and Cox-2 expression between normal and pre-neoplastic tissues of the bladder.*

C.2.1.1. Methods, Procedures, and Data Analysis for SPECIFIC AIM 1:

To evaluate the ability of fluorocoxib A to specifically bind to Cox-2 expressing early stages of bladder cancer we propose to utilize an established mouse model. We propose this mouse model, because dogs show clinical signs of obstruction of the ureters to the bladder or outflow from the bladder due to already developed

TCC carcinoma. The early stages of bladder neoplasia, such as dysplasia and CIS, will be evaluated using well-established carcinogen-induced bladder cancer mouse model that overexpress Cox-2 and responds to the cancer prevention by celecoxib (60, 76). This model mimics human bladder CIS and dysplasia as early stages during tumorigenesis.

Fluorocoxib A will be synthesized in Dr. Marnett's laboratory by Dr. Jashim Uddin from Vanderbilt University according to GLP (4) and shipped to laboratory of PI, Dr. Cekanova to UTCVM overnight on dry ice. Fluorocoxib A will be reconstituted to solution of 5 mg/ml under sterile conditions using a biosafety hood and filtering before administration to animals. The dose 1 mg/kg of fluorocoxib A is safe for use according to our previously published studies. Fluorocoxib A uptake will be detected 24 h after administration.

B6D2F1 Mice: All animal experiments will be performed in accordance with approved UTIACUC protocols. The thirty female 3-4 weeks-old B6D2F1 mice (Taconic, Hudson, NY) will be used to assess fluorocoxib A ability to detect early stages, such as bladder dysplasia and CIS. At 5-week of age, mice will be randomly divided into three groups (n=10/group). Group 1, will be the control that receives only tap water. Two other groups of mice will be exposed to BBN for 12 weeks (Group 2, n=10 mice) and 18 weeks (Group 3, n=10 mice). Carcinogen N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) will be obtained from TCI America (Portland, OR) and will be supplied ad libitum at 0.05% in drinking water to mice from 5 to 12 or 18 weeks of age. CAUSION with CARCINOGEN: Access and handling of BBN carcinogen will be allowed only by personnel who were trained to work with chemical hazardous materials wearing appropriate personal protective equipment in laboratory of Dr. Cekanova. Standard operating procedures for BBN will be developed for Dr. Cekanova's laboratory by the UT chemical safety officer, Dr. Susan Fiscor. Water consumption will be recorded to determine BBN intake and compared between groups. After BBN treatment, fluorocoxib A will be administrated i.v. (1 mg/kg) and followed by imaging of the bladder neoplastic lesions. After performed whole body imaging, the mice will be sacrificed and bladder will be dissected, imaged ex vivo using IVIS system. Bladders (n=3) will be fixed in 10% formalin and processed for histology and immunohistochemistry (IHC) analysis, additional bladders (n=3) from other three mice will be placed to O.C.T. media for immunofluorescence (IF) detection by confocal microscopy and additional bladders isolated from last four mice (n=4) will be kept in RNA later and stored at -80°C freezer until Western blotting (WB) analysis. If the mouse bladders will be of sufficient size to be able to cut them in half, we will randomly divide bladders between each analysis (IHC, IF, and WB).

To evaluate the ability of fluorocoxib A to detect Cox-2-expressing bladder neoplastic lesions, optical imaging will be performed 24 h following the i.v. administration of 1mg/kg fluorocoxib A. A control group will be used together with Group 3 at week 18. Optical imaging using fluorocoxib A in mice: Optical imaging using whole body imaging will be performed in accordance with standard veterinary care and under IACUC protocol. Whole body imaging using IVIS system will be used to detect fluorocoxib A uptake by bladder dysplasia and CIS at 12 and 18 weeks after BBN treatment. Mice will be anesthetized using Ketamine/Xylazine (see details in Vertebrate Animal section). After whole body imaging mice will be euthanized and bladder will be dissected, and imaged ex vivo using Xenogen IVIS Lumina with DsRed filters with excitation 500 to 550 nm and emission 575 to 650 nm and background 460 to 490 nm. Obtained total flux (p/s) and averaged radiance (p/s/cm2/sr) of labeled regions of interest (ROI) of dissected bladder and other tissues (kidney, liver, lung, and pancreas) will be analyzed. The fluorocoxib A uptake will be correlated with the presence of CIS and dysplasia lesions of bladder and intensities of obtained areas will be compared to normal bladder of control group mice. The CIS and dysplasia will be confirmed by a board-certified veterinary pathologist for tumor grading.

To evaluate the expression of Cox-2 and other molecules responsible for bladder carcinogenesis, we will perform IHC, IF, and WB analysis of obtained tissue samples. Immunohistochemistry: IHC analysis for Cox-2 expression will be conducted using formalin-fixed and paraffin-embedded tissue samples from obtained *bladder containing pre-neoplastic lesions.* The tissue samples will be examined for *Up1a (uroplakin),* Cox-2, Cox-1 and other proteins of the arachidonic acid pathway (prostaglandin E receptors), *Ki67, p53,* proteins of receptor tyrosine kinases (RTK) pathways including mitogen activated protein kinases (phospho-Raf-1 and phosphorylated extracellular signal-regulated kinase 1/2, Erk1/2) (39). This technique is well established in laboratory of PI (39, 83). Briefly, the sections will be deparaffinized and after blocking, the primary antibodies will be applied and incubated with the super sensitive detection system (BioGenex). The reaction will be developed using the peroxidase-substrate diaminobenzidine. The nuclei will be counterstained using hematoxylin. Also H&E staining will be performed for histological evaluation. The images of staining slides will be captured by Leica DMRB microscope with an attached DP72 Olympus camera. The stained slides will be reviewed by PI, and the staining intensities of antigen in normal and pre-neoplastic lesions will be scored as either absent (-), low (+, less than 20% of positive tumor cells), moderate (++, 20-50% positive tumor cells), or strong (+++, more than 50% positive tumor cells) for quantitative analysis.

Immunofluorescence (IF) staining of biopsy samples and fluorescence confocal microscopy - The O.C.T. embedded tissue samples will be cut using cryostat at 7µm thickness and will be used for double staining (for Cox-2, and fluorocoxib A uptake) and counterstained with DAPI following the standard protocol for IF staining *in vivo (7),* (84),(85). Briefly, the primary antibody against Cox-2 will be applied and detected using secondary antibody Alexa Fluor488. The nuclei will be counterstained with DAPI staining (blue color). To minimize bleaching of the fluorescence, the tissue samples will be kept in the dark covered by aluminum foil. *The expression levels of Cox-2 (green color) and fluorocoxib A uptake (red color) will be analyzed by fluorescent confocal microscope Leica SP2 as we described and published previously, Cekanova et al., in 2013 (7). The confocal microscope is available to PI in laboratory of Dr. Dunlap at the Center of Environmental Biotechnology in Knoxville UT.*

Western blotting (WB) analysis: The proteins of Cox-2, receptor tyrosine kinases (RTK) signaling pathways will be detected in normal and pre-neoplastic tissues by WB as described by Cekanova et al (39). Briefly, equal amounts of protein (50-100 µg) will be applied to electrophoresis in 8 - 12% polyacrylamide gel. Proteins will be transferred onto nitrocellulose membranes. After blocking, the membrane will be incubated with the primary antibody overnight at 4°C followed by incubation with secondary antibodies. Immunoreactive bands will be detected using a chemiluminescent reaction via autoradiography. Relative densities of the bands will be determined by Scion Image software. We will focus on detection of following proteins: Cox-2, Cox-1, RTKs, EPs, MAPKs, β-catenin signaling pathways, and UP1 markers of bladder cancer (86).

Statistical analysis: Mean values and standard errors from WB densitometric readings from three independent experiments will be analyzed by Student t test. The IHC and fluorocoxib A correlation data will be statistically evaluated using a nonparametric two-tailed paired t test, one-way ANOVA test and Fisher's exact test. Differences will be considered statistically significant in all analysis if a P value is <0.05.

C.2.1.2. Discussion of expected results: *We expect to detect specific uptake of fluorocoxib A by bladder pre-neoplastic tissues and no uptake of fluorocoxib A by normal urothelium. The uptake of fluorocoxib A should correlate with the histologically confirmed bladder pre-neoplastic tissues and by confocal fluorescence imaging overlapping with Cox-2 expression. We expect to detect the uptake of fluorocoxib A in regions of high expression of Cox-2. We expect positive correlation of the Cox-2 expression with other markers, such as RTKs and Ki67 by pre-neoplastic bladder lesions.*

C.2.1.3. Potential pitfalls and alternative approaches: *There are some potential challenges in this approach of proving the specificity of fluorocoxib A uptake by early bladder pre-cancerous lesions. First, we cannot predict the sensitivity of IVIS system to detect very small (1-2 mm) sizes of dysplasia and CIS with over-expressed Cox-2 to produced sufficient accumulation of fluorocoxib A and production of sufficient fluorescent signal. The alternative approaches might be a use of rat BNN-induced bladder carcinogenesis model or increase dose of fluorocoxib A to 2 mg/kg that has been show safe for administration in rodent models. Rat model of carcinogen-induced bladder cancer using Fisher 344 rats (Taconic) is well characterized (8-11).*

C.2.1.4. Personal roles: *We have established a unique multi-disciplinary collaboration to apply knowledge from basic molecular (Dr. Cekanova) and organic chemistry research (Drs. Uddin and Marnett) using optical imaging (Drs. Cekanova, Uddin, Bartges, and Manning) to the veterinary medicine (Dr. Bartges) as a valuable translational team for cancer research. This combination of expertise will optimize the likelihood of developing clinically useful probes for detecting of bladder cancer. For this specific Aim#1, Dr. Cekanova, as PI of this project will be responsible for design, assistance and mentoring the student, analyzing data, and writing the manuscripts and progress reports. PhD/MS student of Dr. Cekanova's lab will be responsible for performing in vitro and in vivo experiments. The PhD/MS student will be enrolled in UTCEM program and will be responsible for presenting the obtained results at a scientific research symposium or meeting, such as AACR, WMIC, UTCEM, and write the initial manuscript. The Ph/MS student will have opportunity to benefit from this multidisciplinary collaboration study and will have opportunity to interact with the experts in this cancer research field. Dr. Uddin (VU) will synthesize and provide fluorocoxib A for in vitro and in vivo experiments. Dr. Uddin will assist us to resolve potential pitfalls of experiments using fluorocoxib A. Dr. Manning (VU consultant) will assist with analysis of obtained images and will help to solve potential pitfalls. The results will be discussed by Drs. Cekanova with PhD/MS student during weekly laboratory meetings. The meeting between PIs of UT and VU will be conducted once per year to plan and discuss the obtained results.*

***C.2.2.SPECIFIC AIM 2:* Use fluorocoxib A to detect K9TCC in a dog cancer model.**

i. *Detect fluorocoxib A uptake during cystoscopy by COX-2-expressing heterogeneous urinary bladder carcinomas that occur naturally in dogs. Compare fluorocoxib A uptake and COX-2 expressions between normal and bladder carcinoma tissues.*

ii. *Determine the ability of fluorocoxib A to predict responses to tyrosine kinase inhibitors and novel COX-2 inhibitors therapies using primary K9TCC in vitro and using COX-2 expressing primary K9TCC xenograft mouse models in vivo.*

Development of new tumor-specific molecular imaging agents is needed for advancing the early detection of cancer. Cancer is usually detected when structural changes in a tissue or organ have occurred. Molecular imaging may allow, in ways not previously possible, the dynamic underlying causes of disease, while providing better treatment monitoring. *Our proposed study using fluorocoxib A in canine cancer models will promote the translation of this optical imaging agent into clinical applications for detection, monitoring the responses to therapy, and identifying the tumor margins of bladder carcinomas in people. The canine bladder cancer at time of diagnosis has predominantly higher grade of invasive tumors that spreads through the bladder wall to lymph nodes and to other organs, such as liver and lung predominantly.*

C.2.2.1. Methods, Procedures, Data Analysis for SPECIFIC AIM 2:

Fluorocoxib A uptake by heterogeneous K9TCC during cystoscopy - All cystoscopy and imaging procedures in client-owned dogs will be conducted by a board-certified veterinary internal medicine specialist (Dr. Bartges) in accordance with standard veterinary care and UT IACUC-approved protocol as described previously (6, 7). The client-owned female dogs (n=15) with symptoms for bladder cancer will be enrolled in our study. The owners will sign a consent form to agree to enroll their pets to this study to evaluate fluorocoxib A uptake in bladder cancer during cystoscopy. Fluorocoxib A will be administrated i.v. 1 mg/kg over 20 minutes using a catheter, followed by up to 24-hour uptake by COX-2--expressing bladder cancers. The concentration of fluorocoxib A (1 mg/kg) was determined on the basis of results from our pharmacokinetic and safety studies (6, 7). Cystoscopy will be performed using 1- or 2.7- mm 0- or 30-degree 18-cm rigid endoscope (Karl Storz Veterinary Endoscopy) attached to a Tricam SLII camera (Karl Storz), and a D-Light AF light source (Karl Storz). Images (still photographs and video) will be captured on an Aida DVD-M (Karl Storz). Cystoscopy will allow us to obtain the biopsy samples that will be used for histological and molecular analysis, as well for establishment of primary K9TCC cell lines to further evaluate the molecular targets for new therapeutic treatments of bladder cancer. The client-owned dogs will be returned to owners after complete recovery from the cystoscopy and anesthesia.

The fluorescent intensity projected from the surface of bladder carcinoma will be analyzed using Image J software. To improve the detection system of fluorocoxib by PDD system of Karl Storz equipment, we purchased and had installed the customer-designed new filters in camera and inside the light source for specific fluorocoxib A wavelengths detection of modified PDD imaging system. We are aware of the limitation of the current endoscopy system as signal is captured only as image (still picture) without any information about radiance or total flux signal information as we can obtain from IVIS whole body imaging system. Saying that, we will be able to correlate and semi-qualify the obtained images from neoplastic vs normal bladder mucosa using Image J software based on densitometry data analysis. We have already performed a pilot study to evaluate the specificity of fluorocoxib A uptake by bladder cancers and confirmed no uptake of fluorocoxib A by normal urothelium as shown in ***Figure 3Aa*** *(7). Our preliminary data show fluorocoxib A uptake by bladder TCC (7); however we still need to answer several questions, such as: Is a time and route of administration for fluorocoxib A uptake optimal? Can we improve the sensitivity of signal with new filters? This study will help us to answer these important questions.*

Isolation, establishment, and characterization of primary K9TCC cells: We will establish primary K9TCC cell lines (n=10) to further evaluate the specificity of fluorocoxib A uptake by COX-2-expressing cancer cells *in vitro*. This technique is well established in the laboratory of PI, Dr. Cekanova and we've successfully established several primary canine carcinoma cell lines *(7)*. Briefly, the tissue samples (5x5x5mm) will be washed in PBS containing antibiotics and then minced using a scalpel. After 10 min digestion by trypsin/EDTA solution, the released cells will be re-suspended into complete media (RPMI-1640 supplemented with 10% FBS, Pen/Strep) and passed through a 100µm-pore size cell strainer. The cells will be washed in complete media and plated into tissue culture flasks containing 10 ml of fresh complete media. The growing cells in monolayer with characteristic epithelial morphology will be maintained continuously *in vitro* and will be further characterized by epithelial markers. *The established primary K9TCC cells will be used to assess the response*

to new therapeutic drugs, such as Masitinib® (AB Sciences, France) and novel NSAIDs by proliferation MTS, WB, and apoptosis assays.

Immunofluorescence staining of biopsy samples and fluorescence immunocytochemistry - The O.C.T. embedded tissue samples will be cut using cryostat at 7µm thickness and counterstained with DAPI and double-stained following the standard protocol for IF staining (84). For fluorescence immunocytochemistry, the established primary cancer cells will be plated on 4-chambers slides, and after reaching 70-80% confluence, the K9TCC cells will be treated with various concentration of fluorocoxib A and fixed in various time points. After fixation, the canine cancer cells will be stained with COX-2 antibodies following the standard IF protocol as described in more detail in the manuscript (85) *(7)*. Briefly, the primary antibody against COX-2 will be applied and detected using secondary antibody Alexa Fluor488. The nuclei will be counterstained with DAPI staining (blue color). The expression levels of COX-2 (green color) and fluorocoxib A uptake (red color) will be analyzed by fluorescent confocal microscopy Leica SP2. To minimize bleaching of the fluorescence, the tissue samples will be kept in the dark covered by aluminum foil.

The effects of receptor tyrosine kinase (RTK) inhibitors (RTKI e.g. *Masitinib*, ZD-1839, GW2016) and novel NSAIDs (new derivatives of indomethacin and celecoxib) on cell proliferation of K9TCC by MTS assay: MTS assay will be used to assess the effect of the several clinically used *RTK inhibitors* alone or in combination with novel derivative of NSAIDs in established K9TCC cells in time- and dose-dependent manners. The most effective treatment will be further evaluated using xenograft nude mice model *in vivo.*

Western blotting (WB) analysis: *Based on our preliminary results using primary K9 oral squamous cell carcinoma cell lines where RTKI affected the expression of COX-2 in vitro, we propose to evaluate the d*etection of the key proteins of COX-2 and RTK signaling pathways after treatment with tyrosine kinase inhibitors and novel derivatives of NSAIDs in a time- and dose-dependent manner using K9TCC cell lines. We will further focus on the expression levels of *β-catenin signaling pathway, and UPs* (86).

K9TCC xenograft tumors using athymic mice model: *Thirty* female athymic nude mice between 3-5 weeks of age will be s.c. inoculated with representative primary *COX-2-expressing K9TCC (n=3)* to monitor early responses to therapy using the most effective treatment of tyrosine kinase inhibitor and/or novel non-steroidal anti-inflammatory drugs based on the results obtained from *in vitro* experiments. After tumors are 7 – 8 mm the mice will be further divided into two groups, as control and treatment group of five mice per group. The treatment *(Masitinib with combination with NSAIDs)* will be administrated i.p. twice per week for three weeks, followed by i.v. administration of fluorocoxib A 1mg/kg and imaged 3-4 hours by IVIS system. Tumor volume will be compared between treated and untreated groups and correlated with fluorocoxib A uptake.

Collection and histologic analysis of tissue: Collected tumors will be further imaged *ex vivo* by IVIS system and a part will be collected for histology analysis and stored in formalin, the second part will be embedded into OCT, the third part will be placed in RNA*later* solution and kept in -80C until further WB analysis. The small piece of tissues will be also taken and analyzed by HPLC to detect fluorocoxib A in tissue to correlate its uptake with COX-2 expression.

HPLC analysis: To quantify the fluorocoxib A uptake, we propose to perform HPLC analysis of fluorocoxib A in biopsies obtained during cystoscopy and in xenograft tumors. The tissue samples will be analyzed via reversed phase HPLC-UV using a Phenomenex 10 x 0.2 cm C18 or a Phenomenex 7.5 x 0.2 cm Synergi Hydro-RP column held at 40°C. This is a well-established procedure by Dr. Uddin.

Statistical analysis: Mean values and standard errors from WB densitometric readings of three independent experiments will be analyzed by Student t test. The IHC and fluorocoxib A correlation data will be statistically evaluated using a nonparametric two-tailed paired t test, one-way ANOVA test and Fisher's exact test. Differences will be considered statistically significant in all analysis if a P value is <0.05.

C.2.2.2. Discussion of expected results: *We expect to detect the COX-2 expressing K9TCC during cystoscopy using fluorocoxib A. Signal generated from fluorocoxib A during cystoscopy and detected by PDD system will be correlated with the expression levels of COX-2 in obtained biopsy to confirm specificity of fluorocoxib A uptake. The established primary K9TCC cells will allow us to assess the response to of bladder cancer therapy in vitro. The xenograft mice model will allow us to evaluate the ability of fluorocoxib A to detect response of K9TCC tumors to therapy in vivo. This approach will assist us to progress with personalized treatment for canine patients diagnosed with TCC as well better translate the use of fluorocoxib A for possible use to detect the responses to therapy in people.*

C.2.2.3. Potential pitfalls and alternative approaches: Inflammatory sites might be detected and give us false-positive signal from fluorocoxib A uptake due to presence of macrophages (inflammation with macrophages), therefore biopsy samples obtained during cystoscopy *of dogs* will be further analyzed to

confirm the histology of obtained samples. *We don't expect problems acquiring sufficient numbers of K9TCC cases based on actual yearly caseload to UTCVM; however we need to keep in mind that some suspected cases of K9TCC may have inflammatory conditions only. If needed we can further advertise our study to increase the case load to have sufficient number of cases enrolled. We don't expect to have a problem detecting fluorocoxib A using PDD system during cystoscopy, however new filter system needs to be evaluated. In case we do not detect the fluorocoxib A in the COX-2 positive bladder carcinomas in dogs after 24 hours, we might increase the fluorocoxib A dose to 2 mg/kg or change the time of fluorocoxib A uptake to 4-6 hours. The other alternative approach will be to change the route of fluorocoxib A administration from systemic i.v. administration to intravesicular bladder administration using catheter. Additional potential approach to this alternation could be modification of uptake time of fluorocoxib A to 30 min after intravesicular administration. We are aware of that not all K9TCC bladder tumors will be positive for COX-2 expression as well the heterogeneity for COX-2 expression by naturally occurring K9TCC may play important role in ability of fluorocoxib A to produce sufficient signal. We have observed the expression pattern of the COX-2 positive K9TCC cells at the periphery of the tumor by IHC, suggesting that fluorocoxib A should be able to detect the margins of the heterogeneous bladder K9TCC. Use of more than one molecular imaging agent ("single bullet") might be more appropriate for detection of heterogeneous bladder cancers; however this study needs to evaluate the specificity and sensitivity of fluorocoxib A to detect K9TCC as single imaging agent before combination with other imaging agents is proposed for future studies.*

C.2.2.4. Personal roles: *For specific Aim#2 the personal roles will be similar as described in details in Specific Aim#1, with addition that Dr. Bartges (veterinary internal medicine specialist) will perform cystoscopy in client-owned dogs diagnosed with bladder K9TCC.*

D. SUMMARY

We propose to use carcinogen-induced urinary bladder tumorigenesis mouse model to investigate the uptake of fluorocoxib A at the early stages of bladder tumorigenesis (8-11). Canine model will allow us to study specificity of fluorocoxib A uptake to detect late stages of COX-2 expressing bladder K9TCC. Our proposed study using fluorocoxib A in mouse and canine TCC models will assist in translating this optical imaging agent into clinical applications for early detection of bladder cancer, as well as for monitoring the responses to therapy, tumor margins, and recurrence of bladder cancer.

E. TIME LINE

This project will take 3 years to complete. The bladder carcinogenesis mouse experiments using fluorocoxib A uptake to detect early stage of bladder cancer will begin in year 1. Analysis of the specific uptake of fluorocoxib A and Cox-2 expressions by bladder lesions of mouse model will be performed in year 1. Additional sequence of studies to detect bladder lesions in dogs using fluorocoxib A, along with collection of biopsy to analyze correlation of fluorocoxib A uptake with COX-2 expression and establishing the primary K9TCC cell lines will be performed in year 1 and 2. Optimization of treatment for K9TCC cells using novel RTK inhibitors and NSAIDs alone or in combination in vitro will be done during year 2. Analysis of K9TCC xenograft nude mice experiments will assess the efficacy of fluorocoxib A to detect responses to therapy in vivo would occur at the end of year 2 and in first half of year 3, followed by analysis. The PIs will communicate by e-mails, phone and we will hold personal or videoconference meetings between PIs of UT and VU once per year to plan and discussed the obtained results. Results will be presented at scientific meetings by the beginning of year 2 and at the end of year 3. Manuscripts will be submitted at the same time frame.

F. IMPACT

Our overall hypothesis is that fluorescence imaging of COX-2 expression will allow earlier detection of tumors, when interventions can be more successful, as well as improved detection of disease margins that may allow complete resection. Improved biomarkers for cancer screenings and tumor surveillance, which can rapidly and cost effectively be deployed into clinical populations are critically needed. The early detection of many types of cancer results in improved patient's prognosis and long-term survival. Despite the prevalence of white light for screening used in the clinic, it is widely assumed that the sensitivity and specificity of cystoscopy can be significantly improved with the use of contrast agents that specifically accumulate in tumors.

Our proposed study address this issue by evaluating fluorocoxib A in rodent and canine cancer models to help to translate this imaging probe into clinical applications for the early detection of *bladder* cancer, as well for monitoring of the early responses to therapy to prolong survival of the cancer patients. In our study we

propose to use the model of *bladder dysplasia using rodent model of bladder carcinogenesis* to monitor the early stages of tumorigenesis and ability of fluorocoxib A to detect the early responses to treatment using canine primary *K9TCC* cancer cells. *The distribution of Fluorocoxib A by Caliper Life Sciences A for preclinical imaging studies is evidence of the intense research interest in imaging COX-2 expression non-invasively. These proposed studies will add information that will complement the clinical trial data and for FDA approval process.* In addition, our work will assist in characterizing the molecular features *bladder* cancer to further develop other imaging agents and therapeutic drugs. Fluorocoxib A is one of the targeted *bladder* cancer specific imaging agents and if approved it will be the first cancer-targeted one for human clinical use.

Although we only evaluated fluorocoxib A in bladder cancer; data obtained from this study can be useful for testing of fluorocoxib A in other types of COX-2-expressing cancers, such as breast, lung, pancreas, head and neck, and prostate carcinomas in dogs and people. In our previously published study, we have shown a specific uptake of fluorocoxib A in canine colorectal adenocarcinomas during colonoscopy (6) *and K9TCC during cystoscopy of dogs in vivo (7).* Exploration of this approach in the dog model provides an opportunity to rapidly translate the use of fluorocoxib A to clinical use in people for detection of COX-2-expressing tumors. Fluorocoxib A may help to improve the visualization of COX-2-expressed *bladder* cancers and help to identify appropriate patients for NSAIDs treatment.

This proposal will lay the framework for enhanced collaborations in translational imaging science between investigators from the University of Tennessee (UT) and Vanderbilt University (VU) and impart a major impact on the scientific atmosphere of the UT. This project will promote scientific environment to attract high-quality student to graduate program to successfully accomplish the proposed research.

G. FUTURE APPROACH

Our collaboration with Drs. Marnett's and Manning's research team at VU with our veterinary clinic at UTCVM allows us to further investigate newly synthesized imaging agents using dogs with naturally-occurring tumors as valuable animal models for preclinical evaluation of imaging agents. The research team of Dr. Marnett's laboratory at VU synthesizes new fluorescent- and radio-labeled derivatives of NSAIDs, as new generation of NIR and PET probes that enable deeper detection and monitoring of tumors. *We have access to PET/CT imaging at our UTGSM facility in Knoxville based on our long-time collaboration with Dr. Jonathan Wall.* PET/CT imaging using NSAID-labeled tracers can be further evaluated to detect the metastatic sites of tumors. The synthesis and evaluation of new generations of stable, COX-2 selective, NSAIDs are currently under intensive investigation at VU, but not available for preclinical testing yet using dog model.

H. STUDENT GRADUATE PROGRAM

Dr. Cekanova developed a multi-disciplinary collaboration with veterinary specialists at UT Veterinary Medical Center in Knoxville and Dr. Marnett's research group and imaging group at VUIIS of VU to evaluate novel imaging agents for detection of tumors in rodent models and naturally-occurring cancers in dogs as a more advance translational animal models. Dr. Cekanova has a suitable environment to successfully accomplish this project. The facility and equipment of Dr. Cekanova are mentioned in details below. The additional facilities available to Dr. Cekanova though VU are mentioned below also in Drs. Manning's and Uddin's (VU) letters of support to provide access to the full resources of the Molecular Imaging Research program at VU which includes all the relevant imaging hardware housed within VUIIS and relevant imaging expertise. Dr. Cekanova has a record of teaching excellence and facilities to successfully mentor additional graduate/undergraduate students. Projects supported by UTCVM and VU in last three years allowed Dr. Cekanova to accept five female undergraduate students and three female DVM summer students to have their first-hands-on research experience and to learn about the molecular and cellular cancer research. Dr. Cekanova has actively trained and co-mentored undergraduate researchers since 2009, Ms. Amanda Carter, an MS student, who has joined health-related program to pursuit MD degree at UT Medical School in Memphis. Dr. Cekanova served as a mentor for not only mentioned undergraduate students and DVM graduate students, but she was assigned as a major PhD mentor and she is a major mentor for the post-doctoral research associate, Dr. Rathore, who joined her laboratory in 2012. The collaboration with VUIIS in Nashville will allow Dr. Cekanova to gain new experiences and to attract high-quality graduate student to perform the propose study. The MS/PhD student will have a chance to interact with several investigators and learn new techniques at UT as well as VU.

Vertebrate Animals

1. Provide a detailed description of the proposed use of the animals in the work outlined in the Research Strategy section. Identify the species, strains, ages, sex, and numbers of animals to be used in the proposed work.

In our study, we propose to evaluate the new optical agent fluorocoxib A in mouse and canine models to detect early stages of bladder cancer. The proposed work involving dogs and mice have been already approved by the University of Tennessee Institutional Animal Care and Use Committee (UT IACUC protocols #1964 and #1892) and are in accordance with NIH guidelines. Additional amendment to #1982 IACUC protocol to use B6D2F1 mice will be submitted after approval of project by NIH as part of JIT request.

EXPERIMENT A: Determine the ability of fluorocoxib A to detect the early bladder neoplasias using a mouse model of nitrosamine-induced bladder cancer.

Species: *Mus musculus*
Strains: *B6D2F1 mice*
Ages: *3-4 weeks-old mice*
Sex: *Female*
Total Numbers of Mice: *N = 30 (F)*

Thirty female 3-4 weeks-old B6D2F1 mice (Taconic, Hudson, NY) will be used to assess fluorocoxib A ability to detect early bladder dysplasia and CIS. At 5-week of age, mice will be randomly divided into three groups (n=10/group). Group 1, will be the control that receives only tap water.Two other groups of mice will be exposed to BBN for 12 weeks (Group 2, n=10 mice) and 18 weeks (Group 3, n=10 mice). Carcinogen N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) will be obtained from TCI America (Portland, OR) and will be supplied ad libitum at 0.05% in drinking water to mice from 5 to 12 or 18 weeks of age. CAUSION with CARCINOGEN: Access and handling of BBN carcinogen will be allowed only by personnel who were trained to work with chemical hazardous materials wearing appropriate personal protective equipment. Standard operating procedures for BBN will be developed for Dr. Cekanova's laboratory by the UT chemical safety officer, Dr. Susan Fiscor. Water consumption will be recorded to determine BBN intake and compared between groups. After BBN treatment, fluorocoxib A will be administrated i.v. (1 mg/kg) and followed by imaging of the bladder neoplasias. A control group will be used together with Group 3 at week 18. Optical imaging using whole body imaging will be performed in accordance with standard veterinary care and under IACUC protocol. Whole body imaging using Xenogen IVIS Lumina with DsRed filters with excitation 500 to 550 nm and emission 575 to 650 nm and background 460 to 490 nm will be used to detect fluorocoxib A uptake by bladder dysplasia and CIS at 12 and 18 weeks after BBN treatment. Mice will be anesthetized using Ketamine/Xylazine (see details below). The fluorocoxib A uptake will be correlated with the presence of CIS and dysplasia lesions of bladder and intensities of obtained areas will be compared to normal bladder of control group mice. The CIS and dysplasia will be confirmed by histologic analysis by a board-certified veterinary pathologist for tumor grading. After whole body imaging by IVIS system, mice will be euthanized at mentioned time points and bladder will be dissected, and imaged ex vivo using. Obtained total flux (p/s) and averaged radiance (p/s/cm2/sr) of labeled regions of interest (ROI) of dissected bladder and other tissues (kidney, liver, lung, and pancreas) will be analyzed. Bladders (n=3) will be fixed in 10% formalin and processed for histology and immunohistochemistry (IHC) analysis, additional bladders (n=3) from other three mice will be placed to O.C.T. media for immunofluorescence detection by confocal microscopy and additional bladders isolated from last four mice (n=4) will be kept in RNA later and stored at -80°C freezer until Western blotting analysis. If the mouse bladders will be of sufficient size to be able to cut them in half, we will randomly divide bladder between each applications (IHC, IF, and WB).

EXPERIMENT B: Detect fluorocoxib A uptake by COX-2-expressing heterogeneous naturally-occurring urinary bladder carcinomas in vivo during cystoscopy.

Species: *Canis lupus familiaris*
Breed: *note limited; at risk for bladder cancer are Scottish terriers, Shetland sheepdogs, Airedale terries, and collies breeds*

Ages: *3-15 years*
Sex: *Female*
Total Numbers of Dogs: *N = 15*

Our clinic at the University of Tennessee, College of Veterinary Medicine will provide unique access to dogs diagnosed with naturally occurring bladder cancer. In our study, we propose to evaluate fluorocoxib A in dogs with spontaneous heterogeneous bladder tumor as a more realistic translational animal model for human cancer. Fifteen female dogs with age between 3-15 years, with no limits for body weight presenting to UTCVM in Knoxville with ultrasound confirmed TCC will be eligible for enrollment in this study. The enrolled female dogs will be removed from any NSAID therapy for at least one week to clear off all residues of NSAIDs from the body before cystoscopy procedures. Female dogs will be excluded from our study if they are poor anesthetic candidates due to pre-existing cardiac or metabolic diseases.

All cystoscopy and imaging procedures in client-owned dogs will be conducted by a board-certified veterinary internal medicine specialist (J.W. Bartges) in accordance with standard veterinary care with UT IACUC-approved protocol 1964. The client-owned female dogs (n=15) with symptoms for bladder cancer will be enrolled in our study. The owners will sign a consent form to agree to enroll their pets to this study to evaluate fluorocoxib A uptake in bladder cancer during cystoscopy. Fluorocoxib A will be administrated i.v. 1 mg/kg over 20 minutes using a catheter, followed by up to 24-hour uptake by COX-2–expressing bladder cancer. The concentration of fluorocoxib A (1 mg/kg) was determined on the basis of results from our pharmacokinetic and safety studies. Cystoscopy will be performed using a 1- or 2.7-mm 0- or 30-degree 18-cm rigid endoscope (Karl Storz Veterinary Endoscopy) attached to a Tricam SLII camera (Karl Storz), and a D-Light AF light source (Karl Storz). Images (still photographs and video) will be captured on an Aida DVD-M (Karl Storz). Cystoscopy will allow us to obtain the biopsy samples that will be used for histological and molecular analysis, as well for establishment of primary carcinoma cell lines to further evaluate the molecular targets for new therapeutic treatments of bladder cancer. The client-owned dogs will be returned to owners after complete recovery from the cystoscopic examination and anesthesia. The fluorescent intensity projected from the surface of bladder carcinoma will be analyzed using Image J software. To improve the currently used filters in PDD system of Karl Storz equipment, we purchased and had installed the customer-designed new filters in camera as well as inside the light source for specific fluorocoxib A wavelengths detection by Karl Storz Company.

EXPERIMENT C: Determine the ability of fluorocoxib A to predict response to tyrosine kinase inhibitors and novel COX-2 inhibitors therapies using COX-2 expressing primary K9TCC xenograft mouse models in vivo.

Species: *Mus musculus*
Strains: *Athymic Nude mice CrTac:NCr-Foxn1^{nu} from Taconic company*
Ages: *4 -5 wks*
Sex: *Female*
Total Numbers of Mice: *N=30 Athymic Nude mice (F)*

Thirty female athymic nude mice between 3-5 weeks of age will be s.c. inoculated with representative primary COX-2-expressing K9TCC (n=3) to monitor early responses to therapy using the most effective treatment of tyrosine kinase inhibitor and/or novel non-steroidal anti-inflammatory drugs based on the results obtained from in vitro experiments. After tumors are 7 – 8 mm the mice will be further divided into two groups, as control and treatment group of five mice per group. The treatment (Masitinib with combination with NSAIDs) will be administrated i.p. twice per week for three weeks, followed by i.v. administration of fluorocoxib A 1mg/kg and imaged 3-4 hours by IVIS imaging system. Tumor volume will be compared between treated and untreated groups and correlated with fluorocoxib A uptake.

Substance(s) Administered (Gavaging, injections):

Substance Administered	Route	Dose	Concentration	Maximum volume/site	Frequency
Primary K9TCC cell lines	s.c., 1ml syringes with 25/27Gx1/2in needle	0.5-1 x10^6	5-10x10^6/ml in PBS, or matrigel, or fibrin 1:1	0.1 ml	Once

Fluorocoxib A	i.v. 5 or 8 Fr Foley catheter catheter (as alternative route for intravesicular administration into bladder)	1mg/kg	0.5mg/ml (mice) 5 mg/ml (dog i.v.) 5 mg/ml (dog, intravesicullarly into bladder)	0.05ml Up to 5 ml Up to 6 ml of Fluorocoxib and further diluted with sterile fluid (saline) to fill up the urinary bladder	Once
Celecoxib (or other NSAIDs, e.g. indomethacin or its derivatives) or vehicle DMSO	i.p. 1ml tuberculin syringes with 25/27Gx1/2in needle	1-2 mg/kg	0.5-1 mg/ml	Up to 0.05ml	Three times per week for three weeks
AG1478 (or other tyrosine kinase inhibitors,) or DMSO	i.p. 1ml tuberculin syringes with 25/7Gx1/2in needle	1-2 mg/kg	0.5-1 mg/ml	Up to 0.05ml	Three times per week for three weeks

2. Justify the use of animals, the choice of species, and the numbers to be used. If animals are in short supply, costly, or to be used in large numbers, provide an additional rationale for their selection and numbers.

EXPERIMENT A: *The early stages of dysplasia and CIS will be evaluated using well-established carcinogen-induced bladder cancer rodent model that overexpress COX-2 and responds to the cancer prevention by celecoxib (59, 76). This model mimics human bladder CIS and dysplasia stage during tumorigenesis. The number of B6D2F1 mice (n=30 total, n=10 mice/per group, n=3 groups) is based on detecting a signal difference (total flux values detected by IVIS in CIS or dysplasia) at a two-sided 0.05 significance level with 80% power assuming the true difference between treatments is 2.03 times the standard deviation. This is a two-treatment parallel-design study (CIS at each time points vs control group).*

EXPERIMENT B: *In our study, we propose to evaluate fluorocoxib A in dogs with spontaneous heterogeneous bladder tumor as a more realistic translational animal model for human cancer. The number of dogs (n=15 control to its own) is based on detecting a signal difference (detected by PDD system in TCC vs normal urothelium of bladder) at a two-sided 0.05 significance level with 80% power assuming the true difference between treatments is 1.6 times the standard deviation. This is also a two-treatment parallel-design study (TCC or pre-neoplastic lesions vs normal urothelium of same dog's bladder) using*

EXPERIMENT C: The athymic nude mice are one of the immunodeficient mouse models that we selected for our study based on the common use of this mice for human and other species xenograft tumor models. Again 5 mice per group will give us sufficient number to detect changes in response to therapy. *The number of athymic nude mice (n=30 total, n=5 mice/per group, n=2 groups (K9TCC after treatment vs control untreated) n=3 K9TCC COX-2 expressing cell lines) is based on detecting a signal difference (total flux values detected by IVIS in K9TCC after treatment vs control untreated) at a two-sided 0.05 significance level with 80% power assuming the true difference between treatments is 3.8 times the standard deviation. This is also a two-treatment parallel-design study (K9TCC after treatment vs control untreated). (http://hedwig.mgh.harvard.edu/sample_size/js/js_parallel_quant.html).*

3. Provide information on the veterinary care of the animals involved.

All animals at our UT CVM animal facility are kept under standard veterinary care and in all regulations with IACUC and Office of Laboratory Animal Care (OLAC) standards. The UTCVM is also an AAALAC-accredited facility (Association for Assessment and Accreditation of Laboratory Animal Care) for animal research activities and we maintain a reputation for excellence in patient care and animal welfare. All procedures in mice will be performed only by trained and approved personnel in accordance with standard veterinary care and the Institutional Animal Care and Use Committee (IACUC) under protocol #1892 and *#1964*.

4. Describe the procedures for ensuring that discomfort, distress, pain, and injury will be limited to that which is unavoidable in the conduct of scientifically sound research. Describe the use of analgesic, anesthetic, and tranquilizing drugs and/or comfortable restraining devices, where appropriate, to minimize discomfort, distress, pain, and injury.

MICE: Anesthesia (inhaled isoflurane) administrated for optical imaging

	Agent	Concentration	Dose (mg/kg)	Volume	Route	Frequency
Anesthetic	*Ketamine/ Xylazine*	*100mg/ml 20mg/ml*	*Ket 80-120 mg/kg Xyl 5-10 mg/kg*	*body weight dependent*	*IP*	*Once*
Anesthetic	Isoflurane	99.9%/ml	To effect	To effect	Inhalation	Up to 3x

a. Reason for administering agent(s): sedation/restraint/pain/optical imaging/euthanasia
b. For which procedures: for inoculation of cancer cells, administration of fluorocoxib A, whole body imaging and for euthanasia
c. Method of monitoring anesthetic depth, including paralyzing drugs: respiration, toe-pinch to monitor any movement responses
d. Methods of physiologic support during anesthesia and recovery: thermoregulatory support
e. Frequency of recovery monitoring: once
f. Specifically what will be monitored: respiration and movement
g. Potential adverse events: None anticipated as using standard anesthetic protocol; possible cardiopulmonary arrest with anesthesia. In the case of cardiac arrest, we will provide epinephrine or other CPR drugs.

DOGS: *Sedatives (butorphanol/acepromazine) will be used, followed by general anesthesia (propofol/inhaled isoflurane) administrated for routine cystoscopy examination with details described in the following table. NOTE: the exact pre-anesthetic and anesthetic protocol will vary and be dependent on the health status of the animal. A typical protocol is given below; however, we reserve the right to change this protocol on a patient-by-patient basis depending on what is deemed appropriate and best for the patient by the anesthesiologist and by the board-certified internal medicine specialist.*

	Agent	Concentration	Dose (mg/kg)	Volume	Route	Frequency
Pre-emptive analgesic	*Buprenorphine*	*0.3 mg/ml*	*0.03*	*0.3ml*	*IV*	*1 to 2 x q8hr; second dose given only if needed*
Pre-anesthetic	*Butorphanol Acepromazine*	*10 mg/ml 10 mg/ml*	*0.2-0.6 0.1-0.2*	*Depends Depends*	*IV IV*	*1 for each anesthetic episodes*

Anesthetic	*Isoflurane*	*99.9%/ml*	*To effect*	*To effect*	*ET*	*Continuous during the anesthesia, 1 for each anesthetic episodes*
Post-procedural analgesics - dogs	*Meloxicam*	*1.5 mg/ml*	*0.1*	*Depends on size of dog*	*PO*	*PRN**
Post-procedural analgesics - cats	*Butorphanol or Buprenorphine*	*5 mg/ tablet 0.3 mg/ml*	*0.3 0.005-0.03 0.005-0.01*	*½-1 tablet or 0.17-1 ml and 0.17-1 ml*	*PO or PO and IMor SQ*	*PRN* PRN* and 1 x for each anesthetic episode*

**Recognition of Pain and Distress SOP*

a. *Reason for administering agent(s): Standard pre-anesthetic and anesthetic agents for client-owned patients in small animal VTH.*
b. *For which procedures: Cystoscopy for optical imaging*
c. *Method of monitoring anesthetic depth, including paralyzing drugs: Heart rate, blood pressure, respiratory rate, response to stimuli, rectal temperature*
d. *Methods of physiologic support during anesthesia and recovery: IV fluid therapy (crystalloids) and possibly colloid support depending on other parameters (hetastarch)*
e. *Frequency of recovery monitoring: Monitored continuously until extubated and then every 5 to 15 minutes.*
f. *Specifically what will be monitored: Heart rate, respiratory rate and effort, rectal temperature, response to stimuli*
g. *Describe any behavioral or husbandry manipulations that will be used to alleviate pain, distress, and/or discomfort: Patients are recovered in anesthesia recovery area in small animal VTH – this area has subdued lighting and restricted noise*
h. *If using paralytic drugs, scientific justification needed: N/A*
i. *Potential adverse events: None are anticipated from anesthesia – potentials include: cardiopulmonary arrest, prolonged recovery, dysphonia or cough due to tracheal intubation. In the case of cardiac arrest, we will provide epinephrine or other CPR drugs.*

5. Describe any method of euthanasia to be used and the reasons for its selection. State whether this method is consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia. If not, include a scientific justification for not following the recommendations.

MICE: Method of euthanasia:

☒ Anesthetic overdose
Drug: **Isoflurane**
Dose: **Saturated air**
Route: **inhaled**
This method is consistent with AVMA recommendations.

***DOGS: Euthanasia IS NOT** a part of this study. All client-owned dogs will be returned to their owners after recovery from cystoscopy.*

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VANDERBILT UNIVERSITY

Office of Sponsored Programs
Main (615-875-6070) Fax (615-343-2447)

Letter of Intent to Establish an NIH Consortium

Title of Application: _Detection of COX-2-Expressed Cancers by Fluorocoxib A

Applicant/Prime Institution: _University of Tennessee-Knoxville_____

Principal Investigator: Maria Cekanova

Cooperating/Subrecipient Institution: _Vanderbilt University Medical Center_____

Co-Investigator: ___Jashim Uddin_____

Costs requested by Cooperating Institution

Proposed Effective Date: _07/01/2014_____

First Budget Year	7/1/14-6/30/15_	Project Period: 7/1/14-6/30/17_____	
Direct Costs:	_$3,185___	Direct Costs:__$9,555__	
F & A Costs:	_$1,815____	F & A Costs:_$5,445_____	
Total:	_$5,000_____	**Total:**_$15,000_____	

The appropriate program and administrative personnel of each institution involved in this grant application are aware of the National Institutes of Health's Consortium Grant Policy and are prepared to establish the necessary inter-institutional agreement consistent with the Guidelines for Establishing and Operating Consortium Grants. Consortium institution hereby certifies that neither it nor its principals nor those performing services under this Agreement are presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded by any Federal department or agency from participation in this transaction and have not, within the 3 year period preceding this application, been convicted of, or had a civil judgment rendered against them or had any public transaction (Federal State or local) terminated for cause or default. The Consortium institution also agrees that it is registered with the Central Contractor Registration (CCR) database, the primary registrant database for the U.S. Federal Government. The amounts shown above appear in the application; the actual amount awarded to the cooperating institution will be determined after an award is made.

Applicant/Prime Institution

_____
Name of Institution

_____
DUNS

_____
Signature of Authorized Official

_____
Name & Title of Authorized Official

_____
Date

Cooperating/Subrecipient Institution

Vanderbilt University Medical Center
Name of Institution

004413456
DUNS


Signature of Authorized Official

Donald Clinton Brown, Director OSP
Name & Title of Authorized Official

10/15/13
Date

THE UNIVERSITY of TENNESSEE
College of Veterinary Medicine

Office of the Associate Dean for Research

October 18, 2013

National Cancer Institute (NCI)
NIH/ Division of Extramural Research and Training

RE: Institutional Support letter for Dr. Maria Cekanova's application for NIH R15 Academic Research Enhancement Award (AREA) supported by NIH/NCI with title: "Detection of COX-2-expressing bladder cancer by fluorocoxib A."

To whom it may concern,

This letter is to provide our support for Dr. Maria Cekanova's application for an Academic Research Enhancement Award (AREA, PA-13-313) from the National Cancer Institute with title: "Detection of COX-2-expressed cancers by fluorocoxib A." This proposal addresses evaluation of a newly synthesized optical imaging agent, fluorocoxib A for COX-2-expressing bladder cancer. There is still need to investigate novel imaging agents and targets for detection of cancer. In addition, fluorocoxib A will assist to identify the individuals who might benefit from the targeted COX-2 inhibition therapy. Currently, these efforts are limited by the lack of appropriate imaging agents. This proposal addresses these concerns.

AREA program funding in support of Dr. Cekanova's research will strengthen the research environment at the College of Veterinary Medicine, University of Tennessee in Knoxville and provide a graduate student exposure to research in the biomedical sciences. Our college offers MS and PhD degrees in the Comparative and Experimental Medicine (CEM) Graduate Program preparing students for teaching and/or research careers in the health sciences. Currently, the CEM program is training three MS students and thirty PhD students. Dr. Cekanova has experience with mentoring students, as shown in her record in mentoring undergraduate students in past three semesters and one PhD graduate student for one year.

Dr. Cekanova joined our Small Animal Clinical Sciences (SACS) Department as a Research Assistant Professor in April 2009. Her translational research focus is in detection of cancer using optical imaging in dogs and cats with naturally-occurring carcinomas. She has established collaborations with Drs. Lawrence Marnett, Jashim Uddin, and Charles Manning from Vanderbilt University in Nashville, whose research team is developing new imaging agents. In addition, her research focus on utilizing the neutron radiography combined with computed tomography of cancer as an emerging and highly innovative technology, which Dr. Cekanova's team in collaboration with scientists at the Oak Ridge National Laboratories has pioneered over the last year.

We will provide adequate resources from the Department of SACS to ensure the success of this important project. As an independent junior investigator, Dr. Cekanova has sufficient resources and two laboratories with office space (1,020 sq. ft.) to conduct her research. Her 100% research position is currently supported by our college with expectations that her future salary and benefits will be covered at least partially by further independent research funding. The college's Center of Excellence in Livestock Diseases and Human Health

2407 River Drive, Room A102 • Knoxville, TN 37996-4550 • Phone 865 974 0227 • Fax 865 974 4773

also granted Dr. Cekanova exclusive funds to pursue her pilot studies, as did the SACS Department through its Milam Grant fund. Dr. Cekanova's other funded grants from private and government agencies as a PI and Co-Investigator are outlined in her biosketch.

Because of Dr. Cekanova's past record and her unlimited future potential, we have no doubt that she will continue to make significant contributions to cancer research. Thus, we give strong support to Dr. Cekanova's application for Academic Research Enhancement Award through the NIH.

Please do not hesitate to contact us if you need additional information.

Sincerely,

Claudia Kirk, DVM, PhD, DACVN, DACVIM
Professor and Head
Small Animal Clinical Sciences
E-mail: ckirk4@utk.edu

Michael McEntee, DVM, DACVP
Associate Dean for Research and Graduate Studies
E-mail: mmcentee@utk.edu

THE UNIVERSITY of TENNESSEE
INSTITUTE of AGRICULTURE
Department of Small Animal Clinical Sciences
COLLEGE OF VETERINARY MEDICINE

October 25, 2013

Maria Cekanova, MS, RNDr, PhD
Research Assistant Professor
Department of Small Animal Clinical Sciences
College of Veterinary Medicine
The University of Tennessee
2407 River Drive
Knoxville, TN 37996-4544

RE: Support letter for AREA award application sponsored by NIH/NCI with title: "Detection of COX-2-expressed cancers by Fluorocoxib A"

Dear Maria:

I am writing in support of your Academic Research Enhancement Award application as a co-investigator on this project. I am happy to serve as a co-investigator and will continue to assist in patient recruitment and management for the project evaluating Fluorocoxib A as a detector of COX-2 expression by cancers. As a board-certified specialist in small animal internal medicine, we have worked together for several years and I have enjoyed performing the endoscopic procedures evaluating Fluorocoxib. I will continue to recruit cases and to manage their diagnostic and medical care. This will continue to provide *in* vivo data. It has been a productive collaboration that has great clinical application to human and veterinary patients. Our work has generated very interesting data and exciting presentations and publications. You are a very creative and talented individual with many great ideas and the skills and knowledge to succeed. You have developed a strong independent research program within the Department of Small Animal Clinical Sciences and at the College of Veterinary Medicine. I appreciate your invitation to continue to be a part of this area of research and look forward to continuing this collaboration for many years.

Please feel free to contact me if you require additional information of if I can answer any questions.

Sincerely,

Joseph W. Bartges, DVM, PhD, DiplACVIM (small animal), DiplACVN
Professor of Medicine and Nutrition
The Acree Endowed Chair of Small Animal Research
Department of Small Animal Clinical Sciences
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Oct 14, 2013

Maria Cekanova, MS, RNDr, PhD
Research Assistant Professor
University of Tennessee,
College of Veterinary Medicine
Small Animal Clinical Sciences,
2407 River Drive, VTH RM C247
Knoxville, TN 37996-4550
Phone: 865-389-5222
E-mail: mcekanov@utk.edu

RE: Support letter for AREA award application sponsored by NIH/NCI with title: "Detection of COX-2-expressed cancers by Fluorocoxib A"

Dear Maria,

I am writing in support to your Academic Research Enhancement Award application to National Institute of Health with title: *Detection of COX-2-expressed cancers by Fluorocoxib A*.

I am happy to be a Co-Investigator for this project. I will synthesize fluorocoxib A and ship it to Dr. Cekanova's laboratory at the University of Tennessee-Knoxville for her use in imaging studies. I request co-authorships in the publications from this study.

We have been working together for approximately five years on a collaborative research project to evaluate newly synthesized imaging agents using animal models with naturally-occurring tumors. We believe that this model with spontaneously occurring cancers will help to progress in translational research of novel imaging agent to human medicine.

My work is focused on development of imaging agents that are targeted to cyclooxygenase-2 (COX-2). COX-2 is overexpressed in inflammatory lesions and in many solid tumors, but its levels are very low in adjacent normal tissue. Thus, it is an ideal target for molecular imaging of premalignant lesions, primary tumors, and metastatic foci. Dr. Marnett and I developed Fluorocoxib A, which is a powerful COX-2–targeted optical imaging agent, the first marketed fluorescent COX-2 selective inhibitor, extensively used for in vivo detection of inflammatory tissues and pre-malignant and malignant tumors expressing elevated levels of COX-2 in rodents and canines. Dr. Lawrence Marnett met Maria during his visit at UT, CVM in 2008, and they discussed some common interests in COX-2 biology. In April 2009, Maria visited us and suggested new collaboration to image COX-2 in naturally-occurring tumors in domestic animals. This was interesting to me, because application to domestic animals would represent an important extension of our work that we would be unable to conduct without a talented collaborator.

I have developed great respect for her talents as a scientist, her drive to conduct high quality research, and her desire to succeed. I feel strongly that she has what it takes to establish an independent research program at College of Veterinary Medicine at the University of Tennessee in Knoxville. This award will help to progress research in field of biomedical sciences at the College of Veterinary Medicine, the University of Tennessee in Knoxville.

We discuss our findings periodically and communicate through the e-mails and we have yearly teleconference meetings to plan further experiments and discuss the findings. We plan to continue in this

collaboration and keeping updating and discussing the research experiments, data analysis and findings by e-mails on monthly bases and meetings through the webcams or personal meetings due to short distance only 2 hours drive between Nashville and Knoxville.

To date, it has been a very productive collaboration. We are getting good results and I am very excited about it. This collaboration would have gone nowhere without Maria's drive and determination. She is not only a good scientist, but she will do whatever is necessary to secure funding, line up equipment, recruit collaborators, etc., to enable good science to be done. This kind of "fire-in-the-belly" is the mark of all successful scientists, especially in this era of contracted budgets and fierce competition for research grants. I am confident that Maria has what it takes to develop a productive, well-funded independent research career.

Please feel free to contact us if you need additional information.

Sincerely,

Md. Jashim Uddin, PhD

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Wednesday, October 23, 2013

Maria Cekanova, RNDr, PhD
Research Assistant Professor
The University of Tennessee
College of Veterinary Medicine
Department of Small Animal Clinical Sciences

RE: "Detection of COX-2-expressed cancers by fluorocoxib A"

Dear Maria,

I am delighted to serve as a consultant on your R15 application entitled, "Detection of COX-2-expressed cancers by fluorocoxib A." My role will be to help you and your colleagues conduct the proposed *in vivo* imaging studies, a portion of which will be conducted in our facility, the Vanderbilt University Institute of Imaging Science (VUIIS). Your proposal is very timely and brings together several important first fruits of many molecular imaging-centric programs at Vanderbilt University. First, Larry Marnett and colleagues from his laboratory at Vanderbilt developed the novel probe you are proposing to study, fluorocoxib A. Larry's COX2 imaging research has been a key driving force behind the success of our NCI-funded In Vivo Cellular and Molecular Imaging of Cancer (ICMIC, P50) program. It is extremely exciting to see Larry's probes moving forward to more advanced preclinical and translational studies, as you are proposing to conduct. The fact that Caliper Life Sciences has recently begun distributing fluorcoxib A for preclinical imaging studies is evidence of the intense research interest in imaging COX2 expression non-invasively and significantly increases the relevance and rapid translation of potential findings that stem from your work.

Finally, through your collaborations with Larry, let me assure you that you will have access to the full resources of the Molecular Imaging Research program at Vanderbilt, which includes all the relevant imaging hardware housed within VUIIS and relevant imaging expertise.

I wish you the best of luck and look forward to these exciting studies.

H. Charles Manning, Ph.D.

Vanderbilt University Institute of Imaging Sciences (VUIIS)
Director of Molecular Imaging Research
Associate Professor of Radiology, Neurosurgery,
Biomedical Engineering, Chemical and Physical Biology
Vanderbilt University Medical School

Resource Sharing Plan

The University of Tennessee is committed to the open and timely dissemination of research outcomes. The investigators are aware of and agreed to abide by the principles for sharing research resources as described by NIH in "Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Resources".

Sharing of data and resources generated by this project is an essential part of our proposed activities and will be accomplished as follows:

- We want to make our results available both to the community of scientists and the public interested in cancer research specifically in assessing fluorocoxib A to selectively detect bladder tumor formation and detect responses to therapy to improve the patient's diagnosis and treatment with bladder cancer.

- This would be accomplished through peer-reviewed publication and communication through scientific meeting abstracts and presentations. Specific target meetings would include the American Association for Cancer Research, and special imaging meetings, such as World Molecular Imaging Congress.