PI: **Onono, Fredrick Onyango**	Title: Intestinal phosphatidylcholine exposure and breast cancer risk	
Received: 10/13/2014	FOA: PAR12-050	Council: 05/2015
Competition ID: FORMS-C	FOA Title: NCI MENTORED RESEARCH SCIENTIST DEVELOPMENT AWARD TO PROMOTE DIVERSITY (K01)	
1 K01 CA197073-01	Dual:	Accession Number: 3747354
IPF: 2793601	Organization: UNIVERSITY OF KENTUCKY	
Former Number:	Department: Saha Cardiovascular Resrch Ctr	
IRG/SRG: NCI-I	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 129,996 Year 2: 129,996 Year 3: 129,996 Year 4: 129,996 Year 5: 129,996	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:*
Fredrick Onono Ph.D	University of Kentucky Research Foundation	PD/PI
Andrew Morris	University of Kentucky Research Foundation	Other Professional-Primary Mentor
Kathleen O'Connor	University of Kentucky College of Medicine	Other Professional-Co-Mentor
Marta Torroella-Kouri Ph.D	University of Miami	Other Professional-Collaborator
Laundette Jones Ph.D	University of Maryland	Other Professional-Collaborator
Andrew Lane	University of Kentucky College of Medicine	Other Professional-Co-mentor
Richard Charnigo	University of Kentucky College of Public Health	Other Professional-Consultant
Arun Sreekuman	Baylor College of Medicine	Other Professional-Consultant

Reference Letters

Susan Smyth	University of Kentucky	10/14/2014
Christoph Reuter	Hannover Medical School	10/13/2014
Alan Daugherty	University of Kentucky	10/13/2014
Hans Spielmann	University of Kentucky	10/13/2014
Zhenyu Li	University of Kentucky	10/13/2014

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

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number
GRANT11758495

5. APPLICANT INFORMATION **Organizational DUNS*:** 9390178770000

Legal Name*: University of Kentucky Research Foundation
Department:
Division:
Street1*: 500 South Limestone
Street2: 109 Kinkead Hall
City*: Lexington
County: Fayette
State*: KY: Kentucky
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 40526-0001

Person to be contacted on matters involving this application
Prefix: Ms. First Name*: Deborah Middle Name: K. Last Name*: Davis Suffix:
Position/Title: Associate Director
Street1*: 500 South Limestone
Street2: 109 Kinkead Hall
City*: Lexington
County:
State*: KY: Kentucky
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 40526-0001
Phone Number*: 859-257-9420 Fax Number: 859-323-1060 Email: ospa@email.uky.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 616033693

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
93.398
TITLE: Cancer Research Manpower

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Intestinal phosphatidylcholine exposure and breast cancer risk

12. PROPOSED PROJECT
Start Date* Ending Date*
07/01/2015 06/30/2020

13. CONGRESSIONAL DISTRICTS OF APPLICANT
KY-006

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: Fredrick Middle Name: O Last Name*: Onono Suffix: Ph.D

Position/Title: Postdoctoral Fellow
Organization Name*: University of Kentucky Research Foundation
Department: Saha Cardiovascular Resrch Ctr
Division: College of Medicine
Street1*: 741 South Limestone
Street2: BBSRB, B348
City*: Lexington
County: Fayette
State*: KY: Kentucky
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 40536-0509
Phone Number*: 859-420-5223 Fax Number: Email*: foon222@uky.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$701,980.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$701,980.00
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: First Name*: Deborah Middle Name: Last Name*: Davis Suffix:

Position/Title*: Associate Director
Organization Name*: University of Kentucky Research Foundation
Department:
Division:
Street1*: 500 South Limestone
Street2: 109 Kinkead Hall
City*: Lexington
County: Fayette
State*: KY: Kentucky
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 40526-0001
Phone Number*: 859-257-9420 Fax Number: 859-323-1060 Email*: ospa@email.uky.edu

Signature of Authorized Representative*
Deborah Davis

Date Signed*
10/13/2014

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:1252-Cover Letter.pdf

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

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

Project/Performance Site Location(s)

Project/Performance Site Primary Location

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

Organization Name:	University of Kentucky Research Foundation
Duns Number:	9390178770000
Street1*:	741 South Limestone
Street2:	B252 Biomedical Biosciences Research Building
City*:	Lexington
County:	
State*:	KY: Kentucky
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	40536-0509
Project/Performance Site Congressional District*:	KY-006

File Name

Additional Location(s)

Funding Opportunity Number: PAR-12-050. Received Date: 2014-10-13T16:35:51.000-04:00

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

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

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: 11-18-2013

Animal Welfare Assurance Number A3336-01

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*	1246-Abstract.pdf
8. Project Narrative*	1247-NARRATIVE.pdf
9. Bibliography & References Cited	1248-Bibliography.pdf
10.Facilities & Other Resources	1249-FACILITIES.pdf
11.Equipment	1250-K01_Onono_Equipment.pdf
12. Other Attachments	1251-List of Referees.pdf

Funding Opportunity Number: PAR-12-050. Received Date: 2014-10-13T16:35:51.000-04:00

ABSTRACT

Diet-dependent obesity is a risk factor for many cancers including postmenopausal breast cancer. Obesity is also associated with more aggressive breast cancer subtypes and poorer clinical outcomes in breast cancer patients. The increasing prevalence of overweight and obese postmenopausal breast cancer patients makes it critical to identify the mechanisms that underlie this link between obesity and breast cancer risk and prognosis. A better understanding of the link between diet, obesity and breast cancer risk might then lead to improvements in healthful nutrition for obese or overweight patients or identify markers for breast cancer risk assessment or targets for pharmacological intervention to mitigate breast cancer risk associated with obesity. The aim of this K01 application is to support mentored training in advanced mass spectrometry/metabolomics and preclinical models that will enable me to become an independent investigator working at the interface of obesity and cancer research. A broad hypothesis to explain the link between obesity and cancer risk is that consumption of a high fat diet and/or increased synthesis and storage of fats is associated with the production of bioactive lipids or lipid-derived molecules that themselves promote disease. I propose to investigate the possibility that intestinal exposure to the phospholipid phosphatidylcholine (PC) constitutes a link between diet and breast cancer risk by promoting the synthesis of a PC derived bioactive lipid, lysophosphatidic acid (LPA). Breast cancer cells are acutely responsive to LPA which stimulates their migration, growth and survival *in vitro*. Genetic and pharmacological targeting of LPA synthesis and signaling attenuate breast cancer tumor growth and metastasis in mouse models. We propose that dietary consumption of foods rich in PC or secretion of PC into the stomach as bile in response to a fatty meal results in uptake of this lipid as its lyso derivative, lysoPC (LPC) that is transported in the blood as a component of intestinally derived lipoproteins where is serves as a substrate for production of LPA by the secreted lysophospholipase D, autotaxin. In support of this idea, we show that plasma LPA levels in mice and humans are acutely sensitive to fasting and re-feeding and that, in mice, dietary PC can be converted to LPA in the blood. To address our hypothesis, we will use mass spectrometry to monitor metabolism of dietary PC in mice and develop mouse diets with defined PC composition that we propose will lead to alterations in plasma LPA levels. The information in this aim will then be exploited to determine the impact of manipulations of dietary PC and pharmacological inhibition of the LPA generating enzyme autotaxin on breast cancer metastasis in mouse models. Completion of these studies will provide important new information about how a specific dietary constituent could contribute to breast cancer metastasis in humans and might also provide an impetus for future translational studies exploring the possibility that pharmacological inhibition of LPA synthesis and signaling could be a viable strategy to mitigate human breast cancer risk, particularly in obese subjects.

NARRATIVE

Being obese or overweight dramatically increases cancer risk, particularly in women, and obese or overweight cancer patients often experience worse clinical outcomes than normal weight individuals. However, the basis for this association of obesity with cancer risk and prognosis is not well understood. This proposal requests funds to support research training and career development for a promising underrepresented minority scientist who proposes to investigate a potential mechanism linking obesity to cancer risk and clinical outcomes.

LITERATURE CITED

1. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. 2003. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 348:1625-38. PMCID: 12711737
2. Azrad M, Demark-Wahnefried W. 2014. The association between adiposity and breast cancer recurrence and survival: A review of the recent literature. *Curr Nutr Rep* 3:9-15. PMCID: 3921906
3. Jensen MD, Ryan DH, Apovian CM, Ard JD, Comuzzie AG, Donato KA, Hu FB, Hubbard VS, Jakicic JM, Kushner RF, Loria CM, Millen BE, Nonas CA, Pi-Sunyer FX, Stevens J, Stevens VJ, Wadden TA, Wolfe BM, Yanovski SZ. 2013. 2013 AHA/ACC/TOS Guideline for the Management of Overweight and Obesity in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. *Circulation*. PMCID: 24239920
4. Roberts DL, Dive C, Renehan AG. 2010. Biological mechanisms linking obesity and cancer risk: new perspectives. *Annu Rev Med* 61:301-16. PMCID: 19824817
5. Panupinthu N, Lee HY, Mills GB. 2010. Lysophosphatidic acid production and action: critical new players in breast cancer initiation and progression. *Br J Cancer* 102:941-6. PMCID: Pmc2844037
6. Han X, Stevens J, Truesdale KP, Bradshaw PT, Kucharska-Newton A, Prizment AE, Platz EA, Joshu CE. 2014. Body mass index at early adulthood, subsequent weight change and cancer incidence and mortality. *Int J Cancer* 135:2900-9. PMCID: 24771654
7. Nelson ER, Wardell SE, Jasper JS, Park S, Suchindran S, Howe MK, Carver NJ, Pillai RV, Sullivan PM, Sondhi V, Umetani M, Geradts J, McDonnell DP. 2013. 27-Hydroxycholesterol links hypercholesterolemia and breast cancer pathophysiology. *Science* 342:1094-8. PMCID: 3899689
8. Warner M, Gustafsson JA. 2014. On estrogen, cholesterol metabolism, and breast cancer. *N Engl J Med* 370:572-3. PMCID: 24499217
9. Choi JW, Herr DR, Noguchi K, Yung YC, Lee CW, Mutoh T, Lin ME, Teo ST, Park KE, Mosley AN, Chun J. 2010. LPA receptors: subtypes and biological actions. *Annu Rev Pharmacol Toxicol* 50:157-86. PMCID: 20055701
10. van Meeteren LA, Moolenaar WH. 2007. Regulation and biological activities of the autotaxin-LPA axis. *Prog Lipid Res* 46:145-60. PMCID: 17459484
11. Moolenaar WH, Hla T. 2012. SnapShot: Bioactive lysophospholipids. *Cell* 148:378-.e2. PMCID: 3645273
12. Xiang SY, Dusaban SS, Brown JH. 2013. Lysophospholipid receptor activation of RhoA and lipid signaling pathways. *Biochim Biophys Acta* 1831:213-22. PMCID: 4076288
13. Kitayama J, Shida D, Sako A, Ishikawa M, Hama K, Aoki J, Arai H, Nagawa H. 2004. Over-expression of lysophosphatidic acid receptor-2 in human invasive ductal carcinoma. *Breast Cancer Res* 6:R640-6. PMCID: 1064082
14. Chen M, Towers LN, O'Connor KL. 2007. LPA2 (EDG4) mediates Rho-dependent chemotaxis with lower efficacy than LPA1 (EDG2) in breast carcinoma cells. *Am J Physiol Cell Physiol* 292:C1927-33. PMCID: 17496233
15. Houben AJ, Moolenaar WH. 2011. Autotaxin and LPA receptor signaling in cancer. *Cancer Metastasis Rev* 30:557-65. PMCID: 22002750
16. Chen M, O'Connor KL. 2005. Integrin alpha6beta4 promotes expression of autotaxin/ENPP2 autocrine motility factor in breast carcinoma cells. *Oncogene* 24:5125-30. PMCID: 15897878
17. Du J, Sun C, Hu Z, Yang Y, Zhu Y, Zheng D, Gu L, Lu X. 2010. Lysophosphatidic acid induces MDA-MB-231 breast cancer cells migration through activation of PI3K/PAK1/ERK signaling. *PLoS One* 5:e15940. PMCID: 3012724
18. O'Connor KL, Chen M, Towers LN. 2012. Integrin alpha6beta4 cooperates with LPA signaling to stimulate Rac through AKAP-Lbc-mediated RhoA activation. *Am J Physiol Cell Physiol* 302:C605-14. PMCID: 3287157
19. Harrison SM, Knifley T, Chen M, O'Connor KL. 2013. LPA, HGF, and EGF utilize distinct combinations of signaling pathways to promote migration and invasion of MDA-MB-231 breast carcinoma cells. *BMC Cancer* 13:501. PMCID: 3819718
20. Moolenaar WH, Perrakis A. 2011. Insights into autotaxin: how to produce and present a lipid mediator. *Nat Rev Mol Cell Biol* 12:674-9. PMCID: 21915140

21. Rancoule C, Dusaulcy R, Treguer K, Gres S, Guigne C, Quilliot D, Valet P, Saulnier-Blache JS. 2012. Depot-specific regulation of autotaxin with obesity in human adipose tissue. *J Physiol Biochem* 68:635-44. PMCID: 22644624
22. van Meeteren LA, Ruurs P, Stortelers C, Bouwman P, van Rooijen MA, Pradere JP, Pettit TR, Wakelam MJ, Saulnier-Blache JS, Mummery CL, Moolenaar WH, Jonkers J. 2006. Autotaxin, a secreted lysophospholipase D, is essential for blood vessel formation during development. *Mol Cell Biol* 26:5015-22. PMCID: 1489177
23. Euer N, Schwirzke M, Evtimova V, Burtscher H, Jarsch M, Tarin D, Weidle UH. 2002. Identification of genes associated with metastasis of mammary carcinoma in metastatic versus non-metastatic cell lines. *Anticancer Res* 22:733-40. PMCID: 12014644
24. Popnikolov NK, Dalwadi BH, Thomas JD, Johannes GJ, Imagawa WT. 2012. Association of autotaxin and lysophosphatidic acid receptor 3 with aggressiveness of human breast carcinoma. *Tumour Biol* 33:2237-43. PMCID: 22922883
25. Liu S, Umezu-Goto M, Murph M, Lu Y, Liu W, Zhang F, Yu S, Stephens LC, Cui X, Murrow G, Coombes K, Muller W, Hung MC, Perou CM, Lee AV, Fang X, Mills GB. 2009. Expression of autotaxin and lysophosphatidic acid receptors increases mammary tumorigenesis, invasion, and metastases. *Cancer Cell* 15:539-50. PMCID: 4157573
26. Gaetano CG, Samadi N, Tomsig JL, Macdonald TL, Lynch KR, Brindley DN. 2009. Inhibition of autotaxin production or activity blocks lysophosphatidylcholine-induced migration of human breast cancer and melanoma cells. *Mol Carcinog* 48:801-9. PMCID: 2736327
27. Samadi N, Gaetano C, Goping IS, Brindley DN. 2009. Autotaxin protects MCF-7 breast cancer and MDA-MB-435 melanoma cells against Taxol-induced apoptosis. *Oncogene* 28:1028-39. PMCID:
28. Samadi N, Bekele RT, Goping IS, Schang LM, Brindley DN. 2011. Lysophosphatidate induces chemo-resistance by releasing breast cancer cells from taxol-induced mitotic arrest. *PLoS One* 6:e20608. PMCID: 3103588
29. Boucharaba A, Serre CM, Gres S, Saulnier-Blache JS, Bordet JC, Guglielmi J, Clezardin P, Peyruchaud O. 2004. Platelet-derived lysophosphatidic acid supports the progression of osteolytic bone metastases in breast cancer. *J Clin Invest* 114:1714-25. PMCID: 535068
30. Baker DL, Fujiwara Y, Pigg KR, Tsukahara R, Kobayashi S, Murofushi H, Uchiyama A, Murakami-Murofushi K, Koh E, Bandle RW, Byun HS, Bittman R, Fan D, Murph M, Mills GB, Tigyi G. 2006. Carba analogs of cyclic phosphatidic acid are selective inhibitors of autotaxin and cancer cell invasion and metastasis. *J Biol Chem* 281:22786-93. PMCID: 3505596
31. Boucharaba A, Serre CM, Guglielmi J, Bordet JC, Clezardin P, Peyruchaud O. 2006. The type 1 lysophosphatidic acid receptor is a target for therapy in bone metastases. *Proc Natl Acad Sci U S A* 103:9643-8. PMCID: 1480460
32. Boucharaba A, Guillet B, Menaa F, Hneino M, van Wijnen AJ, Clezardin P, Peyruchaud O. 2009. Bioactive lipids lysophosphatidic acid and sphingosine 1-phosphate mediate breast cancer cell biological functions through distinct mechanisms. *Oncol Res* 18:173-84. PMCID:
33. David M, Ribeiro J, Descotes F, Serre CM, Barbier M, Murone M, Clezardin P, Peyruchaud O. 2012. Targeting lysophosphatidic acid receptor type 1 with Debio 0719 inhibits spontaneous metastasis dissemination of breast cancer cells independently of cell proliferation and angiogenesis. *Int J Oncol* 40:1133-41. PMCID: 3584523
34. Marshall JC, Collins JW, Nakayama J, Horak CE, Liewehr DJ, Steinberg SM, Albaugh M, Vidal-Vanaclocha F, Palmieri D, Barbier M, Murone M, Steeg PS. 2012. Effect of inhibition of the lysophosphatidic acid receptor 1 on metastasis and metastatic dormancy in breast cancer. *J Natl Cancer Inst* 104:1306-19. PMCID: 3611817
35. Benesch MG, Tang X, Maeda T, Ohhata A, Zhao YY, Kok BP, Dewald J, Hitt M, Curtis JM, McMullen TP, Brindley DN. 2014. Inhibition of autotaxin delays breast tumor growth and lung metastasis in mice. *Faseb j* 28:2655-66. PMCID: 24599971
36. Leblanc R, Lee SC, David M, Bordet JC, Norman DD, Patil R, Miller D, Sahay D, Ribeiro J, Clezardin P, Tigyi GJ, Peyruchaud O. 2014. Interaction of platelet-derived autotaxin with tumor integrin alphaVbeta3 controls metastasis of breast cancer cells to bone. *Blood*. PMCID:
37. Sanders LM, Zeisel SH. 2007. Choline: Dietary Requirements and Role in Brain Development. *Nutr Today* 42:181-6. PMCID: 2518394

38. Hofmann AF. 2007. Biliary secretion and excretion in health and disease: current concepts. *Ann Hepatol* 6:15-27. PMCID: 17297425
39. Labonte ED, Kirby RJ, Schildmeyer NM, Cannon AM, Huggins KW, Hui DY. 2006. Group 1B phospholipase A2-mediated lysophospholipid absorption directly contributes to postprandial hyperglycemia. *Diabetes* 55:935-41. PMCID: 2048981
40. Zierenberg O, Grundy SM. 1982. Intestinal absorption of polyenephosphatidylcholine in man. *J Lipid Res* 23:1136-42. PMCID: 7175371
41. Kumar K, Sachdanandam P, Arivazhagan R. 1991. Studies on the changes in plasma lipids and lipoproteins in patients with benign and malignant breast cancer. *Biochem Int* 23:581-9. PMCID:
42. Rodrigues Dos Santos C, Fonseca I, Dias S, Mendes de Almeida JC. 2014. Plasma level of LDL-cholesterol at diagnosis is a predictor factor of breast tumor progression. *BMC Cancer* 14:132. PMCID: 3942620
43. Qiu Y, Zhou B, Su M, Baxter S, Zheng X, Zhao X, Yen Y, Jia W. 2013. Mass spectrometry-based quantitative metabolomics revealed a distinct lipid profile in breast cancer patients. *Int J Mol Sci* 14:8047-61. PMCID: 3645730
44. Guo S, Wang Y, Zhou D, Li Z. 2014. Significantly increased monounsaturated lipids relative to polyunsaturated lipids in six types of cancer microenvironment are observed by mass spectrometry imaging. *Sci Rep* 4:5959. PMCID: 4121604
45. Salous AK, Panchatcharam M, Sunkara M, Mueller P, Dong A, Wang Y, Graf GA, Smyth SS, Morris AJ. 2013. Mechanism of rapid elimination of lysophosphatidic acid and related lipids from the circulation of mice. *J Lipid Res* 54:2775-84. PMCID: 3770090
46. Baker DL, Morrison P, Miller B, Riely CA, Tolley B, Westermann AM, Bonfrer JM, Bais E, Moolenaar WH, Tigyi G. 2002. Plasma lysophosphatidic acid concentration and ovarian cancer. *JAMA* 287:3081-2. PMCID: 12069669
47. Bjorklund MM, Hollensen AK, Hagensen MK, Dagnaes-Hansen F, Christoffersen C, Mikkelsen JG, Bentzon JF. 2014. Induction of atherosclerosis in mice and hamsters without germline genetic engineering. *Circ Res* 114:1684-9. PMCID:
48. Albers HM, Dong A, van Meeteren LA, Egan DA, Sunkara M, van Tilburg EW, Schuurman K, van Tellingen O, Morris AJ, Smyth SS, Moolenaar WH, Ovaa H. 2010. Boronic acid-based inhibitor of autotaxin reveals rapid turnover of LPA in the circulation. *Proc Natl Acad Sci U S A* 107:7257-62. PMCID: 2867685
49. Hausmann J, Kamtekar S, Christodoulou E, Day JE, Wu T, Fulkerson Z, Albers HM, van Meeteren LA, Houben AJ, van Zeijl L, Jansen S, Andries M, Hall T, Pegg LE, Benson TE, Kasiem M, Harlos K, Kooi CW, Smyth SS, Ovaa H, Bollen M, Morris AJ, Moolenaar WH, Perrakis A. 2011. Structural basis of substrate discrimination and integrin binding by autotaxin. *Nat Struct Mol Biol* 18:198-204. PMCID: 3064516
50. Wu T, Kooi CV, Shah P, Charnigo R, Huang C, Smyth SS, Morris AJ. 2014. Integrin-mediated cell surface recruitment of autotaxin promotes persistent directional cell migration. *Faseb j* 28:861-70. PMCID: 3898650
51. Gierse J, Thorarensen A, Beltey K, Bradshaw-Pierce E, Cortes-Burgos L, Hall T, Johnston A, Murphy M, Nemirovskiy O, Ogawa S, Pegg L, Pelc M, Prinsen M, Schnute M, Wendling J, Wene S, Weinberg R, Wittwer A, Zweifel B, Masferrer J. 2010. A novel autotaxin inhibitor reduces lysophosphatidic acid levels in plasma and the site of inflammation. *J Pharmacol Exp Ther* 334:310-7. PMCID:
52. Heppner GH, Miller FR, Shekhar PM. 2000. Nontransgenic models of breast cancer. *Breast Cancer Res* 2:331-4. PMCID: 138654
53. Kim JB, Urban K, Cochran E, Lee S, Ang A, Rice B, Bata A, Campbell K, Coffee R, Gorodinsky A, Lu Z, Zhou H, Kishimoto TK, Lassota P. 2010. Non-invasive detection of a small number of bioluminescent cancer cells in vivo. *PLoS One* 5:e9364. PMCID: 2826408

FACILITIES & OTHER RESOURCES

Laboratory: Dr Onono has assigned bench space within an open plan laboratory of ~2000 sq. feet of laboratory space on the 3rd floor of the Biomedical Biosciences Research Building (BBSRB 306) at the University of Kentucky that includes dedicated rooms for tissue culture and animal surgery. Some of the research will be conducted using instrumentation housed in a 1000 square foot purpose configured laboratory that houses the University of Kentucky Small Molecule Mass Spectrometry Core Laboratory (directed by Dr Morris). This laboratory space is on the same floor as the space assigned to Co-mentor, Dr O'Connor and adjacent to the Biopharmacy building that houses Co-mentor Dr Lane's research laboratory. Other shared resources that are important for the proposed research are detailed in the institutional environment section of the proposal.

Office: Dr Onono has desk space within laboratory space assigned to Dr Morris on the 3rd floor of the Biomedical Biosciences Research Building (BBSRB 306) at the University of Kentucky.

Computer: Dr Onono has access to networked computers for data processing, archiving, sharing and analysis.

Animal Resources: All animals used in the proposed research will be housed in the University of Kentucky Division of Laboratory Animal Medicine operated vivarium in the basement of the Biomedical Biosciences Research Building. This includes fully equipped for state-of-the art animal surgery and monitoring, including heart rate and temperature monitoring and bench top equipment to measure blood gas, blood chemistry, and blood counts. Additionally, state-of-the art equipment for measuring lean and fat body composition and whole body metabolism in rodents are available to the investigators through core facilities operated by the Vice President for Research and the Center for Research on Obesity and Cardiovascular disease.

Clinical: Not applicable.

MAJOR EQUIPMENT and other Resources available for Dr. Onono's Research

Cell Culture: Two laminar flow hoods, four CO_2 incubators, one phase (Nikon TS100) and one fluorescent (Zeiss) inverted microscope.

General Laboratory Equipment: Refrigerated table-top and micro- centrifuges and ultracentrifuges, acrylamide/agarose gel electrophoresis and electroblotting equipment, power supplies, gel-dryer, Odyssey Infrared Imaging System, UV transilluminator, digital camera for gel documentation, analytic balances, Pharmacia dual pump FPLC system with controller and UV monitor, single and multi-channel peristaltic pumps, in-line UV monitor, chart recorders, three fraction collectors, chromatography columns, three thermocyclers, electroporator, rotary evaporator, vacuum apparatus, pH meter, orbital shakers, refrigerator and freezers (-70ºC and -20ºC), fume hoods, centrifugal evaporator/concentrator, rotary evaporator, thin layer chromatography tanks, bacterial incubators, probe and bath sonicators. BioTek Powerwave 200 absorbance and Flx800 fluorescence plate readers with computer control and software for data acquisition and analysis, LiCor Odyssey Infrared imager, Packard Tri-Carb Liquid Scintillation Counter.

Confocal and Wide Field Microscope: Nikon TE 2000 fully motorized inverted motorized microscope with 20, 40, 60 X objectives, DIC capabilities and a Nikon A1R resonance scanning four laser confocal system. This microscope also has a wide field fluorescence illuminator with fluorescence filter sets and a Cascade CCD camera. Computer running Nikon Elements software for data acquisition and analysis.

Small Animal Surgical Resources: A dual-headed surgical dissecting microscope (Leica M691) with digital recording system, 2 dissecting microscopes (Nikon SMZ 800 with camera port), Doppler Processing Work Station (Indus) with 10 and 20 M-Hx pulsed Doppler, perivascular flow meter with 0.5PSB, 0.7PSB, and 1.5 PSL probes (Transonics T400), THM 100 temperature and EKG board (Indus Instruments), mouse tail cuff blood pressure systems CODA (Kent Scientific), pressure-volume conductance system (Millar Instruments), mouse pulse oximeter (Starr), I-STAT system for blood gas and blood chemistry analysis, ABC vet Analyzer for complete blood counts, surgical equipment, instrument sterilizer, bipolar hand coagulator with footswitch (Kirwan Surgical Products), thermocouple thermometer for monitoring rectal temperature (WPI); inhaled isoflurane anesthetic mixing chamber for rodents, small animal respirator. The lab has a small animal imaging system (Vevo 770 biomicroscopy system with 30 and 40 MHz probes from Visual Sonics) and equipment for hyperinsulinemic-euglycemic clamps, including tether system (INSTECH), multi-infusion pump (Harvard Apparatus), and dual infusion pump (Harvard Apparatus).

Multiplex analyzers: Biorad Bioplex 200 suspension array reader and Luminex MAGPIX analyzers with dedicated computers running manufacturer's software for instrument control and data analysis.

Common Equipment: Real-time PCR Systems (ABI 7500 and BioRad iQ), FacsCalibur flow cytometer (BD Sciences), scintillation counter and gamma counter, deionized water system, and dark room/film developer. Available through the Center for CV Disease and Obesity: EchoMRI, DEXA-IR system, LabMaster TSE system.

Small Molecule Mass Spetrometry Core Laboratory

Two ABSciex 4000 Q-TRAP Hybrid Triple Quadrupole Ion Trap Mass spectrometers with electrospray ionization, atmospheric pressure ionization and photoionization sources. One of these instruments is additionally equipped with an ABSciex Flashquant MALDI source. The other instrument is additionally equipped with an Advion Triversa Nanomate robotic chip-based electrospray ionization source. Both systems are supplied by Parker Balston zero air and N_2 gas generators. Both instruments can be connected to Shimadzu binary pump HPLC systems with autosamplers, solvent degassers and control interfaces. Both systems are interfaced with computers running ABSciex "Analyst" and "Multi Quant" software for instrument control data acquisition and analysis.

AB Sciex 5600 "Triple TOF" hybrid quadrupole time of flight high resolution mass spectrometer. This instrument is equipped with electrospray and nanospray ion sources, is equipped with a Parker Balston gas generator and interfaced with a Shimadzu binary pump HPLC system and autosampler configured for both LC MS and automated flow infusion. This instrument can also be operated with an Eksigent microflow HPLC system configured for calipiliary chromatography or direct sample infusion. This system is connected to a computer running ABSciex "Analyst TOF" and "Multi Quant" software for instrument control and data analysis.

Offline data analysis. 64 Bit Windows Workstation running a variety of software packages for data processing, metabolite identification and data comparisons. This includes Markerview and Lipidview (ABSciex),) and several academic/not for profit software packages including MAVEN, LipidXplorer.

Agilent 6890 gas chromatograph equipped with Agilent 5975 inert mass selective detector and electron capture detector. This instrument also has a headspace autosampler and is connected to a computer running Agilent Chemstation software for instrument control and data acquisition and analysis.

Agilent 7890 gas chromatograph with Agilent 7000C triple quadrupole mass spectrometer. This instrument has two autosamplers, two inlets (standard split/splitless and multimode) and a column oven with fast heating and integrated fluidics cartridges to operate two columns with outflow directed to a flame ionization detector, an electron capture detector or the mass spectrometer. The instrument is connected to a computer running Agilent Chemstation/Masshunter software for instrument control, data acquisition and analysis.

Equipment for Sample preparation: The laboratory also has two Gilson Gradient HPLC systems with absorbance, fluorescence and evaporative light scattering detectors. Other equipment in the laboratory for sample preparation for HPLC/MS includes two N-Evap N_2 evaporator systems, two Biotage Turbo Vap evaporators (one configured for 4 ml vials, the other for 96 well plates), a LabConco centrifugal evaporator/concentrator, two Supelco Solid Phase extraction systems, Matrix Scientific Well Mate multiwell plate dispenser, Gilson 215 Robotic Liquid Handling System configured for multiwell plate and cartridge based Solid Phase Extraction with workstation computer running Gilson Trilution Software, multi sample vortexer and heating blocks for lipid phosphate analysis and chemical derivatization.

List of Referees:

1. Christoph W. Reuter, MD, Dept. of Hematology, Oncology and Stem Cell Transplantation, Hannover Medical School, Germany.

2. H. Peter Spielmann, PhD, College of Medicine, Dept. of Molecular and Cellular Biochemistry, University of Kentucky, KY

3. Alan Daugherty, PhD, DSc, Saha Cardiovascular Research Center, Dept. of Internal Medicine, University of Kentucky, KY

4. Zhenyu Li, MD, PhD, Saha Cardiovascular Research Center, Dept. of Internal Medicine, University of Kentucky, KY

5. Susan S. Smyth, MD, Ph.D., Professor and Chief of Cardiovascular Medicine, University of Kentucky College of Medicine, Lexington KY

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*: Fredrick Middle Name O Last Name*: Onono Suffix: Ph.D

Position/Title*:	Postdoctoral Fellow
Organization Name*:	University of Kentucky Research Foundation
Department:	Saha Cardiovascular Resrch Ctr
Division:	College of Medicine
Street1*:	741 South Limestone
Street2:	BBSRB, B348
City*:	Lexington
County:	Fayette
State*:	KY: Kentucky
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	40536-0509

Phone Number*: 859-420-5223 Fax Number: E-Mail*: foon222@uky.edu

Credential, e.g., agency login: FREDRICK.ONONO

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

Degree Type: Ph.D. Degree Year: 2009

	File Name
Attach Biographical Sketch*:	1235-K01_Onono_Biosketch.pdf
Attach Current & Pending Support:	

Tracking Number: GRANT11758530 Funding Opportunity Number: PAR-12-050 . Received Date: 2014-10-13T16:35:51.000-04:00

PROFILE - Senior/Key Person

Prefix: Prof. First Name*: Andrew Middle Name J Last Name*: Morris Suffix:

Position/Title*:	Professor
Organization Name*:	University of Kentucky Research Foundation
Department:	Internal Medicine
Division:	
Street1*:	741 South Limestone
Street2:	
City*:	Lexington
County:	Fayette
State*:	KY: Kentucky
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	40536-0509

Phone Number*: 859 323 3749 Fax Number: E-Mail*: a.j.morris@uky.edu

Credential, e.g., agency login: AMorris

Project Role*: Other Professional Other Project Role Category: Primary Mentor

Degree Type: Ph.D. Degree Year: 1988

	File Name
Attach Biographical Sketch*:	1236-Morris S10 Biosketch Sept 2014 ononoR.pdf
Attach Current & Pending Support:	1237-Morris Other Support NIH October 2014R.pdf

PROFILE - Senior/Key Person

Prefix: Prof. First Name*: Kathleen Middle Name Last Name*: O'Connor Suffix:

Position/Title*:	Professor
Organization Name*:	University of Kentucky College of Medicine
Department:	Biochemistry
Division:	Markey Cancer Center
Street1*:	741 South Limestone
Street2:	
City*:	Lexington
County:	Fayette
State*:	KY: Kentucky
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	40506-0509

Phone Number*: 859-323-7534 Fax Number: E-Mail*: kloconnor@uky.edu

Credential, e.g., agency login: kloconnor

Project Role*: Other Professional Other Project Role Category: Co-Mentor

Degree Type: Ph.D. Degree Year: 1996

	File Name
Attach Biographical Sketch*:	1238-O'Connor Bio.pdf
Attach Current & Pending Support:	1239-K01_Onono_O'Connor_Funding.pdf

PROFILE - Senior/Key Person

Prefix: Prof. First Name*: Marta Middle Name Last Name*: Torroella-Kouri Suffix: Ph.D

Position/Title*:	Associate Professor
Organization Name*:	University of Miami
Department:	Microbiology and Immunology
Division:	Miller School of Medicine
Street1*:	1600 NW 10th Ave
Street2:	Rosentiel Medical School Building room 3123A
City*:	Miami
County:	
State*:	FL: Florida
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	33136-1015

Phone Number*: (305) 243-6260 Fax Number: (305) 243-5522 E-Mail*: mtorroella@med.miami.edu

Credential, e.g., agency login: mtorroella

Project Role*: Other Professional Other Project Role Category: Collaborator

Degree Type: Ph.D. Degree Year: 1985

	File Name
Attach Biographical Sketch*:	1240-Torroella-Kouri Biosketch.pdf
Attach Current & Pending Support:	

PROFILE - Senior/Key Person

Prefix: Prof. First Name*: Laundette Middle Name Patrice Last Name*: Jones Suffix: Ph.D

Position/Title*:	Assistant Professor
Organization Name*:	University of Maryland
Department:	Pharmacology
Division:	School of Medicine
Street1*:	685 West Baltimore
Street2:	Rm 580F
City*:	Baltimore
County:	
State*:	MD: Maryland
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	21201-1509

Phone Number*: 410-706-7331 Fax Number: 410-706-0032 E-Mail*: Ljones@som.umaryland.edu

Credential, e.g., agency login: LPJones

Project Role*: Other Professional Other Project Role Category: Collaborator

Degree Type: Ph.D. Degree Year: 2000

	File Name
Attach Biographical Sketch*:	1241-LJones Biosketch F.O. (1).pdf
Attach Current & Pending Support:	

PROFILE - Senior/Key Person

Prefix: Prof. First Name*: Andrew Middle Name N Last Name*: Lane Suffix:

Position/Title*:	Professor
Organization Name*:	University of Kentucky College of Medicine
Department:	Toxicology
Division:	Markey Cancer Center
Street1*:	525 Biopharm Building
Street2:	789 S. Limestone
City*:	Lexington
County:	Fayette
State*:	KY: Kentucky
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	40536-0596

Phone Number*: 859 218 2868 Fax Number: E-Mail*: andrew.lane@uky.edu

Credential, e.g., agency login: ANLANE01

Project Role*: Other Professional Other Project Role Category: Co-mentor

Degree Type: Ph.D. Degree Year: 1979

	File Name
Attach Biographical Sketch*:	1242-Biosketch_Lane_Oct2014_K no page numbers.pdf
Attach Current & Pending Support:	1243-Lane OS.pdf

PROFILE - Senior/Key Person

Prefix: First Name*: Richard Middle Name Last Name*: Charnigo Suffix:

Position/Title*:	Professor
Organization Name*:	University of Kentucky College of Public Health
Department:	Statisics, Biostatistics
Division:	
Street1*:	311 MDS Building
Street2:	
City*:	Lexington
County:	Fayette
State*:	KY: Kentucky
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	40536-0082

Phone Number*: 859 218 2072 Fax Number: E-Mail*: richard.charnigo@uky.edu

Credential, e.g., agency login: RICH.CHARNIGO

Project Role*: Other Professional Other Project Role Category: Consultant

Degree Type: Ph.D. Degree Year: 2003

	File Name
Attach Biographical Sketch*:	1244-CharnigoBiosketch_NIH_Oct14_AM.pdf
Attach Current & Pending Support:	

PROFILE - Senior/Key Person

Prefix:	First Name*: Arun	Middle Name	Last Name*: Sreekuman	Suffix:

Position/Title*:	Associate Professor
Organization Name*:	Baylor College of Medicine
Department:	Mol. and Cellular Biology
Division:	
Street1*:	Mail Stop BCM-130, One Baylor Plaza
Street2:	
City*:	Houston
County:	
State*:	TX: Texas
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	77030-3411

Phone Number*: 717 798 3305 Fax Number: E-Mail*: sreekuma@bcm.edu

Credential, e.g., agency login: asreekum

Project Role*: Other Professional Other Project Role Category: Consultant

Degree Type: Ph.D. Degree Year: 2000

	File Name
Attach Biographical Sketch*:	1245-Sreekumar BIOGRAPHICAL SKETCH.pdf
Attach Current & Pending Support:	

BIOGRAPHICAL SKETCH

NAME OF FELLOWSHIP APPLICANT	POSITION TITLE
Fredrick Onyango Onono	Postdoctoral Fellow
eRA COMMONS USER NAME (credential, e.g., agency login) FREDRICK.ONONO	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Jomo Kenyatta University of Agriculture and Technology (JKUAT), Kenya	B.Sc.	04/1997	Biochemistry and Chemistry
University of Nairobi, Kenya	M.Sc.	09/2004	Biochemistry
Hannover Medical School, Germany	PhD	06/2009	Molecular Medicine
University of Kentucky, Lexington, KY	Postdoc Fellow	11/2009-present	Biochemistry

A. Personal Statement

My overall goal for seeking a K01 Mentored Research Scientist Development Award is to acquire the necessary training, practical skills and expertise to become an independently funded investigator in the field of cancer metabolism. I have been in academic research for more than 10 years and have gained valuable experience. My initial research (MSc thesis) focused on vaccine development for East Coast fever, a parasitic disease of cattle. This work resulted in 2 co-author publications in *Proceedings of the National Academy of Sciences of the USA* and *Infection and Immunity* and a launch of field trials for the vaccine candidates we identified. After graduation with an MSc. degree in biochemistry I moved to Germany and joined Dr. Christoph Reuter lab at Hannover Medical School (HMS). Here at HMS, I devoted myself to understanding mechanisms of lipid-dependent protein posttranslational modifications (prenylation) with the long-term goal of identifying novel therapeutic targets for cancer treatment. My research at HMS resulted in 4 publications including 2 first author papers in *Molecular and Cellular Proteomics* and *Journal of Molecular Medicine*. I also reported data from my studies in several national and international meetings and won travel awards. After completion of my PhD in June, 2009, I took a 5 month break to attend to family problems back in my native country Kenya and then in November 2009 joined Dr. H Peter Spielmann lab at the University of Kentucky and focused my research on the mechanisms by which cancer cells metabolize exogenous isoprenols. Because isoprenoids are also intermediates of cholesterol biosynthesis, I won a prestigious postdoctoral fellowship award from the American Heart Association to conduct my research, indicating my potential for future successful NIH and other foundation funding. We discovered that an alternative source of providing or recycling mevalonate pathway intermediates is significantly active and regulated independently of the mevalonate pathway in breast cancer cells. Findings from these studies were published in the *Journal of Biological Chemistry.* My accomplishments during this time also include establishing mass spectrometry based methods to quantitate series of natural and unnatural mevalonate pathway intermediates and monitoring utilization and incorporation of these substrates into proteins. This research trajectory has led to my growing expertise in lipid metabolism in cancer.

Metastasis is the main cause of most cancer deaths. In order to develop effective cancer treatment options that could increase the survival of cancer patients, it is important to understand the mechanisms that promote cancer invasion and metastasis. I am interested in using the mass spectrometry and biochemical skills I have acquired in lipid metabolism to test a hypothesis about how lipids in diet increase the risk of cancer. I will use innovative mass spectrometry and biochemical methods to test the hypothesis that higher intestinal levels of phosphatidylcholine promotes metastatic cancerous phenotype by increasing blood plasma levels of the signaling molecule, lysophosphatidic acid (LPA). The current application builds on my ongoing training and will provide me with a unique set of skills and a knowledge base that will further my career as an innovative and productive researcher in cancer metabolism. Currently I am a T32 trainee in the laboratory of Dr. Andrew Morris, a well-established researcher in the lipid metabolism field. My research focuses on testing the hypothesis that bioactive lysophospholipids generated from intestinal exposure to phosphatidylcholine in food and bile contributes to obesity-associated disease risks. This career development proposal will offer me a unique opportunity to bridge my learning experience with the expertise of my mentors and collaborators. I have

made significant progress towards an independent academic career by developing capabilities in mass spectrometry using multistage instruments and conducting studies that have also generated some of the preliminary data presented in this proposal. This has laid the groundwork for my studies demonstrating that LPA levels are altered during fasting and re-feeding in human subjects and rodents. These groundbreaking findings, as well as my past achievement illustrate that I as a New investigator have the necessary skills, motivation, dedication and ambition to achieve the studies proposed in this K01. I feel strongly that the acknowledgement of my potential in the form of an award from the NIH will encourage me to continue my research goals with even greater fervor.

B. Positions and Honors

ACTIVITY/OCCUPATION	BEGINNING DATE (mm/yy)	ENDING DATE (mm/yy)	FIELD	INSTITUTION/COMPANY	SUPERVISOR/ EMPLOYER
Graduate Fellow	01/00	01/02	Immunology	International Livestock Research Institute	Tony Musoke
Research Technologist	02/02	12/04	Vaccine Development	International Livestock Research Institute	Duncan Mwangi
Research Assistant	01/05	06/09	Hematology and Oncology	Hannover Medical School	Christoph Reuter
Postdoc	11/09	Present	Biochemistry	University of Kentucky	H. Peter Spielmann & Andrew J.Morris

Other Academic and Professional Honors.

NIH Ruth L. Kirschstein National Research Service Award (T32) – 2014

Federation of American Societies for Experimental Biology – MARC- Travel Award - 2013

American Society for Biochemistry and Molecular Biology – Postdoctoral travel Award - 2013

American Heart Association – Postdoctoral fellowship, 2012

Southeastern Regional Lipid Conference – Travel Award, 2010

American Society of Hematology (ASH) – Travel Award, 2009

Merck Sharp & Dohme Limited GMBH – Travel Grant, 2008

German Academic Exchange Program (DAAD) – Merit PhD support funding, 2007

Hannover Medical School, Germany – Merit PhD fellowship, 2005 – 2009

International Livestock Research Institute (ILRI), Nairobi, Kenya – Training grant, 2003

International Livestock Research Institute (ILRI), Nairobi, Kenya – Graduate fellowship, 2000 - 2002

Gandhi Smarak Nidhi Fund – Support for undergraduate studies, 1996

Government of Kenya – Sponsorship for undergraduate studies, 1993 - 1996

Memberships in professional societies:

American Society for Biochemistry and Molecular Biology

International Society of Experimental Hematology

American Heart Association

C. Publications

Research papers:

1. Subramanian T, Ren H, Subramanian KL, Sunkara M, **Onono FO,** Morris AJ and Spielmann HP. **2014** Design and synthesis of non-hydrolyzable homoisoprenoid α-monofluorophosphonate inhibitors of PPAPDC family integral membrane lipid phosphatases. Bioorg Med Chem Lett. 24:4414-7. PMID: 25150376

2. Matveev SV, Spielmann HP, Metts BM, Chen J, **Onono F**, Zhu H, Scheff SW, Walker LC, LeVine H 3rd. **2014** A Distinct Subfraction of Aβ is Responsible for the High-Affinity Pittsburgh Compound B (PIB) Binding Site in Alzheimer's Disease Brain. J Neurochem. PMID: 24995708 (Accepted article)

3. Reuter CWM*, Krauter J*, **Onono FO**, Bunke T, Damm F, Thol F, Wagner K, Göhring G, Schlegelberger B, Heuser M, Ganser A and Morgan MA. **2014**. Lack of non-canonical *RAS* mutations in cytogenetically normal acute myeloid leukemia. **Ann Hematol.** 93:977-82 PMID: 24737308 *Equal 1st authors

4. **Onono FO,** Subramanian T, Sunkara M, Subramanian KL, Spielmann HP, and Morris AJ**. 2013**. Efficient use of exogenous isoprenols for protein isoprenylation by MDA-MB-231 cells is regulated independently of the mevalonate pathway. ***J Biol Chem***. 288:27444-55. PMID: 23908355

5. Subramanian T, Subramanian KL, Sunkara M, **Onono FO**, Morris AJ and Spielmann HP. **2013** Synthesis Of Deuterium Labeled Prenyldiphosphate And Prenylcysteine Analogues For in vivo Mass Spectrometric Quantification. ***J. Label. Compd. Radiopharm***. 56: 370-375. PMID: 24285475

6. Morgan MA*, **Onono FO***, Spielmann HP, Subramanian T, Scherr M, Venturini L, Dallmann I, Ganser A and Reuter CWM. **2012**. Modulation of anthracycline-induced cytotoxicity by targeting the prenylated proteome in myeloid leukemia cells**. *J Mol Med*** 90:149-61. PMID 21915711 *Equal 1st authors

7. **Onono FO**, Morgan MA, Spielmann HP, Andres DA, Subramanian T, Ganser A, Reuter CW. **2010**. A tagging-via-substrate approach to detect the farnesylated proteome using two-dimensional electrophoresis coupled with Western blotting. ***Mol Cell Proteomics***. 9:742-51. PMID: 20103566

8. Divchev D, Grothusen C, Luchtefeld M, Thoenes M, **Onono F**, Koch R, Drexler H, Schieffer B. **2008**. Impact of a combined treatment of angiotensin II type 1 receptor blockade and 3-hydroxy-3-methyl-glutaryl-CoA-reductase inhibition on secretory phospholipase A2-type IIA and low density lipoprotein oxidation in patients with coronary artery disease. ***Eur Heart J*** 29: 1956-65. PMID: 18565968

9. Graham SP, Pellé R, Yamage M, Mwangi DM, Honda Y, Mwakubambanya RS, de Villiers EP, Abuya E, Awino E, Gachanja J, Mbwika F, Muthiani AM, Muriuki C, Nyanjui JK, **Onono FO**, Osaso J, Riitho V, Saya RM, Ellis SA, McKeever DJ, MacHugh ND, Gilbert SC, Audonnet JC, Morrison WI, van der Bruggen P, Taracha EL. **2008**. Characterization of the fine specificity of bovine CD8 T-cell responses to defined antigens from the protozoan parasite Theileria parva. ***Infect Immun***. 76: 685-94. PMID: 18070892

10. Graham SP, Pellé R, Honda Y, Mwangi DM, Tonukari NJ, Yamage M, Glew EJ, de Villiers EP, Shah T, Bishop R, Abuya E, Awino E, Gachanja J, Luyai AE, Mbwika F, Muthiani AM, Ndegwa DM, Njahira M, Nyanjui JK, **Onono FO**, Osaso J, Saya RM, Wildmann C, Fraser CM, Maudlin I, Gardner MJ, Morzaria SP, Loosmore S, Gilbert SC, Audonnet JC, van der Bruggen P, Nene V, Taracha EL. **2006**. Theileria parva candidate vaccine antigens recognized by immune bovine cytotoxic T lymphocytes. ***Proc Natl Acad Sci U S A***. 103, 3286-91. PMID: 16492763

Research Support

Completed Research Support

12POST9420010 (Onono)

American Heart Association 1/1/2012-12/31/2013

Physiological role of isoprenols in sterol synthesis and protein isoprenylation

This postdoctoral fellowship award supported my training in lipid metabolism and signaling in the context of isoprenoid utilization by cells in normal and pathophysiology state.

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
Andrew J. Morris	Professor
eRA COMMONS USER NAME (credential, e.g., agency login) AMorris	

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 Bristol	B.Sc.	1981-84	Biochemistry
University of Birmingham	Ph.D.	1984-88	Biochemistry
University of North Carolina, Chapel Hill, NC	Post-Doctoral	1988-91	Pharmacology

A. Personal Statement

Lipid phosphates are key molecules in the synthesis of sterols, neutral and phospholipids and in some cases serve as extracellular signaling molecules with actions on cell surface receptors. The broad theme of my research program is to use approaches of biochemistry, genetics, chemical, cell and molecular biology to gain fundamental insights into the metabolism of this class of molecules and to then apply this information in preclinical and clinical models to explore their potential roles in physiological and disease processes. As part of this research, my laboratory has become invested in the development and implementation of tandem mass spectrometry based methods for quantitation and profiling of lipids. Over the past 20 years, a measure of my commitment to the career development of pre- and post- doctoral fellows and early career stage investigators is that individuals receiving training in my laboratory have competed successfully for individual support from the American Heart Association (8 pre or Post-Doctoral Fellowship Awards, three Beginning Grant in Aids and two Scientist Development Grants) as well as individual F32 and K99/R00 awards from the NIH and gone onto successful careers as independent investigators in academia and industry. As part of my role as director of a mass spectrometry core laboratory I am also particularly focused on training students in biomedical applications of mass spectrometry. I will serve as primary mentor for this project,

B. Positions and Honors

Positions and Employment

1991-1993: Res. Assistant Professor, Pharmacology, UNC-Chapel Hill, Chapel Hill, NC
1993-1999 Assistant Professor, Pharmacology, SUNY-Stony Brook, Stony Brook, NY.
1999-2001 Associate Professor, Pharmacology, SUNY-Stony Brook, Stony Brook, NY.
2001-2005 Associate Professor, Cell Biology, UNC-Chapel Hill, Chapel Hill, NC
2005-Present Endowed Professor, Cardiovascular Medicine & Pharmacology University of Kentucky, & Investigator, Lexington Veterans Affairs Medical Center, Lexington KY

Other Experience and Professional Memberships

1999, 2001 Co-Chair, FASEB Conference on Phospholipase D
2000-2005, 2010- Present Editorial Board, Journal of Biological Chemistry
2001-2010 Associate Editor, The Biochemical Journal
2004 Chair, FASEB Conference on Phospholipases
2005 & 2007 Co-Chair, FASEB Conference on Lysophospholipids and Related Mediators

Honors

1983-87: SERC CASE Predoctoral Fellowship Award
1990: American Heart Association Young Investigator Award
1990-92: Patrick J. Mitchell Fellowship, American Heart Association North Carolina Affiliate
1994: NIH Director's Shannon Award.

C. Selected recent publications (past 3 years) demonstrating expertise in small molecule mass spectrometry (from 213 total peer reviewed publications).

1. Albers HM, Dong A, van Meeteren LA, Egan DA, Sunkara M, van Tilburg EW, Schuurman K, van Tellingen O, Morris AJ, Smyth SS, Moolenaar WH, Ovaa H. 2010. Boronic acid-based inhibitor of autotaxin reveals rapid turnover of LPA in the circulation. ***Proc Natl Acad Sci U S A*** 107:7257-62. PMCID: 2867685
2. Pihlajamaki J, Lerin C, Itkonen P, Boes T, Floss T, Schroeder J, Dearie F, Crunkhorn S, Burak F, Jimenez-Chillaron JC, Kuulasmaa T, Miettinen P, Park PJ, Nasser I, Zhao Z, Zhang Z, Xu Y, Wurst W, Ren H, Morris AJ, Stamm S, Goldfine AB, Laakso M, Patti ME. 2011. Expression of the splicing factor gene SFRS10 is reduced in human obesity and contributes to enhanced lipogenesis. ***Cell Metab*** 14:208-18. PMCID: 3167228
3. Hausmann J, Kamtekar S, Christodoulou E, Day JE, Wu T, Fulkerson Z, Albers HM, van Meeteren LA, Houben AJ, van Zeijl L, Jansen S, Andries M, Hall T, Pegg LE, Benson TE, Kasiem M, Harlos K, Kooi CW, Smyth SS, Ovaa H, Bollen M, Morris AJ, Moolenaar WH, Perrakis A. 2011. Structural basis of substrate discrimination and integrin binding by autotaxin. ***Nat Struct Mol Biol*** 18:198-204. PMCID: 3064516
4. Breart B, Ramos-Perez WD, Mendoza A, Salous AK, Gobert M, Huang Y, Adams RH, Lafaille JJ, Escalante-Alcalde D, Morris AJ, Schwab SR. 2011. Lipid phosphate phosphatase 3 enables efficient thymic egress. ***J Exp Med*** 208:1267-78. PMCID: 3173249
5. Huang H, Gao Q, Peng X, Choi SY, Sarma K, Ren H, Morris AJ, Frohman MA. 2011. piRNA-associated germline nuage formation and spermatogenesis require MitoPLD profusogenic mitochondrial-surface lipid signaling. ***Dev Cell*** 20:376-87. PMCID: 3061402
6. Golan K, Vagima Y, Ludin A, Itkin T, Cohen-Gur S, Kalinkovich A, Kollet O, Kim C, Schajnovitz A, Ovadya Y, Lapid K, Shivtiel S, Morris AJ, Ratajczak MZ, Lapidot T. 2012. S1P promotes murine progenitor cell egress and mobilization via S1P1-mediated ROS signaling and SDF-1 release. ***Blood*** 119:2478-88. PMCID: 22279055
7. Mendoza A, Breart B, Ramos-Perez WD, Pitt LA, Gobert M, Sunkara M, Lafaille JJ, Morris AJ, Schwab SR. 2012. The transporter Spns2 is required for secretion of lymph but not plasma sphingosine-1-phosphate. ***Cell Rep*** 2:1104-10. PMCID: 3616498
8. Xiang B, Zhang G, Guo L, Li XA, Morris AJ, Daugherty A, Whiteheart SW, Smyth SS, Li Z. 2013. Platelets protect from septic shock by inhibiting macrophage-dependent inflammation via the cyclooxygenase 1 signalling pathway. ***Nat Commun*** 4:2657. PMCID: 24150174
9. Mitra MS, Chen Z, Ren H, Harris TE, Chambers KT, Hall AM, Nadra K, Klein S, Chrast R, Su X, Morris AJ, Finck BN. 2013. Mice with an adipocyte-specific lipin 1 separation-of-function allele reveal unexpected roles for phosphatidic acid in metabolic regulation. ***Proc Natl Acad Sci U S A*** 110:642-7. PMCID: 3545773
10. Singh S, Chang A, Helmich KE, Bingman CA, Wrobel RL, Beebe ET, Makino S, Aceti DJ, Dyer K, Hura GL, Sunkara M, Morris AJ, Phillips GN, Jr., Thorson JS. 2013. Structural and Functional Characterization of CalS11, a TDP-Rhamnose 3'-O-Methyltransferase Involved in Calicheamicin Biosynthesis. ***ACS Chem Biol*** 8:1632-9. PMCID: 3875630
11. Li X, Zhou Q, Sunkara M, Kutys ML, Wu Z, Rychahou P, Morris AJ, Zhu H, Evers BM, Huang C. 2013. Ubiquitylation of phosphatidylinositol 4-phosphate 5-kinase type I gamma by HECTD1 regulates focal adhesion dynamics and cell migration. ***J Cell Sci*** 126:2617-28. PMCID: 3687698
12. Salous AK, Panchatcharam M, Sunkara M, Mueller P, Dong A, Wang Y, Graf GA, Smyth SS, Morris AJ. 2013. Mechanism of rapid elimination of lysophosphatidic acid and related lipids from the circulation of mice. ***J Lipid Res*** 54:2775-84. PMCID: 3770090
13. Herzog BH, Fu J, Wilson SJ, Hess PR, Sen A, McDaniel JM, Pan Y, Sheng M, Yago T, Silasi-Mansat R, McGee S, May F, Nieswandt B, Morris AJ, Lupu F, Coughlin SR, McEver RP, Chen H, Kahn ML, Xia L. 2013. Podoplanin maintains high endothelial venule integrity by interacting with platelet CLEC-2. ***Nature*** 502:105-9. PMCID: 3791160
14. Onono F, Subramanian T, Sunkara M, Subramanian KL, Spielmann HP, Morris AJ. 2013. Efficient use of exogenous isoprenols for protein isoprenylation by MDA-MB-231 cells is regulated independently of the mevalonate pathway. ***J Biol Chem*** 288:27444-55. PMCID: 3779739
15. Wu T, Kooi CV, Shah P, Charnigo R, Huang C, Smyth SS, Morris AJ. 2014. Integrin-mediated cell surface recruitment of autotaxin promotes persistent directional cell migration. ***Faseb j*** 28:861-70. PMCID: 3898650

D. Research Support

Active

BX001984-01 (Morris) 11/05/12-11/04/16
VA BLR&D Merit Review**: Association of a Common Variant of PPAP2B gene with cardiovascular disease**
The major goals of this application are to test specific hypotheses about the mechanistic basis for the strong association of a common polymorphism in the PPAP2B gene with cardiovascular disease. Role: PI, no overlap

5I01BX001014-03 (Smyth) 10/1/2010-9/30/2018
VA BLR&D Merit Review: **Regulation of adipose cells by autotaxin / lysophosphatidic acid signaling**
The goal of this project is to test the hypothesis that signaling pathways involved in the synthesis and metabolism of lysophosphatidic acid contribute to diet-induced thermogenesis and regulate the development of obesity. Role Co-PI. No overlap.

1R01HL120507-01 (Morris, Smyth, MPI) 01/01/2015 – 12/30/2019
NIH/NHLBI **Lipid phosphate phosphatase 3 as a novel atherosclerosis suppressor**
The goal of this study is to use pre-clinical models to identify the cell types and mechanisms involved in protective effects of lipid phosphate phosphatase 3 against cardiovascular disease. Note, this received a priority score of 1.2 and a percentile ranking of 1% so we anticipate it will be approved for funding.
Role: PI

5R01HL078663-07 (Smyth) 09/24/2004-06/30/2015
NIH/NHLBI: **Lysolipid Signaling in Cardiovascular Disease**
The broad goal of this project is to identify roles for LPA signaling and specific LPA receptors in vascular injury responses and regulation of vascular tone. Role: Co-PI. No overlap

8P20GM103527-05 (Cassis) 09/08/08-06/30/2019
NIH/NIGMS: **Center of Research in Obesity and Cardiovascular Disease: Analytical Core**
I direct an analytical core of this center grant and serve as a mentor to junior faculty investigators supported by this award. Role: Core director, mentor. No overlap.

5P42ES007380-16 (Hennig) 04/07/97-03/31/19
NIEHS: **Superfund Basic Research Program: Research Support Core**
I direct this core which provides Bioanalytical and Bioinformatics support to investigators of the University of Kentucky Superfund basic research program. Role: Core director. No overlap.

5P42ES007380-16 (Hennig) 04/07/97-03/31/19
NIEHS: **Superfund Chemicals, Nutrition, and Endothelial Cell Dysfunction**
The goal of this study is to identify mechanisms by which environmental pollutants impair vascular endothelial cell function to promote cardiovascular disease. Role: Co-PI. No overlap.

1R01HL112788 (Rataczjak) 03/01/2013-02/28/2017
University of Louisville (NIH flow through): **Bioactive lipids in stem cell mobilization and homing**
The goal of this proposal is to test the hypothesis that sphinogsine 1 phosphate and ceramide 1 phosphate regulate bone marrow mobilization and homing of hematopoietic stem cells. Role: Co-PI. No overlap

1R01ES023470-01 (Zhou) 09/26/2013 – 06/30/2018
NIH/NIEHS: **Endocrine disruptor mediated activation of PXR causes dyslipidemia**
The goal of this study is to define the role of endocrine disrupting environmental toxins as regulators of pathological hyperlipidemia and cardiovascular disease. Role: Co-PI. No overlap.

1R56HL124266-01 (Abdel-Latif) 09/01/2014-08/31/2015
NIH/NHLBI: **Role of Bioactive Lipids in Stem Cell Mobilization and Homing in Cardiac Ischemia**
The goal of this study is to test the hypothesis that bioactive sphingophospholipids promote mobilization from the bone marrow and homing to the myocardium of stem cells that mediate beneficial effects on recovery from myocardial ischemia and to validate therapeutic approaches to promote this process in animal models. Role: Co-PI. No overlap.

Recently completed relevant prior research support.

3R01GM050388-16S2 (Morris) 5/15/10-5/14/11
NIH/NIGMS: **Role of Lipid Phosphatases in Cholesterol and Triglyceride Synthesis**
This ARRA supplement funded the acquisition of upgraded mass spectrometry equipment to be used for the identification and quantitation of lipids and other small molecule metabolites

3R01GM066152-07 (Spielmann)
NIH/NIGMS 5/15/10-5/14/11
Synthetic probes of Protein Prenylation
This ARRA supplement supported acquisition of upgraded mass spectrometry equipment to be used for the identification and quantitation of lipids and other small molecule metabolites by high throughput compatible MALDI mass spectrometry.

1R56HL124266-01 (Abdel-Latif)
NIH/NHLBI
Role of Bioactive Lipids in Stem Cell Mobilization and Homing in Cardiac Ischemia

S10 RR026884 (Morris) 05/31/11-6/30/12
NIH/NCRR
Advion Triversa Nanomate/ABSciex Nanospray III Ion Source for Targeted Lipidomics
This award provided funds for acquisition of a robotic chip-based nano electrospray ion source for mass spectrometry of lipids and other small molecule metabolites.

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

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
Kathleen L. O'Connor, PhD	Professor, Department of Molecular and Cellular Biochemistry
eRA COMMONS USER NAME (credential, e.g., agency login) kloconnor	

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
James Madison University, Harrisonburg, VA	BS	05/1988	Biology
Case Western Reserve University, Cleveland, OH	PhD	09/1996	Molecular Biology
Harvard Medical School, BIDMC, Boston, MA	Postdoctoral	12/2001	Cancer Cell Biology

A. Personal Statement

My lab focuses on the contributions of integrin signaling to the invasion and metastasis of breast cancer with specific emphasis on the integrin α6β4. Integrin α6β4 confers an invasive and metastatic phenotype in many types of carcinomas. Dissecting the pathways altered by integrin α6β4 has given and will continue to contribute great insight into the processes that perpetuate an invasive and metastatic phenotype. I originally discovered links between integrin signaling and the cAMP pathway, and also determined that integrin α6β4 could stimulate the small GTPase RhoA leading to lamellae formation. My lab continues to expand upon these findings in the context of chemotactic migration and invasion toward tumor-relevant attractants such as LPA, EGF and HGF with a concentration on how RhoA signaling is regulated and contributes to invasion. We have further expanded our work to include investigations on how integrin α6β4 modifies the transcriptome toward an invasion signature. Through these studies, we have identified the first transcriptional targets for NFAT1 and NFAT5 in cancer and provided evidence that integrin α6β4 can stimulate select demethylation of the promoters of pro-metastatic genes, including S100A4, amphiregulin and epiregulin. The overarching goal of our work is to better understand the contributions of integrin α6β4 to tumor invasion and understand how this "oncogenic" function differs from its normal functions in order to better target integrin α6β4 and pathways it influences for therapeutic intervention. My knowledge and expertise in the fields of breast cancer; signal transduction from integrin α6β4, from Rho family GTPases, and in tumor cell dissemination; and in transcriptional control of an invasive phenotype and in breast cancer will be an asset to the proposed study. Furthermore, I serve as the Associate Director or Cancer Education where I mentor many students, post-docs and junior faculty. Notably, I have a strong track record of mentoring junior faculty toward independent funding. I am also the Co-Director of the Breast Translational Group, which is a multidisciplinary group of scientists, clinicians and bioinformaticists dedicated to translation cancer research in breast cancer. My expertise in breast cancer, my mentorship and the resources through the Breast Translational Group are available to Dr. Onono throughout his career development.

B. Positions and Honors

Positions and Employment

1988-1990 Research Assistant, Department of Pathology, University of Texas Health Sciences Center, Houston, TX
1996-2001 Research Fellow, Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA
2002-2009 Scientist, Sealy Center for Cancer Cell Biology, University of Texas Medical Branch, Galveston, TX
2002-2006 Assistant Professor, Departments of Surgery and Biochemistry & Molecular Biology, The University of Texas Medical Branch, Galveston, TX
2006-2009 Associate Professor, Departments of Surgery and Biochemistry & Molecular Biology, The University of Texas Medical Branch, Galveston, TX (tenured in 2009)
2005-2009 Director, Cancer Cell Biology Track within Biochemistry and Molecular Biology, Cell Biology and

Pharmacology/Toxicology Graduate Programs, University of Texas Medical Branch, Galveston, TX
2009-2013 Associate Professor, Markey Cancer Center and the Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY (tenured)
2010-present Associate Director for Cancer Education, Markey Cancer Center, University of Kentucky, Lexington, KY
2013-present Professor, Markey Cancer Center and the Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY (tenured)

Honors

1991-1995 National Research Service Award, Predoctoral Fellowship under National Institutes of Health Aging Training Grant (AG00105)
1997-2001 Department of Defense Breast Cancer Research Program Postdoctoral Fellowship (DAMD17-98-1-8033)
2000 Invited Speaker, Department of Defense, Era of Hope Breast Cancer Meeting, Atlanta, GA
2004 Invited Speaker, Genomic Based Drug Discovery, Florence, Italy
2004 Invited Speaker, American Association for Cancer Research, Orlando, FL
2005 UTMB Representative at the Association of American Medical Colleges (AAMC) Early Career Women Faculty Professional Development Seminar, Santa Fe, NM
2006 Nominated for the UTMB Graduate Student Organization's Distinguished Faculty Teaching Award; 2007, UTMB Graduate Student Organization Student Advocacy Award
2010 Wethington Award, University of Kentucky
2013 Wethington Award, University of Kentucky
2013 Distinguished Achievement Award in Pancreatic Cancer Research, Shanghai International Conference on Pancreatic Cancer
2014 Wethington Award, University of Kentucky

Study Section Service

2009-present Regular Member of the American Cancer Society Cell Structure and Metastasis Peer Review Committee
2010 Ad Hoc member of NIH Tumor Progression and Metastasis Study Section (February and November)
2012 Ad Hoc member of NIH Tumor Progression and Metastasis Study Section (June)
2011 Ad Hoc Vice-Chair, American Cancer Society Cell Structure and Metastasis Peer Review Committee
2012-present Chair, American Cancer Society Cell Structure and Metastasis Peer Review Committee
2012 Ad Hoc, NIH Special Emphasis panel ZRG1 FO9-P(20) (F32 review);
2013 ZCA1 SRLB-1 NCI Omnibus Biology Study Section (July and November)
2013 DOD BC13 TRN-PBY Panel Member
2013 NCI-A RTRB-L R1 Workgroup 005 NCI Cancer Center Support Grant (P30) cancer center review and site visit
2014 Temporary Members NCI IRG Subcommittee A Parent Committee
2014 NCI-A RTRB-L R1 Workgroup 005 NCI Cancer Center Support Grant (P30) cancer center review and site visit
2014 ZCA1 SRLB-1 NCI Omnibus Biology Study Section (March and November)

C. Selected Peer-Reviewed Publications (39 publications to date; 10 in the last three years)

1. Yang X, Yin Y, Deng X, Baldwin L, Hoff J, Erfani S, Lefringhouse J, Rucker E, **O'Connor K**, Liu C, Wu Y, and Zhou PB. CD151 Represses Mammary Gland Development by Maintaining the Niches of Progenitor Cells. Cell Cycle. *In Press*.
2. Chen M, Knifley T, Subramanian T, Spielmann HP, **O'Connor KL.** Use of synthetic isoprenoids to target protein prenylation and Rho GTPases in breast cancer invasion. PLoS ONE 9:e89892, 2014. PMCID: PMC3935959
3. **O'Connor KL**, Chen M. Dynamic functions of RhoA in tumor cell migration and invasion. Small GTPases 4:1-7, 2013. PMCID: PMC3976970
4. Chen M, Bresnick AR, **O'Connor KL**. Coupling S100A4 to Rhotekin alters Rho signaling output in breast cancer cells. Oncogene 32:3754-3764, 2013. PMCID: PMC3525797

5. Harrison SM, Knifley T, Chen M, **O'Connor KL**. LPA, HGF, and EGF utilize distinct combinations of signaling pathways to promote migration and invasion of MDA-MB-231 breast carcinoma cells. BMC Cancer 13:501, 2013. PMCID: PMC3819718
6. Zaytseva YY, Rychahou PG, P Gulhati P, Elliott, VA, Mustain WC, **O'Connor K**, Morris AJ, Sunkara M, Weiss HL, Lee EY, Evers BM. Inhibition of fatty acid synthase attenuates CD44-associated signaling and reduces metastasis in colorectal cancer. Cancer Res 72:1504-17, 2012. PMCID: PMC3596828
7. **O'Connor KL**, Chen M, Towers LN. Integrin α6β4 cooperates with LPA signaling to stimulate Rac1 through AKAP-Lbc-mediated RhoA activation. Am J Physiol Cell Physiol 302:C605-14, 2011. PMCID: PMC328
8. Chen M, Sastry SK, **O'Connor KL.** Src kinase pathway is involved in NFAT5-mediated S100A4 induction by hyperosmotic stress in colon cancer cells. Am J Physiol Cell Physiol 300:C1155-63, 2011.
9. Gulhati P, Bowen KA, Liu J, Stevens PD, Rychahou PG, Chen M, Lee EY, Weiss HL, **O'Connor KL**, Gao T Evers BM. mTORC1 and mTORC2 regulate EMT, motility and metastasis of colorectal cancer via RhoA and Rac1 signaling pathways. Cancer Res 71:3246-56, 2011. PMCID: PMC3085654
10. Paulucci-Holthauzen AA, Vergara LA, Bellot LJ, Canton D, Scott JD, and **O'Connor KL**. Spatial distribution of PKA activity during cell migration is mediated by A Kinase Anchoring Protein AKAP-Lbc. J Biol Chem 284:5956-67, 2009. PMCID: PMC2645839
11. Chen M, Sinha M, Luxon BA, Bresnick AR, **O'Connor KL**. Integrin α6β4 controls the expression of genes associated with cell motility, invasion and metastasis including S100A4/metastasin. J Biol Chem 284:1484-94, 2009. PMCID: PMC2615501
12. Cruz-Monserrate Z, **O'Connor KL.** Integrin α6β4 promotes the migration and invasion of pancreatic cancer cells through the upregulation of Tiam-1 and subsequent activation of Rac. Neoplasia 10:1-10, 2008. PMCID: PMC2373869
13. Chen M, Towers LN, and **O'Connor KL**. LPA2 (EDG4) mediates Rho-dependent chemotaxis with lower efficacy than LPA1 (EDG2) in breast carcinoma cells. Am J Physiol Cell Physiol 292:C1927-33, 2007.
14. Cruz-Monserrate Z, Qiu S, Evers BM, and **O'Connor KL**. Upregulation and redistribution of integrin α6β4 expression occurs at an early stage in pancreatic adenocarcinoma progression. Mod Pathol 20:656-67, 2007.
15. Chen M, **O'Connor KL**. Integrin α6β4 promotes expression of autotaxin/ENPP2 autocrine motility factor in breast carcinoma cells. Oncogene 24:5125-5130, 2005.

D. Research Support

Ongoing

R01 CA109136 (PI: O'Connor, KL) 07/01/04-03/31/17
NIH
"Novel Mechanisms of Carcinoma Cell Migration"
Goals: To define the macromolecular complex that couples β1 integrins to PKA activation thereby limiting RhoA activity at the leading edge; to determine how integrin α6β4 leads to the activation of RhoA; and to elucidate how RhoA function is altered to promote lamellae formation.
Role: PI

T32 CA165990 (PI: Rangnekar, V) 04/01/13-03/31/16
NCI
"Interdisciplinary Research Training in Cancer Biology"
Goals: To develop a cadre of future scientists who can become leaders in integrative team approaches to understand the complex issue of cancer as it relates to potential prevention and treatment strategies.
Role: Co-PI

T32 CA160003 (PI: Evers, BM) 07/01/11-06/30/16
NIH
"Oncology Research Training for Surgeon Scientists"
Goals: To provide intensive and interdisciplinary basic science research training for a minimal two-year period for qualified individuals who are pursuing a career in academic oncologic surgery.
Role: Co-PI

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 Torroella-Kouri, Marta	Associate Professor
eRA COMMONS USER NAME (credential, e.g., agency login) mtorroella	

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 Havana, Cuba	B.S.	1973	Biology
Nat. Center Sc. Res. (CENIC), Havana, Cuba	M.S.	1978	Microbial Genetics
Czechoslovak Acad Science, Prague, Czech Rep	M.S.	1984	Genetic Toxicology
Czechoslovak Acad Science, Prague, Czech Rep	Ph.D.	1985	Genetics
Academy Science Berlin Buch, Germany	Postdoc	1987	Breast cancer
Karolinska. Hospital, Stockholm, Sweden	Postdoc	1988	oncogenes and tumor suppressor genes.

A. Personal Statement

I have the expertise and motivation necessary to successfully carry out the projected work as a collaborator on this proposal, effectively interacting with the PI. Obesity has been recognized as a significant health problem in US, although consuming high fat diets is more common than being obese. Either being obese or consuming a high fat diet significantly induces inflammation and increases cancer risk.

I have extensive experience in various areas of cancer research as cancer prevention, genetic toxicology, molecular epidemiology, tumor immunology, inflammation and cancer, and *in vitro/in vivo* models of cancer, spanning the last 40 years. I started focusing on the interplay between macrophages and tumors in 1999, particularly in the way by which tumors modify macrophages and differently impact them on diverse tissue locations, especially the tumor microenvironment. With the solid experience attained working in macrophages, inflammation and cancer, I became interested in the last years in the potential promoting role of breast adipose tissue in breast cancer, particularly in the context of obesity or upon ingestion of a high fat diet, a hypothesis in which I started working four years ago and in which I have been funded by NIH. I have built a very solid infrastructure for these studies, both in mice and humans, with internationally well-known collaborators in the field of cancer and obesity and also within our university, with highly experienced and motivated colleagues. As PI of two recently awarded NIH grants in this new subject, and also recognized by our institution with several other grants in the field, I believe that I have laid the groundwork for these studies, which have been considered very relevant. In summary, I believe I have a record of successful and productive research projects in areas of high relevance to the present study, and my expertise and experience have prepared me to successfully collaborate with the PI of the proposed project.

B. Positions and Honors

Professional Appointments:

1974-81 Investigator, National Center for Scientific Research (CENIC), Havana, Cuba.
1981-86 Aggregate Investigator, CENIC, Havana, Cuba.
1986-91 Auxiliary Investigator, National Institute of Oncology and Radiobiology (INOR), Havana, Cuba.
1991-94 Senior Research Investigator, INOR, Havana, Cuba.
1992-94 Adjunct Professor, University of Havana, Cuba.
1994-96 Endowed Chair (Patrimonial Chair of Excellence, CONACyT, Mexico), Invited Research Professor, National Institute of Cancer (INCAN), Mexico City, Mexico.
1995-96 Visiting Faculty, Johns Hopkins School of Hygiene and Public Health, Dept. of Molecular Microbiology and Immunology, Baltimore, MD.
1997-98 Visiting Scientist, University of Miami School of Medicine, Dept. of Pathology, Miami, FL
1998-99 Assistant Professor, University of Miami School of Medicine, Dept. of Pathology, Miami, FL.
1999-2012 Assistant Professor, Dept. of Microbiology and Immunology, University of Miami School of Medicine, Miami, FL.
2008-present Graduate faculty, University of Miami Miller School of Medicine.

2012: Associate Professor, Departments of Microbiology and Immunology and Epidemiology/Public Health, University of Miami Miller School of Medicine, Miami, FL.
2013: Associate Member, Cancer Biology Graduate Program, Univ. Miami Miller Sch. of Medicine.

Training and academic visits:

- Laboratorio di Mutagenesi e Differenziamento, Pisa, Italy, 1976.
- Institute of Hygiene, Prague, Czechoslovakia, 1982-83 and 1984-85.
- Institute of Hygiene and Institute of Molecular Genetics of the Academy of Sciences, Prague, Czechoslovakia, 1984-85.
- International Agency for Research on Cancer (IARC), Lyon, France, 1985 and 1992.
- Center for Molecular Biology of the Academy of Sciences, Berlin Buch, Berlin, Germany, 1987.
- Department of Clinical Genetics, Karolinska Hospital, Stockholm, Sweden, 1988.
- Karolinska Hospital and Institute, Stockholm, Sweden, 1992.
- Institute Curie and Pasteur Institute and Hospital, Paris, France, 1992.
- Research Center for Advances Studies (CINVESTAV), Mexico City, Mexico,1993-94.
- Institute of Cancerology (INCAN), Mexico City, Mexico, 1994-96.
- Johns Hopkins School of Hygiene and Public Health, Dept. Molecular Microbiology and -Immunology, Baltimore, MD, US, 1995-96.
- Digene Diagnostics, Inc, Silver Spring, MD, US, 1995.
- Department of Microbiology and Immunology, Weill Medical College at Cornell University, New York, US, 2002-03.
- Venezuelan Institute for Scientific Research, Experimental Medicine Center, Caracas, Venezuela, 2008.

Honors and Awards:

1976 Research Fellowship Award from the Italo-Latin-American Institute (IILA), Italy.
1982-85 Research Fellowships from Polytechna/Czech Ministry of Higher Education, Czech Republic
1994-96 Endowed Chair for Research (Catedra Patrimonial de Excelencia) from the Mexican National Commission for the Development of Science and Technology, Mexico.
1995 Research Fellowship from the Population Council Office, Mexico City, Mexico
1996 Research Fellowship from the Pan American Health Organization, Mexico City, Mexico
2000 AACR/NCI Minority Scholar Award
2002 AACR/NCI Minority Scholar Award
2003 AACR/NCI Minority Scholar Award
2008 AACR/NCI Minority-Serving Institution (MSI) Faculty Scholar in Cancer Research Award.
2010 AACR/NCI Minority-Serving Institution (MSI) Faculty Scholar in Cancer Research Award.
2010 Women's Cancer Association of the University of Miami 2010 endowment.
2012 AACR/NCI Minority-Serving Institution (MSI) Faculty Scholar in Cancer Research Award.
2012 Minorities in Cancer Research Council (MICR) award in recognition of academic excellence and research achievements.

C. Publications:

Books and monographs published:

• Torroella-Kouri M and Villa Trevino S (1998) Genetic Basis of Cancer, book. Fondo de Cultura Economica (eds), Mexico.
• Torroella-Kouri M (1994) Molecular Mechanisms in Human Carcinogenesis, booklet. Instituto Tecnologico y de Estudios Superiores de Monterrey, Mexico.

Some relevant papers in the field of Tumor Immunology (selected from a total of 50+ peer reviewed publications):

1. **Torroella-Kouri M**, J Keith, M Ivanova and D Lopez, (2003) IL-11 induced reduction of C/EBP transcription factor binding may contribute to the IL-12 downregulation in tumor-bearing mice, *International J. Oncology*, 22 (2): 439-48.
2. **Torroella-Kouri M** and DM Lopez, (2003), Mammary tumor-derived TGF-beta1 impairs crucial innate immune responses in tumor hosts, *J of Immunology and Immunopathology*, 5 (1): 31-38.
3. **Torroella-Kouri M**, Herbert LM, Perry G and Lopez DM (2004) Altered IL-12 Signaling Pathways Contribute to the Deficient IFN-gamma production by T Splenocytes from Tumor-BearingMice, *Cancer Genomics and Proteomics* 1:345-354.
4. DiNapoli M, **Torroella-Kouri M,** Perry G and Lopez DM (2005), Diminished PKC activity and decreased binding of transcription factors are involved in the impaired production of nitric oxide by macrophages from

tumor-bearing mice, *International J Molecular Med*, 15:503-11.
5. **Torroella-Kouri M,** Ma X, Perry G, Ivanova M, Cejas PJ, Owen J, Iragavarapu-Charyulu, V and Lopez DM (2005), Diminished expression of transcription factors NFkB and C/EBP underlie a novel tumor evasion mechanism affecting macrophages of mammary tumor-bearing mice, *Cancer Research*, 65: (22): 10578-10584.
6. Owen J, Lopez, D, Guthrie K, Herbert, LM, Grosso, JF, **Torroella-Kouri M** and Iragavarapu-Charyulu,V, (2005), The expression of CCL-2 by T lymphocytes of mammary tumor bearers: Role of tumor-derived factors, *Cellular Immunology* 235:122-135.
7. Owen JL, **Torroella-Kouri M**, Handel-Fernandez ME, and Iragavarapu-Charyulu V. (2007), GM-CSF up-regulates the expression of CCL2 by T lymphocytes in mammary tumor bearing mice, *International J. of Molecular Medicine*, 20:129-136.
8. Calderon,CL **Torroella-Kouri M,** DiNapoli MR, and Lopez DM. (2008), Involvement of protein kinase C and not of NFκB in the modulation of macrophage nitric oxide synthase by tumor-derived phosphatidyl serine, *International. J of Oncology,* 32: 713-721.
9. Owen JL, **Torroella-Kouri M** and Iragavarapu-Charyulu V. (2008), Molecular Events Involved in the Increased Expression of MMP-9 by T Lymphocytes of Mammary Tumor Bearing Mice, *International J. of Molecular Medicine*, 21:125-134.
10.**Torroella-Kouri M,** Silvera R, Rodriguez D, Caso R, Shatry A, Opiela S, Ilkovitch D, Schwendener RA, Vijaya Iragavarapu-Charyulu, Yoslayma Cardentey, Natasa Strbo and Diana M. Lopez, (2009), Identification of a new subpopulation of macrophages that are neither M1 nor M2 in mammary tumor-bearing mice and are less differentiated, *Cancer Research*, 69 4800-4809.
11. Perry G, Iragavarapu-Charyulu V, Harjah EW and **Torroella-Kouri M** (2010), The role of proteasome in the downregulation of transcription factors NF□B and C/EBP in macrophages from tumor hosts, *Oncology Reports* 23:875-881.
12. Caso, R. R. Silvera, R.Carrio, V. Iragavarapu-Charyulu, R. R. Gonzalez-Perez and **M.Torroella-Kouri**, Blood monocytes from mammary tumor-bearing mice: early targets of tumor-induced immune suppression? *International J. Oncology*, 2010, 37: 891-900.
13. Carrio, R., **M.Torroella-Kouri**, V.Iragavarapu-Charyulu and D. M. Lopez, Tumor-induced thymic atrophy: Alteration in interferons and Jak/Stats signaling pathways, *International J. Oncology*, 2011, 38:547-553.
14. Owen, JL, M.F. Criscitiello, S. Libreros, R.Garcia-Areas, K. Guthrie, **M. Torroella-Kouri** and V.Iragavarapu-Charyulu, Expression of inflammatory chemokines CCL2, CCL5 and CXCL2 and the receptors CCR1-3 and CXCR2 in T lymphocytes from mammary tumor-bearing mice, *Cellular Immunology*, 270(2):172-82, 2011.
15. Carrio, R., **M.Torroella-Kouri**, S.Libreros, R.A. García-Areas, V.Iragavarapu-Charyulu, and D.M. López, Decreased accumulation of immune regulatory cells is correlated to the antitumor effect of IFN-γ overexpression in the tumor, *International J. Oncology*, 2011, Dec;39(6):1619-1627.
16**.** Libreros, S., R.Garcia-Arenas, Y. Shibata, R. Carrio, **M. Torroella-Kouri** and V. Iragavarapu-Charyulu, Induction of proinflammatory mediators by CHI3L1 is reduced by chitin treatment: decreased tumor metastasis in a breast cancer model, *International Journal of Cancer*, 2012, July 15; 131 (2): 377-86.
17. Guo, S. M.Liu, G. Wang, **M.Torroella-Kouri**, and R.R. Gonzalez-Perez, Oncogenic role and therapeutic target of leptin signaling in breast cancer and cancer stem cells, Review Article, accepted for publication in *Biochimica et Biophysica Acta BBA - Reviews on Cancer,* April; 1825 (2): 207-22, 2012.
18. Cornet, I., Gheit, T., Franceschi, S., Vignat, J., Burk, R.D., Sylla, B.S., Tommasino, M., Clifford, G.M., Hammouda, D., Loria, D., Matos, E., Alihonou, E., Rios-Dalenz, J.L., Eluf-Neto, J., Ghadirian, P., Ferreccio, C., Luzoro, A., Ojeda, J.M., Prado, R., Aristizabal, N., Tafur, L.A., Molano, M., Posso, H., **Torroella, M**., Alibegashvili, T., Kordzaia, D., Keita, N., Koulibaly, M., Rajkumar, T., Rajkumar, R., Lee, D.-H., Shin, H.R., Bayo, S., Chaouki, N., Thomas, J.O., Okolo, C., Adewole, I., Meijer, C.J.L.M., Snijders, P.J.F., De Los Rios, E.D., Rolon, P.A., Caceres, E., Santos, C., Ngelangel, C., Zatonski, W., Moodley, D., Gichangi, P., de Vuyst, H., de Sanjose, S., Castellsague, X., Kitinya, J.N., Chichareon, S., Sukvirach, S., Tunsakul, S., Wabinga, H.R., Human papillomavirus type 16 genetic variants: Phylogeny and Classification Based on E6 and LCR, Journal of Virology,86: 6855-6861 (2012).
19. Carrio, R., T.Koru-Sengul, F.Miao, S.Glück, O.Lopez, Y. Selman, C.Alvarez, C.Milikowski, C.Gomez, M.Jorda, M.Nadji and **M.Torroella-Kouri,** Macrophages as independent prognostic factors in small T1 breast cancers, *Oncology Reports* 29: 141-148, 2013.
20. Rodriguez, D., R. Silvera, R. Carrio, M.Nadji, R.Caso, G.Rodriguez, V.Iragavarapu-Charyulu and **M.Torroella-Kouri**, Tumor microenvironment profoundly modifies functional status of macrophages: peritoneal and tumor-associated macrophages are two very different subpopulations, accepted for publication, *Cellular Immunology*, 283: 51-60, 2013.

21. Battle, M., C.Gillespie, T.McGlothen, **M.Torroella-Kouri** and R.R. Gonzalez-Perez**.** Obesity induces leptin-Notch signaling in breast cancer*, Int. J Cancer*, 2013 Sep 30. doi: 10.1002/ijc.28496. [Epub ahead of print]. 2013.
22. **Torroella-Kouri M,** D. Rodríguez and R. Caso, Alterations in macrophages and monocytes from tumor-bearing mice: evidence of local and systemic immune impairment, *Immunologic Research*, Volume 57, Issue 1 (2013), 86-98.
23. Santander, AM, Lopez-Ocejo, O, Casas, O. Agostini, T., Sanchez, L., Lamas-Basulto, E., Carrio, R., Cleary, MP., Gonzalez-Perez RR. and Torroella-Kouri, M., Paracrine interactions between adipocytes and tumor cells recruit and modify macrophages to the mammary tumor microenvironment: the role of obesity and inflammation in the breast adipose tissue (submitted for publication).

D. Research Support:

Active:

1) NIH-NCI-R21 CA176055: Role of obesity and breast fat tissue inflammation in breast cancer promotion" for
PAR-12-096 Exploratory Grant Award to Promote Workforce Diversity in Basic Cancer Research (R21).
PI: Torroella-Kouri, Marta. 9/1/13-8/31/15.

2) NIH-NCI- R21 CA176055 Research Supplement under PI's current R21 grant " Role of obesity and breast fat tissue inflammation in breast cancer promotion" for PA-12-149 Research Supplements to Promote Diversity in Health-Related Research (Admin Supp).
PI: Torroella-Kouri, Marta 8/1/14-7/31/15.

Past:

1) NIH-NCI/R21: Breast cancer health disparity: mammary fat tissue and tumor macrophages interplay PAR-09-162 Exploratory Grant Award to Promote Workforce Diversity in Basic Cancer Research
4/1/11- 3/31/14. PI: Torroella-Kouri, Marta.
2) The effects of obesity and adipose tissue inflammation on the progression of breast cancer in the mouse, UM's 2011 Interdisciplinary Research Development Initiative (IRDI). 8/1/11- 3/31/13. PI: Torroella-Kouri, Marta.
3) NIH-NCI/KO1: Tumor-mediated impairment of IL-12 gene expression 7/2006-6/2012. (Career Development Award) PI: Torroella-Kouri, Marta.
4) The role of fat, estrogen and macrophages in breast cancer prognosis in African American and Latinas: a preliminary study, Sylvester Braman Family Developmental Grant 10/2010-09/2012. PI: Torroella-Kouri, Marta.
5) University of Miami Sylvester Comprehensive Cancer Center, Women's Cancer Association 2010 endowment. PI: Torroella-Kouri, Marta.
6) Molecular mechanisms involved in the impaired production of IL-12 and its receptor in tumor bearing animals. 6/1/01-05/31/02. PI: Torroella-Kouri, Marta. American Cancer Society Institutional Grant.
7) Tumor-mediated impairment of IL-12 and its receptor. 2/1/03- 1/31/04. 2002 UM/Sylvester Comprehensive Cancer Center Developmental Research Grant. PI: Torroella-Kouri, Marta.

Pending:

1) NIH/NCI: "Intrinsic inflammatory effects of a high fat diet in breast cancer progression" for PAR-12-096 "Exploratory Grant Award to Promote Workforce Diversity in Basic Cancer Research" (R21).
PI: Torroella-Kouri, Marta.
2) DoD Breast Cancer Era of Hope Scholar Award:" Diabetes and obesity increase breast cancer metastasis through RAGE-ligand signaling".
PI: Hudson, Barry; Co-investigator: Torroella-Kouri, Marta.
3) Bankhead-Coley Cancer Research Program, Discovery Science program: "The role of RAGE-ligand signaling in obesity-related breast cancer".
PI: Hudson, Barry; Co-investigator: Torroella-Kouri, Marta.

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
Jones, Laundette Patrice	Assistant Professor
eRA COMMONS USER NAME (credential, e.g., agency login) LPJones	

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
Morgan State University, Baltimore, MD	B.S.	06/92	Chemistry
Johns Hopkins University, Baltimore, MD	Ph.D.	05/00	Environ. Health Sciences/Toxicology
Laboratory of Experimental Carcinogenesis, National Cancer Institute, NIH, Bethesda, MD	Postdoc	06/00	Experimental Carcinogenesis
Georgetown University, Washington, DC	Postdoc	07/02	Tumor Biology

A. Personal Statement

Experiments in our research program work together towards a common goal: To translate our mechanistic basic research to the clinic by identifying early biomarkers which are associated with the development of breast cancer that could yield new targets for prevention of this disease. We seek to gain a better understanding of the etiology of breast cancer, including how changes in endogenous and exogenous factors impact the ultimate fate of whether a mammary epithelial cell develops normally or turns cancerous. I have over 14+ years of experience in mammary carcinogenesis and studying the mechanisms of breast tumor development in preclinical mouse models of breast cancer. Our lab has also made contributions towards understanding how the combination of genetic and environmental risk factors impacts the development of breast cancer, including the novel gene-environment paradigm of breast cancer etiology. I am also collaborating with several clinicians and basic science faculty in the University of Maryland's Hormone Responsive Cancers Research program. This program is comprised of basic science and clinical investigators dedicated to understanding the biology, diagnosis, treatment, and prevention of breast and prostate cancers through basic and translational research. Taken together, I believe that my past experiences in breast cancer research and collaborations with clinical faculty are well-suited to assist in the professional development of Mr. Fredrick Onono.

B. Positions and Honors

Positions and Employment

Assistant Director, Biotechnical Institute of Maryland, Baltimore, MD
2001-2002 Adjunct Assistant Professor, Department of Medical Research and Technology, University of Maryland School of Medicine, Baltimore, MD
2002-2005 Instructor, Biotechnology Part-Time Graduate Programs, Johns Hopkins University, Krieger School of Arts and Sciences, Baltimore, MD
2005- Pres. Assistant Professor, University of Maryland School of Medicine, Department of Pharmacology, Baltimore, MD
2005-Pres. Member, University of Maryland School of Medicine Program in Oncology, Hormone Responsive Cancers Program, Marlene and Stewart Greenbaum Cancer Center
2013-Pres. Global Breast Cancer Advisory Board, Amgen Pharmaceuticals

Other Experience and Professional Memberships

1998-Present Member, American Association for Cancer Research
2006-2009 Reviewer, Susan G. Komen Breast Cancer Foundation Scientific Review Panel
2006-Pres. Member, Congressionally Directed Biomedical Research Grants, Department of Defense (DOD) Breast and Prostate Cancer Review Panel
2010-2011 Executive Committee Member, Sister's Network Baltimore Chapter
2011 Member, American Cancer Society Institutional Research Grant Review Committee

2013 Study section member, National Cancer Institute (NCI) Special Emphasis Panel NCI Small Grants Program (R21/R03) for Cancer Research

Honors

1993-1998 Individual National Research Service Award, National Cancer Institute, National Institutes of Health

1998, 2000, 2003, 2004 Minority Scholar in Cancer Research Award, American Association for Cancer Research

2003 Minority Trainee Research Forum Award, Temple University, Philadelphia, PA

2004 Federation of American Societies for Experimental Biology (FASEB) Program Poster/Oral Presentation Travel Award

2006 The Henry C. Welcome Fellowship Grant; Maryland Higher Education Commission

C. Selected Peer-reviewed Publications

1. Snyderwine, E.G., Yu, M, Schut, H.A., **Knight-Jones, L**., and Kimura, S.(2002) Effect of CYP1A2 deficiency on heterocyclic amine DNA adduct levels in mice. *Food Chem Toxicol*. 40, 1529-1533. PMID: 12387319

2. Snyderwine, E.G., Yoon, H.S., **Knight-Jones, L.P**., Tran, M., Schut, H.A., and Yu, M. (2003) Mutagenesis and DNA adduct formation in the mouse mammary gland exposed to 2-hydroxyamino-1-methyl-6-phenylimidazo-[4,5-b]pyridine in whole organ culture. *Mutagenesis.* 18, 7-12. PMID: 12473729

3. **Jones, L.P.,** Li, M., Halama, E., Ma, Y., Lubet, R., Grubbs, C.J., Deng, C-X, Rosen, E., Furth, P.A. (2005) Promotion of mammary cancer development by tamoxifen in a mouse model of Brca1-mutation related breast cancer. *Oncogene* 24: 3554-3562. PMID: 15750629

4. Ma, Y, Katiyar, P, **Jones, LP,** Fan, S, Zhang, Y, Furth, PA and Rosen, EM. (2006) The Breast Cancer Susceptibility Gene BRCA1 Regulates Progesterone Receptor Signaling in Mammary Epithelial Cells. Molecular Endocrinology, *Molecular Endocrinology*, 20:14-34. PMID: 16109739

5. **Jones, L.P**.*, Frech, M.S.*, and Furth P.A. (2006) Validation of transgenic models of breast cancer: Ductal Carcinoma In Situ (DCIS) and Brca1 mutation related breast cancer. *Breast Cancer Online*, Vol. 8, Issue 8 *(*equal contribution).*

6. Herschkowitx, JI, Simin, K, Weigman, VJ, Mikaelian, I, Usary, J, Hu, Z, Rasmussen, KE, **Jones, LP,** Assfnia, S, Chandrasekharan, S., Backlund, MG, Yin, Y., Khramtsov, AI, Bastein, R, Quac,enbush, J, Glazer, RI, Brown, PH, Green, JE, Kopelovich, L., Furth, PA, Palazzo, JP, Olopade, OI, Bernard, PS, Churchhill, GA, Dyke, TV, and Perou, CM. (2007) Identification of conserved gene expression features between murine mammary carcinoma models and human breast tumors. *Genome Biology*, 8(5):R76. PMID: 17493263

7. **Jones, L. P.**, Tilli, M. T., Assefnia, S., Torre, K., Halama, ED., Parrish, A., Rosen, E. M., Furth, P. A. Activation of estrogen signaling pathways collaborates with loss of Brca1 to promote development of ERalpha-negative and ERalpha-positive mammary preneoplasia and cancer. *Oncogene.* 27:794-802. PMID: 17653086

8. Tilli, M.T., Parrish, A.R., Cotarla, I., **Jones, L.P.** Johnson, M. and Furth, PA. (2008) Comparison of mammary gland imaging techniques and applications: reflectance confocal microscopy, GFP imaging, and ultrasound. *BMC Cancer*. **8**:21. PMID: 18215290

9. Bodreddigari, S*, **Jones, L*.**, Egner, P, Groopman, J, Sutter, C, Roebuck,B, Guengerich, F., Kensler, T, and Sutter, T. (2008) Protection Against Aflatoxin B_1-induced Cytotoxicity by Expression of the Cloned Aflatoxin B_1-aldehyde Reductases Rat AKR7A1 and Human AKR7A3. **(equal contribution) Carcinogenesis*, 20, 1215-1223. PMID: 18416522

10. **Jones, L.P*.,** Sampson, A., Kang, H.J., Kim, HJ, Yong-Weon, Y, Kwone, S.Y., Babus, J.K., Bae, I. (2010) Loss of BRCA1 leads to an increased sensitivity to Bisphenol A. *Toxicology Lett*. 199:261-268. PMID: 20868731. **Co-Corresponding Senior Author.*

11. **Jones, LP*,** Stefansson, S, Kim, M, Ahn, SN. (2011) Comparison of Radioimmuno and Carbon Nanotube Field-Effect Transistor Assays for Measuring Insulin-Like Growth Factor-1 in a Preclinical Model of Human Breast Cancer. *J. Nanobiotechnology, 9:36.* PMID: 21888628 **Co-Corresponding Senior Author.*

12. **Jones LP***, Buelto D, Tago E, Owusu-Boaitey K. (2011) Abnormal mammary adipose tissue environment of Brca1 mutant mice show a persistent deposition of highly vascularized multilocular adipocytes. Journal of Cancer Science & Therapy. S2 http://dx.doi.org/10.4172/1948-5956.S2-004. **Corresponding Senior Author.*

13. Cao Q, Hersl J, La H, Smith M, Jenkins J, Goloubeva O, Vasken Dilsizian,Tkaczuk K, Chen W, **Jones L.** (2014) A Pilot Study of FDG PET/CT Detects a Link Between Brown Adipose Tissue and Breast Cancer. *BMC-Cancer* 14:126.

D. Research Support

Research Grants
Current

1R21 CA167268 (PI: Jones) 09/21/2012-08/31/2015
NIH/NCI
Plasticity of Mammary Adipose and Breast Cancer Development
This proposal examines a novel paradigm of tumor development, namely whether a sustained multilocular, brown adipocyte phenotype in mammary adipose can contribute to the progression of breast cancer and be used as an early biomarker for disease.

1R01ES021483 (PI: Jaiswal) 05/1/2013-12/30/2017
NIH/NIGMS
Quinone Oxidoreductases and mammary Toxicity/Carcinogenencity
The overall goal of the proposed studies are to investigate the hypothesis that quinone oxidoreductases [NAD(P)H:quinone oxidoreductase 1 (NQO1) and NRH:quinone oxidoreductase 2 (NQO2)] are endogenous factors in protection against mammary toxicity/cancer and metastasis
Role: Co-Investigator

R01 CA157779 (PI: Zhou) 07/1/2013-6/30/2018
NIH/NCI
Shikinine and Nrf2 Chemoprevention
This project will identify the novel agent Shikinine can protect estrogen induced DNA damage in breast cancer.
Role: Co-Investigator

Completed Research Support

Amgen, Incorportated (PI: Jones) 01/01/2012-05/31/2014

Role of RANKL Inhibition in the Management of Brca1-associated Mammary Tumorigenesis.
The goal of this study is to determine whether selective pharmacological inhibition of RANKL can attenuate mammary preneoplasia and adenocarcinoma in Brca1 mutant mice.

1R21 CA162273-01 (PI: Webb) 08/1/2011-7/31/2013
NIH/NCI
Role of BRCA-1 on NKT cell-Induced Immune Responses to Breast Cancer
The hypothesis to be tested in this proposal is that the loss of functional BRCA1 expression results in a loss of NKT cell function, which impedes anti-tumor immune responses. We propose to test our hypothesis by examining the following specific aims: (1) Determine the mechanism by which BRCA-1 regulates NKT cell proliferation and cytokine production (2) Investigate the phenotype and function of NKT cells in BRCA-1 mutant mice, compared to wildtype controls.
Role: Co-Investigator

1R01CA062483 (PI: Brodie, Angela) 07/01/2010-06/30/2012
NIH/NCI PA-08-190
Aromatase and Breast Cancer
The goal of this study is a) to identify and quantify key circulating and tissue specific hormonal and metabolic perturbations (i.e. hormones, cytokines/adipokines) influenced by aromatase inhibitors in the mammary gland; and (2) to identify specific pathophysiological mechanisms and hormone responsiveness to aromatase inhibitors in mammary glands with loss of BRCA1 function.
Role: Co-Investigator

Clinical Research Unit of Maryland:1006541 (PI:Jones) 09/01/2009 – 08/31/2010
Nutrition Obesity Research Center (NORC) of Maryland Pilot Grant
Is BRCA1 a novel regulator of adiposity and energy balance?
The goal of this study is to determine whether BRCA1 deficiency in mammary epithelial cells affects the differentiation of adipose tissue in the mammary gland.

UM:10004467 (PI:Jones) 02/01/2009 – 08/03/2010
Maryland Industrial Partnerships
Breast Cancer Biomarker Study using CNT Sensor
Funding initiative to collaborative R&D projects between companies and University System of Maryland faculty.
The goal of this study is to utilize and test an ultrasensitive nanotechnology-based antigen detection system for detecting candidate biomarkers of breast cancer.
Role: co-PI with Saeyoung Nate Ahn, PhD, CEO & Director of R&D, Fuzbien Technology Institute

University of Maryland Statewide Health Network Field Outreach 06/05/07 – 12/31/08
(PI: Baquet)
The Role of Environment Estrogens in African American Women Breast Cancers
The study seeks to determine whether a synergistic effect exists between low dose environmental estrogen activity and breast cancer risk in African American women.
Role: Co-Investigator

K12 HD043489 (PI: Langenberg) 07/01/05- 06/30/08
NICHD
Maryland's Organized Research Effort in Women's Health.
This mentored research scientist development program award is designed to foster interdisciplinary research in women's health among junior faculty Scholars working together with senior faculty mentors to bridge the gap between specialized training and independent research careers. Role: Faculty Scholar

Program Director/Principal Investigator (Last, First, Middle):

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
LANE Andrew N.	**Professor Department of Toxicology**
eRA COMMONS USER NAME (credential, e.g., agency login) ANLANE01	

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 College London	BSc	1972-1975	Biochemistry
University College London	PhD	1975-1979	Biochemistry
Biozentrum, Universitaet Basel, CH	Post doc	1979-1983	Biophysics
Stanford University, CA	Post doc	1983-1986	Biophysics

A. Personal Statement

I have nearly thirty years of experience in the application and development of NMR methods for macromolecular NMR and probing metabolism, especially related to human cancers. I am able to develop and implement methods as needed and to schedule instrument time for specific projects. I have a background in biochemistry and biophysics and an interest in the functional and physical properties of macromolecules, their interactions and dynamics, as well as the dynamics of metabolic networks. I am also an Associate Director of the Center Environmental Systems Biochemistry (CESB), which is responsible for developing and applying new methods in metabolic studies. My background in biochemistry, enzyme kinetics and NMR is particularly appropriate for designing stable isotope resolved metabolomics (SIRM) experiments, their execution and biological interpretation of results. With Drs. Teresa Fan and Richard Higashi of CESB, I have been developing the stable isotope tracer approach for cancer metabolomics, especially by 2D NMR. I have developed quantitative techniques for isotopomer analysis, and implementing 2D NMR experiments for stable isotope-based cancer metabolism. Such metabolic studies form the basis of the translational NCI program project grant on non-small cell lung cancer which I direct, and I am also a PI on an NIH Regional Metabolomics Center to promote metabolic analyses of human diseases.

B. Positions and Honors

Positions and Employment

1986-1994	Staff Scientist, National Institute for Medical Research, London
1994-2001	Senior Staff Scientist, National Institute for Medical Research, London
2001-2013	Professor of Medicine, University of Louisville Director, JG Brown NMR Facility Program Leader, Structural Biology JG Brown Cancer Center
2002-2013	Joint appointments as Professor of Chemistry and of Biochemistry, University of Louisville
2005-2013	Associate Director, Center for Regulatory and Analytical Metabolomics, U. Louisville.
2013-	Professor of Toxicology, University of Kentucky

Other Experience and Professional Memberships

Editorial Board, Nucleic Acids Research, 2000-present
Editorial Board, J. Structural and Functional Genomics, 1999-2007
Committee British Biophysical Society, 1997-2001
Editor, Biophysics, Central European Journal of Biology since 2006
Editorial Board, *Metabolomics* from 2011
Editorial Board, *Cancer & Metabolism* from 2012
NCRR Study Section, August 2005.
American Heart Association Study Section, April 2008, 2009, 2012

NIGMS R13 study section, March 2010, 2011
NIH CSR (SIG) study section, June 2012
Advisory board for NCI R25 Cancer Education Program, UofL, 2012-2013
NCI Omnibus Special Emphasis Panel March 11-12, 2013
NCI Omnibus Special Emphasis Panel June 2014

Honors

Honorary Research Fellow, Department of Biochemistry and Molecular Biology, University College London
EMBO Fellow at Stanford University 1983-1985
James Graham Brown endowed chair of Structural Biology, University of Louisville, 2002-2013
Member, NIGMS Metabolomics Network
Carmen L. Buck endowed chair, University of Kentucky

C. Representative Peer-reviewed Publications (Selected from 195 peer-reviewed publications)

1. Fan, T.W-M., Higashi, R.M., **Lane, A.N.** & Jardetzky, O. (1986) Combined Use of ^{1}H NMR and GC-MS for Monitoring Metabolites and *in vivo* ^{1}H NMR*. Biochim. Biophys. Acta* 882 154-167
2. Fan, T. W-M., Bandura, L.L., **Lane**, **A.N.** & Higashi, R.M. (2005) Metabolomics-Edited Transcriptomics Analysis of Se Anticancer Action in human lung cancer cells. *Metabolomics* **1**, 325-339
3. Thornburg, J.M., Nelson, K.K., **Lane, A.N.,** Arumugam, S., Simmons, A. Eaton, J.W., Telang, S., & Chesney, J. (2008) Targeting Aspartate Aminotransferase in Breast Cancer *Breast Cancer Res*. **10**:R84
4. **Lane AN,** Fan TW-M, Xie X, Moseley HN, Higashi RM. (2009) Stable isotope analysis of lipid biosynthesis by high resolution mass spectrometry and NMR**.** *Anal Chim Acta* **651**:201-8; PMC2757635.
5. Yalcin A, Clem BF, Simmons S, **Lane AN,** Nelson KK, Clem AL, Brock E, Siow D, Wattenberg B, Telang S, Chesney J. (2009) Nuclear targeting of 6-phosphofructo-2-kinase(PFKFB3) increases proliferation via cyclin-dependent kinases. *J Biol Chem* **284**:24223-32; PMC 2782016.
6. Fan, T.WM., **Lane**, **A.N**., Higashi, R.M., Farag, M.A., Gao, H., Bousamra, M. & Miller, D.M. (2009) Altered Regulation of Metabolic Pathways in Human Lung Cancer Discerned by ^{13}C Stable Isotope-Resolved Metabolomics (SIRM). *Molecular Cancer*. **8**:41. PMCID: PMC2717907
7. Fan, T. W-M., **Lane, A.N**., Higashi, R.M., Yan, J. (2011) Stable Isotope Resolved Metabolomics of Lung Cancer in a SCID Mouse Model. *Metabolomics* **7**, 257-269 PMC3109995
8. Moseley, H.N.B., **Lane, A.N.**, Belshoff, A.C, Higashi, R.M. Fan, W. W-M. (2011) Non-Steady State Modeling of UDP-GlcNAc Biosynthesis Enabled by Stable Isotope Resolved Metabolomics. *BMC Biology*. **9**:37. PMC3126751
9. Le, A., **Lane, A.N.,** Hamaker, M., Bose, S., Gouw, A., Barbi, J., Tsukamoto, T., Rojas, C.J., Slusher, B.S., Zhang, H., Zimmerman, L.J., Liebler, D.C., Slebos, R.J.C., Lorkiewicz, P.K., Higashi, R.M., Teresa W. M. Fan, T.W-M., Dang, C.V. (2012) MYC induction of hypoxic glutamine metabolism and a glucose-independent TCA cycle in human B lymphocytes. *Cell Metabolism*. **15**, 110-121. PMC3345194
10. Fan, T.W-M., Lorkiewicz, P., Sellers, K., Moseley, H.N.B., Higashi, R.M., **Lane**, **A.N.** (2012). Stable Isotope-resolved metabolomics: past, present, and future. *Pharmacology & Therapeutics.* **133**:366-391 PMC3471671
11. Fan, T.W-M., Higashi, R.M. & **Lane, A.N**. (2012) "*The Handbook of Metabolomics"* Methods in Pharmacology and Toxicology, vol. 17" Humana Press
12. Liu, W., Le, A., Fan, T. W-M., **Lane, A.N**., Dang, C.V., Phang, J.M. (2012) The reprogramming of proline and glutamine metabolism contributes to the proliferative and metabolic responses to c-MYC**.** *PNAS* **109**:8983-8988. PMC3384197
13. Yang, Y., **Lane**, **A.N**., Ricketts, C.R., Wei, M-H., Wu, M., Roualt, T.A., Boros, L.G., Fan, T.W-M., Linehan, W.M**.** (2013) Metabolic Reprogramming for Producing Energy and Reducing Power in Fumarate Hydratase Null Cells from Hereditary Leiomyomatosis Renal Cell Carcinoma. *PlosOne* **8**:e72179 PMCID:PMC3744468
14. Reynolds, M.R., **Lane, A.N**., Kemp, S., Liu, Y., Hill, B., Dean, D.C., Clem, B.F. (2014) Control of Glutamine Metabolism By the Tumor Suppressor Rb. *Oncogene* **33**(5):556-66. PMC3918885
15. Xie, H., Hanai, J-i., Ren, J-G., Kats, L., Burgess, K., Bhargava, P., Signoretti, S., Billiard, J., Duffy, K.J., Grant, A., Wang, X., Lorkiewicz, P.K., Schatzman, S., Bousamra, M. II, **Lane**, **A.N.,** Higashi, R.M., Fan, T. W-M., Pandolfi, P.P., Sukhatme, V.P., and Seth, P. (2014) Targeting lactate dehydrogenase-A (LDH-A) inhibits tumorigenesis and tumor progression in mouse models of lung cancer and impacts tumor initiating cells. *Cell Metabolism* **19**, 795–809. PMCID:PMC4096909.

D. Research Support

Ongoing Research Support

R01ES022191-01 T.W-M. Fan (PI) 09/01/2012-08/30/2017
Technology Development to Enable Large Scale Metabolomics Analyses (R01)
This project designs and synthesizes chemoselective reagents for targeting metabolites bearing specific functional groups, incorporating a permanent positive change and a stable isotope for increasing sensitivity and identification and quantification of classes of metabolites by mass spectrometry and NMR.
Role: co-I

5R01ES022191-04 T.W-M. Fan (PD) 04/26/2014-08/30/2017
Integrated Chemoselective and Informatic Platform for Large-Scale Metabolomics. Administrative supplement from Targeted Opportunity
This is a joint project with NCI on pre-clinical characterization of metabolic abnormalities in tumors by stable isotope resolved metabolomics for novel target identification. This involved SIRM studies on cells, tissue slices and animal models as well as hyperpolarization NMR in prostate and renal carcinomas driven by germline mutations.
Role: multiple PI

P01CA163223-01A1 A.N. Lane (PD) 3/1/13-2/28/18
Systems Biochemistry in Lung Cancer: Toward a Mechanistic Understanding of NSCLC
The program comprises three project areas utilizing stable isotope resolved metabolomics to gain a mechanistic understanding of NSCLC in situ. The projects combine cell culture, animal models and human subjects to define the influence of the tumor microenvironment on cancer progression.
Role: PD

1 U24 DK097215-01A1 R.M.Higashi (PD) 9/11/13-8/31/18
Resource Center for Stable Isotope-Resolved Metabolomics
This regional center for metabolomics was established at UK to develop and support stable isotope resolved metabolomics.
Role: multiple PI, analytical core director

3U24DK097215-02S1 R.M.Higashi (PD) 9/1/14-3/31/15
Resource Center for Stable Isotope-Resolved Metabolomics
Administrative Supplement for a standards Ring Trial
Role: multiple PI, analytical core director

SBCR Pty Ltd. T.W-M. Fan (PD) 4/1/14-11/30/14
Plasma lipid biomarker(s) for breast cancer diagnosis.
The contract is to valdidate lipid biomarkers in plasma from women with early stage breast cancer
Role: multiple PI

2 R01 DK054921-15 subcontract from U.Minnesota (J. Albrecht, PI) 5/1/14-4/30/16
Cyclin D1/CDK4 Complex in Hepatocyte Proliferation
This project will use SIRM methods to determine the metabolic changes controlled by Cyclin D1 dependent cell cycling in hepatocytes.
Role: co-I

Completed Research Support (last 3 years)

J. G. Brown Chair in Structural Biology A.N. Lane (PI) 01/05/2002-2013
Kentucky's Research Challenge Trust Fund and The James Graham Brown Foundation.
Support for research and activities in any area related to cancer biology.
Role: PI.

Program Director/Principal Investigator (Last, First, Middle):

American Cancer Society RSG-13-139-01-CNE B.F. Clem (PI) 7/1/13-6/30/17
(Role on this grant completed prior to leaving University of Louisville and affiliating with University of Kentucky 09/09/2013.) "Control of Glucose and Glutamine Metabolism by the Retinoblastoma Protein"
Goals: My role in this proposal is to provide NMR-based stable isotope resolved metabolomics to the metabolism of cells controlled by Rb.
Role: Co-I

NCI 1R01 CA166327-01A B.F. Clem (PI) 6/1/13-5/31/18
(Role on this grant completed prior to leaving University of Louisville and affiliating with University of Kentucky 09/09/2013.)"Regulation of Tumor Metabolism by Retinoblastoma Protein"
Goals: My role in this proposal is to supply stable isotope tracing analysis of cells with Rb knockouts, to determine the metabolic regulatory functions of Rb.

1R01CA140991-02 S. Telang (PI) 07/01/2009-04/30/2014
"Targetting Glucose Metabolism in Cancer"
The major goal of this project is to establish the role of PFKFB4 in the invasiveness of transformed cells. My role is to provide NMR support.
Role: Investigator

KLCRP T.W-M. Fan (PI) 12/01/2010-11/30/2013
Stable isotope-resolved metabolomics to elucidate the mechanism of a tumor-associated cytochrome in lung cancer growth and metabolism
The goal is to use metabolomics to characterize exosomal lipids and Pgrmc1 levels in blood from lung cancer patients as biomarkers of therapeutic response.
Role: Co-I

1R01CA118434-03 T.W-M. Fan (PI) 09/01/2008-08/30/2013
Biochemical Mechanisms of Se Anticancer Activity in Lung
This proposal examines the biochemical and cell biological effects of different selenium compounds on lung cancer cells both in vitro and in mouse xenograft models.
Role: Co-I

American Cancer Society S. Telang (PI) 07/01/2009–06/30/2013
Role 6- Phosphofructo-2- Kinase Isoform 4 in NSCLC
My role is to provide NMR-based metabolomics support.
Role: Investigator

5P20RR018733-08 D.M. Miller (PI) 08/01/2003-06/30/2013
Center of Biomedical Research in Molecular Targets
Core Director, NMR Facility. My role is to provide support for protein expression and NMR in projects supported by the grant.
Role: Investigator

1R21CA133688-02 A.N. Lane (PI) 07/31/2009-05/31/2012
"Stable isotopomer analysis of anabolic metabolic pathways in breast cancer"
This project aims to relate effects of nutrient supply and hypoxia on the metabolism of breast cancer cells in culture to the metabolism of the same cells in a mouse xenograft model in discovering the influence of the tumor microenvironment
Role: PI

5RC2GM092729-02 R. Kaddurah-Daouk (PI) 09/01//2009-08/31/2011
Metabolomics Network for Drug Response Phenotype
This grant was concerned with metabolomics readout of drug response in several diseases. My role is to consult on the metabolic biochemistry.
Role: Consultant

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
Charnigo, Richard (Jr.)	Professor of Statistics, Professor of Biostatistics
eRA COMMONS USER NAME (credential, e.g., agency login) RICH.CHARNIGO	

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
Case Western Reserve University, Cleveland OH	B.S.	05/97	Mathematics
Case Western Reserve University, Cleveland OH	M.S.	08/99	Mathematics
Case Western Reserve University, Cleveland OH	Ph.D.	08/03	Statistics

A. Personal Statement

Dr. Charnigo is part of the Biostatistics and Epidemiology Research Design key function of the NIH-funded (U54) Center for Clinical and Translational Science and a mentor to junior investigators in the NIH-funded (P20) Center of Research in Obesity and Cardiovascular Disease at the University of Kentucky. Dr. Charnigo was principal investigator on an NSF project (2007-2011) that developed statistical theory and methodology for nonparametric regression along with engineering methodology for nanoparticle characterization, and he was principal investigator on a related project funded by the United States Army Research Office (2012-2013). He has been the dissertation advisor to eight Ph.D. students in Statistics (three completed) and two Ph.D. students in Epidemiology and Biostatistics, the capstone advisor to two M.P.H. students in Biostatistics (both completed), and the supervisor to nine graduate and two undergraduate research assistants (one current). His experiences equip him to serve as statistical mentor on the proposed project, not only on account of his technical knowledge but also because of his particular interest in cardiovascular research.

B. Positions and Honors

Positions and Employment

2003-2009	Assistant Professor, University of Kentucky, Lexington KY
2009-2013	Associate Professor, University of Kentucky, Lexington KY
2013-	Professor, University of Kentucky, Lexington KY

Other Experience and Professional Memberships

2010-2014	Advisory Board, Quantitative Initiative for Policy and Social Research
2011-	Member, Delta Omega Honorary Society in Public Health
2011-2014	University of Kentucky Senate
2012-	Editor-in-Chief, *Journal of Biometrics and Biostatistics*
2012-	President, Kentucky Chapter of American Statistical Association
2012-2014	Reviewer, Natural Sciences and Engineering Research Council of Canada

Honors

2006; 2008; 2009; 2010; 2011; 2012; 2013; 2014	Wethington Award, University of Kentucky
2007; 2008; 2009; 2010; 2011; 2012; 2013; 2014	Marquis Who's Who in America
2008; 2009; 2010; 2011; 2012; 2013; 2014; 2015	Marquis Who's Who in the World
2010	Golden Apple Teaching Award, University of Kentucky
2014-2015	University Research Professor, University of Kentucky

C. Publications (selected from 124)

Charnigo, Richard; Srinivasan, Cidambi (2014). “A Multivariate Generalized C_p and Surface Estimation.” *Biostatistics*. (Accepted manuscript) PMID: 25187530

Huang, Bin; Guo, Jing; Charnigo, Richard (2014). “Statistical Methods for Population-Based Cancer Survival in Registry Data.” *Journal of Biometrics and Biostatistics*: Volume 5, Article e129.

Wu, Tao; Vander Kooi, Craig; Huang, Cai; Shah, Pritom; Charnigo, Richard; Smyth, Susan; Morris, Andrew (2014). “Integrin-Mediated Cell Surface Recruitment of Autotaxin Promotes Directional Cell Migration.” *The FASEB Journal*: Volume 28, pp. 861-870. PMID:24277575

Seratnahaei, A.; Leung, S.; Charnigo, Richard; Cummings, M.; Sorrell, V.; Smith, M. (2014). “The Changing ‘Face’ of Endocarditis in Kentucky: A Rise in Tricuspid Cases.” *American Journal of Medicine*: Volume 127, pp. 786.e1-6. PMID: 24769025

Rateri, Debra; Davis, F.; Balakrishnan, A.; Howatt, D.; Moorleghen, J.; O’Connor, W.; Charnigo, Richard; Cassis, Lisa; Daugherty, Alan (2014). “Angiotensin II Induces Region-Specific Medial Disruption during Evolution of Ascending Aortic Aneurysms.” *American Journal of Pathology*: Volume 184, pp. 2586-2595. PMID: 25038458

Platt, Kristen; Charnigo, Richard; Pearson, Kevin (2014). “Adult Offspring of High-Fat Diet-Fed Dams Can Have Normal Glucose Tolerance and Body Composition.” *Journal of Developmental Origins of Health and Disease*: Volume 5, pp. 229-239. PMID: 24901663

Whitbeck, Matthew; Charnigo, Richard; Shah, J.; Morales, G.; Leung, S.; Fornwalt, B.; Bailey, A.; Ziada, K.; Sorrell, V.; Zegarra, M.; Thompson, J.; Hosn, N.; Campbell, C.; Gurley, J.; Anaya, P.; Booth, D.; DiBiase, L.; Natale, A.; Smyth, Susan; Moliterno, David; Elayi, Claude (2014). “QRS Duration Predicts Death and Hospitalization among Patients with Atrial Fibrillation Irrespective of Heart Failure: Evidence from the AFFIRM Study.” *EP Europace*: Volume 16, pp. 803-811. PMID: 24368753

Whitbeck, M.; Charnigo, Richard; Khairy, P.; Ziada, K.; Bailey, A.; Zegarra, M.; Shah, J.; Morales, G.; Macaulay, T.; Sorrell, V.; Campbell, C.; Gurley, J.; Anaya, P.; Nasr, H.; Bai, R.; DiBiase, L.; Booth, D.; Jondeau, G.; Natale, A.; Roy, D.; Smyth, S.; Moliterno, D.; Elayi, C. (2013). “Increased Mortality among Patients Taking Digoxin - Analysis from the AFFIRM Study.” *European Heart Journal*: Volume 34, pp. 1481-1488. PMID: 23186806

Chen, X.; Rateri, D.; Howatt, D.; Balakrishnan, A.; Moorleghen, J.; Morris, Andrew; Charnigo, Richard; Cassis, Lisa; Daugherty, Alan (2013). “Amlodipine Reduces AngII-Induced Aortic Aneurysms and Atherosclerosis in Hypercholesterolemic Mice.” Public Library of Science One: Volume 8, Article 81743. PMID: 24244746

Selim, Samy; Sunkara, Manjula; Salous, Abdel; Berdyshev, Evgeny; Bailey, Alison; Campbell, Charles; Charnigo, Richard; Morris, Andrew; Smyth, Susan (2011). “Plasma levels of sphinosine 1 phosphate are strongly correlated with hematocrit but variably restored by red blood cell transfusions.” Clinical Science: Volume 121, pp. 565-572. PMID: 21749329

Oestreich, Julie; Holt, John; Dunn, Steven; Smyth, Susan; Campbell, Charles; Charnigo, Richard; Akers, Wendell; Steinhubl, Steven (2009). “Considerable Variability in Platelet Activity among Patients with Coronary Artery Disease in Response to an Increased Maintenance Dose of Clopidogrel.” Coronary Artery Disease: Volume 20, pp. 207-213. PMID: 19318928

Pamuklar, Zehra; Lee, Jin; Cheng, Hsin-Yuan; Pantcharam, Manikandan; Steinhubl, Steven; Morris, Andrew; Charnigo, Richard; Smyth, Susan (2008). “Individual Heterogeneity in Platelet Response to Lysophosphatidic Acid: Evidence for a Novel Inhibitory Pathway.” Arteriosclerosis, Thrombosis, and Vascular Biology: Volume 28, pp. 555-561. PMID: 18202325

D. Research Support

Ongoing Research Support

1. 1l01 CX000975 Tannock (PI) 4/1/14 – 3/31/18
VA-ORD
THE ASSOCIATION OF SAA WITH APOB LIPOPROTEINS AFFECTS CARDIOVASCULAR RISK
The central hypothesis of this grant is that the shift of SAA from HDL to apoB-containing lipoproteins in insulin resistant conditions such as Metabolic syndrome and diabetes contributes to the increased atherosclerosis and cardiovascular disease observed in these populations. Role: Statistician.

2. 1l01 CX000773-A2 Webb (PI) 1/1/14–12/31/17
VA-ORD
HDL REMODELING IN THE METABOLIC SYNDROME
The central hypothesis of this proposal is that TG enrichment of HDL in MetS predisposes the particle to remodeling by intravascular factors and impedes selective lipid uptake by SR-BI. Consequently, TG-enriched HDL in MetS is more susceptible to rapid clearance and less capable of supporting reverse cholesterol transport. Role: Statistician.

3. 1R01ES023470-01 Zhou (PI) 09/26/13 - 06/30/18
NIH/NIEHS
ENDOCRINE DISRUPTOR MEDIATED ACTIVATION OF PXR CAUSES DYSLIPIDEMIA
The goal of this project is to investigate a novel mechanism linking endocrine disrupting chemical (EDC) exposure and hyperlipidemia.
Role: Co-Investigator

4. 1R01DK100892-01 Graf (PI) 09/20/13 - 08/31/17
NIH
THE ROLE OF HEPATIC INSULIN RESISTANCE ON SR-BI DEPENDENT HDL CHOLESTEROL UPTAKE AND METABOLISM
The overall objective of this proposal is to examine the extent to which impaired insulin signaling alters HDL-mediated reverse cholesterol transport (RCT).
Role: Co-Investigator

5. ISSBRIL0171 Smyth (PI) 4/23/13 - 10/30/14
AstraZeneca Pharmaceuticals
TARGETING PLATELETS IN PNEUMONIA
This human clinical study will assess the effects of Brilinta on platelet function among patients with pneumonia.
Role: Co-Investigator

6. 1R01HL111040-01A1 Bruemmer (PI) 9/27/12 - 6/30/16
NIH/NHLBI
EPIGENETIC REGULATION OF INFLAMMATORY GENE EXPRESSION BY TELOMERASE
This application will investigate novel mechanisms that control the activation of inflammation during atherosclerosis formation. The results of these studies may ultimately characterize novel pathways contributing to vascular diseases and lead to new therapeutic opportunities.
Role: Co-Investigator

7. 1DP5OD012132-01 Fornwalt (PI) 9/25/12 - 8/31/17
NIH/Office of the Director
EXPLORING THE ROLE OF DYSSYNCHRONY IN PEDIATRIC HEART DISEASE WITH MRI
The long-term goal of this research program is to address the problem of pediatric heart failure by adapting a relatively new, highly successful therapy for adult heart failure called cardiac resynchronization therapy into a treatment option to improve the health of children with heart failure.
Role: Co-Investigator

8. Lorch (PI) 3/1/12 – 6/30/15
Institute of Education Sciences
A NARRATIVE COMPREHENSION INTERVENTION FOR ELEMENTARY SCHOOL CHILDREN AT-RISK FOR ATTENTION-DEFICIT HYPERACIVITY DISORDER
Children with ADHD often fall behind their comparison peers in narrative comprehension ability due to deficits in story domains that are important for comprehension; the purpose of the proposed project is to develop a supplemental afterschool intervention for 2nd and 3rd grade children at-risk for ADHD.
Role: Co-Investigator

9. CTSA 1U54RR031263-01A1 Kern (PI) 7/1/11 - 6/30/16
NIH
KENTUCKY CENTER FOR CLINICAL AND TRANSLATIONAL SCIENCE
The University of Kentucky Center for Clinical and Translational Science is dedicated to growing the clinical and translational science research teams of the future, to providing the infrastructure needed to foster collaborations between basic and clinical scientists to facilitate research translation, and to enhancing outreach pathways to confront chronic health issues in rural Appalachia.
Role: Program Faculty, Biostatistics

10. 1R21HD068844-01 Swanson (PI) 5/1/11 - 4/30/15
NIH/CHHD
COMBATING DISPARITIES IN PRODUCE CONSUMPTION: AN APPALACHIAN FARM TO SCHOOL INTERVENTION
Located in rural Appalachian Kentucky, a region with low fruit and vegetable intake and high rates of diet related diseases, the proposed project examines the feasibility of using a farm to school program, which provides locally-raised produce to the school cafeteria, as a health intervention.
Role: Co-Investigator

11. 2P20GM103527-06 Cassis (PI) 9/08/08 – 7/31/18
NIH/NCRR
CENTER OF RESEARCH IN OBESITY AND CARDIOVASCULAR DISEASE
The objective of this Center is to identify mechanisms linking the epidemic of obesity to the high incidence of cardiovascular diseases in the obese population and to develop promising junior project investigators.
Role: Mentor to junior project investigators

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
Arun Sreekumar	Associate Professor
eRA COMMONS USER NAME asreekum	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Indian Institute of Science; Bangalore, India	PhD	2000	Biochemistry
University of Poona; India	MS	1994	Biochemistry
University of Mysore; India	BS	1992	Biochemistry, Botany & Zoology

A. Personal Statement

I am an Associate Professor and Director of Metabolomics for the Alkek Center for Molecular Discovery at Bayor College of Medicine. I completed my postdoctoral fellowship training at the Michigan Center for Translational Pathology within the Department of Pathology at the University of Michigan School of Medicine. In 2008, I was appointed as an Assistant Professor at the University of Michigan Medical School. During this period, I got my first RO1 and my team was selected by the Multiple Myeloma Reseach Foundation, as one of the 3 labs that they funded in the US for Myeloma Research. In 2009, I moved as an Assistant Professor and Georgia Cancer Coalition Scholar to the Medical College of Georgia Cancer Center (MCGCC), where I established a state-of-the-art metabolomics platform, obtained the ARRA Challenge grant for Bioinformatics and employed metabolomics to study prostate and bladder cancer. In 2011, I was awarded the Susan Komen Foundation grant to study metabolomic alterations in breast cancer subtypes. In 2011, I joined Baylor College of Medicine (BCM), as a tenured Associate Professor and Director of the Metabolomics Program at the Alkek Center of Molecular Discovery. At BCM, over the past two years, I have established a strong prostate and breast cancer metabolomics program funded by National Cancer Institute (UO1), Department of Defense (IDEA grant), National Science Foundation (Investigator grant), Susan Komen Foundation (Investigator grant), Helis Foundation and Alkek Foundation. I also established the CPRIT funded metabolomics program for Cancer Research Community. Thus over the short span of 5 years, I have established myself as funded, tenured associate professor. I have over 42 publications, many of which are in top tier journals. My research in the area of health disparity is focused in the area of prostate and breast cancer where we examine the metabolic profiles associated with African American tumors to understand the biology underlying racial disparity and develop racially exclusive biomarkers for prognosis of the tumors. I have three graduate students, 4 post doctoral trainees and a clinical trainee in my lab. One of the post-doctoral trainees is supported by a Minority Supplement Grant from the Center for Reducing Cancer Health Disparity at NIH. In the past I have trained 7 post doctoral trainee, many of whom have started their own independent laboratories in the US and abroad.

B. Positions and Honors

Positions

1994-1999	Ph.D. Fellow; Indian Institute of Science, Bangalore, India
1999-2003	Research Fellow; Department of Pathology, University of Michigan Medical School, Ann Arbor, MI
2003-2004	Research Associate II; Department of Pathology, University of Michigan Medical School, Ann Arbor, MI
2004-2006	Research Investigator; Department of Pathology, University of Michigan Medical School, Ann Arbor, MI
2006-2008	Research Assistant Professor; Department of Pathology and Michigan Center for Translational Pathology, University of Michigan Medical School, Ann Arbor, MI
2008-2009	Assistant Professor; Department of Pathology and Michigan Center for Translational Pathology, University of Michigan Medical School, Ann Arbor, MI
2009-2011	Assistant Professor; Medical College of Georgia Cancer Center and Georgia Cancer Coalition Distinguished Scientist, Medical College of Georgia, Augusta, GA
2011-pres.	Associate Professor, Tenured; Baylor College of Medicine, Houston, TX

2011-pres. Director; Metabolomics, Alkek Center for Molecular Discovery, Baylor College of Medicine, Houston, TX

Honors

1. Invited speaker at the International Conference on “Chip to Hits 2002” held in Philadelphia, USA from Oct 27-31, 2002, presented the work entitled “Profiling of Cancer Cells Using Protein Microarrays: Discovery of Novel Radiation-Regulated Proteins.”
2. Invited to write a review article on Protein Microarrays in Current Opinions in Molecular Therapeutics.
3. Invited to write a review article on Protein Microarrays in Frontiers in Bioscience.
4. Invited to edit a book on Protein Microarrays to be published by Mercel and Dekker publishers, New York.
5. Contributed a chapter titled “Humoral Response Profiling Using Protein Microarrays” for the book entitled “Functional Protein Microarrays in Drug Discovery” to be published by Taylor and Francis group.
6. Invited to write a chapter on “Antibody Microarrays” for the book on “Protein Microarray Technology” to be published by BIOS.
7. Invited as a reviewer for the journal TARGETS, a Drug Discovery Today Publication.
8. Awarded Georgia Cancer Coalition Distinguished Scientist in 2009.
9. Medical College of Georgia Emerging Scientist Award 2010.
10. Associate Member of American Association for Cancer Research since 2002 (Membership No. 80004).
11. Member Comprehensive Cancer Centre, University of Michigan, Ann Arbor, MI.
12. Reviewer for the Scientific Peer Advisory and Review Services division of the American Institute of Biological Sciences.
13. Peer Reviewer for DOD, Susan Komen and NCI extramural grants.

C. Selected Peer-Reviewed Publications Relevant to this Proposal (from a total of 42 publications).

1. Stashi E, Lanz RB, Mao J, Michailidis G, Zhu B, Kettner NM, Putluri N, Reineke EL, Reineke LC, Dasgupta S, Dean A, Stevenson CR, Sivasubramanian N, **Sreekumar A,** Demayo F, York B, Fu L, O'Malley BW. SRC-2 Is an Essential Coactivator for Orchestrating Metabolism and Circadian Rhythm. ***Cell Rep.*** 2014 Feb 27;6(4):633-45. doi: 10.1016/j.celrep.2014.01.027. Epub 2014 Feb 13. PMID: 24529706.
2. Kaushik AK, Vareed SK, Basu S, Putluri V, Putluri N, Panzitt K, Brennan CA, Chinnaiyan AM, Vergara IA, Erho N, Weigel NL, Mitsiades N, Shojaie A, Palapattu G, Michailidis G, **Sreekumar A**. Metabolomic Profiling Identifies Biochemical Pathways Associated with Castration-Resistant Prostate Cancer. ***J Proteome Res.*** 2013 Dec 31. [Epub ahead of print] PMID: 24359151.
3. Kommagani, R.Szwarc, MM.Kovanci, E.Gibbons, WE,.Putluri, N. Suman, M.Creighton, CJ.**Sreekumar, A.**DeMayo, FJ.Lydon, JP.O'Malley, BW.Acceleration of the Glycolytic Flux by Steroid Receptor Coactivator-2 is Essential for Endometrial Decidualization. ***PLoS Genet***. 2013 Oct;9(10):e1003900. doi: 10.1371/journal.pgen.1003900. Epub 2013 Oct 24.PMID: 24204309
4. Terunuma, A. Putluri, N. Mishra, P. Mathé, EA. Dorsey, TA. Yi, M. Wallace, TA. Issaq, J. Zhou, M. Killian, JK. Stevenson, HS. Karoly, ED. Chan, K. Samanta, S. Hsu, TYT. Kurley, SJ. Putluri, V.Edelman, DC. Wulff, J. Starks, AM. Yang, Y. Kittles, RA. Yfantis, HG. Lee, DH. Ioffe, OB. Schiff, R. Stephens, RM. Meltzer, PS. Veenstra, TD. Westbrook, TF. **Sreekumar, A***. and Ambs, S*. MYC-driven 2-Hydroxyglutarate Associates with Poor Prognosis in Breast Cancer. ***J Clin Invest***. 2014 Jan 2;124(1):398-412. doi: 10.1172/JCI71180. Epub 2013 Dec 9 PMID: 24316975 . * Co-Corresponding Author
5. Khan AP, Rajendiran TM, Ateeq B, Asangani IA, Athanikar J, Yocum AK , Mehra R, Siddiqui J, Palapattu G, Wei JT, Michailidis M, **Sreekumar A** and Chinnaiyan AM. The role of sarcosine metabolism in prostate cancer progression. ***Neoplasia***, 2013 May;15(5):491-501.
6. Poisson LM, **Sreekumar A,** Chinnaiyan AM, Ghosh D. Pathway-directed weighted testing procedures for the integrative analysis of gene expression and metabolomic data. ***Genomics***. 2012 May;99(5):265-74. Epub 2012 Apr 2. PMID:22497771
7. Putluri, N., Shojaie, A., Vasu, V., Nalluri, S., Vareed, S., Putluri, V., Vivekanandan-Giri, A., Byun, J., Pennathur, S., Sana, T., Fischer, S.M., Palapattu, G.S., Creighton, C.J., Michailidis, G., **Sreekumar, A.** Metabolomic Profiling Reveals A Role for Androgen in Activating Amino Acid Metabolism and Methylation in Prostate Cancer Cells. ***PLoS One.*** 2011;6(7):e21417. Epub 2011 Jul 18. PMID: 21789170.
8. Putluri N, Vasu V, Shojaie A, Vareed SK, Putluri V, Butler C, Giri J, Sana TR, Fischer SM, Terris MK, Michailidis G and **Sreekumar A.** Metabolomic Profiling Reveals Impaired Detoxification

9. Mechanism in Bladder Cancer. ***Cancer Research*** 2011, October 11 PMID: 21990318. Vareed SK, Bhat VB, Thompson C, Vasu VT, Fermin D, Creighton CJ, Gayatri S, Lan L, Putluri N, Thangjam GS, Kaur P, Shabahang M, Cashikar AG, Giri JG, Nesvizhskii AI, Asea AAA, Rao A, McLoughlin J and **Sreekumar A.** Metabolites of Purine Nucleoside Phosphorylase (NP) in Serum have the Potential to Delineate Pancreatic Adenocarcinoma. ***PLoS One.*** 2011 Mar 23;6(3):e17177. PMID:21448452
10. Vellaichamy A, Dezso Z, JeBailey L, Chinnaiyan AM, **Sreekumar A,** Nesvizhskii AI, Omenn GS, Bugrim A. "Topological significance" analysis of gene expression and proteomic profiles from prostate cancer cells reveals key mechanisms of androgen response. ***PLoS One***. 2010 Jun 3;5(6):e10936.PMID: 20532174
11. **Sreekumar A**, Poisson LM, Rajendiran TM, Khan AP, Cao Q, Yu J, Laxman B, Mehra R, Lonigro RJ, Li Y, Nyati MK, Ahsan A, Kalyana-Sundaram S, Han B, Cao X, Byun J, Omenn, GS, Ghosh D, Pennathur S, Alexander DC, Berger A, Shuster JR, Wei JT, Varambally S, Beecher C and Chinnaiyan AM. Metabolomic Profiles Delineate Potential Role for Sarcosine in Prostate Cancer Progression. ***Nature*** 2009 Feb12; 457(7231):910-4, PMID: 19212411
12. Vellaichamy A, **Sreekumar A***, Strahler J, Rajendiran TM, Yu J, Varambally S, Li Y, Omenn G, Chinnaiyan AM and Nesvizhskii A*. Proteomic Interrogation of Androgen Action in Prostate Cancer Cells Reveals Roles of Aminoacyl tRNA Synthetases. ***PLoS One***. 2009 Sep 18;4(9):e7075.PMID:19763266 * joined Corresponding Authors.
13. Khan AP, Poisson LM, Bhat V, Fermin D, Zhao R, Kalyana-Sundaram S, Michailidis G, Nesvizhskii AI, Omenn GS, Chinnaiyan AM, **Sreekumar A.** Quantitative proteomic profiling of prostate cancer reveals role for miR-128 in prostate cancer. ***Mol Cell Proteomics***. 2009 Nov 9. PMID: 19955085
14. **Sreekumar A,** Laxman B, Rhodes DR, Bhagavathula S, Giacherio G, Ghosh D, Sanda MG, Rubin MA, Chinnaiyan AM. (2004) Protein Microarray Analysis Reveals that Prostate Cancer Patients Elicit a Humoral Immune Response to α-Methylacyl-CoA Racemase. ***Journal of National Cancer Institute***, 96(10) 834-843. PMID: 15173267.
15. Wang X, Yu J, **Sreekumar A**, Varambally S, Shen R, Giacherio D, Mehra R, Montie J, Pienta K, Sanda M, Kantoff P, Rubin M, Wei J, Ghosh D, Chinnaiyan, A. Autoantibody Signatures in Prostate Cancer. ***New England Journal of Medicine***: Sep 22;353(12):1224-35. PMID: 16177248.

ACTIVE

1) W81XWH-12-1-0130 (Sreekumar) 09/01/2012 – 08/31/2015
DOD
Metabolomic profiling to distinguish racially distinct prognostic markers in prostate cancer

Goals: specifically address metabolic profiles associated with low/high Gleason grade prostate cancer in AA and EA men and to develop tissue microarray based enzymatic signatures for low or high risk prostate cancer.

2) DMS 1161759 (Michailidis and Sreekumar) 08/01/2012 – 07/31/2016
NSF
Collaborative research: statistical methodology for network based integrative analysis of omics data
Goals: develop statistical methods for network based pathway-centric integration of OMICs data sets in prostate cancer. The identified pathways from the integrative analysis will be verified using functional invitro and invivo studies.

3) CPRIT (Edwards) RP120092 12/01/2011 – 11/30/2016
Tumor Metabolomics Core Facility
Goals: To establish a cancer metabolomics core facility at Baylor College of Medicine. To support metabolomics studies of investigators at Baylor College of Medicine in the area of Cancer Research.

4) U01 CA167234 (Sreekumar) 07/01/2012 – 07/31/2017
NCI
Metabolomic profiling and biologic basis of racial disparity in prostate cancer
Goals: definitively define and compare the PCa metabolome of AA and EA men and uncover the biological mechanism in an ancestry-verified subset of AA and EA prostate cancers. To functionally characterize the race-associated metabolic pathways and evaluate the pathway-associated metabolites in urine specimens from AA and EA men with prostate cancer.

5) 3U01 CA167234-02S1 (Diversity Supplement, Sreekumar) 08/29/2013 – 07/31/2015
NCI
Metabolomic profiling and biologic basis of racial disparity in prostate cancer
Goals: To train the postdoctoral trainee Stacey Lloyd on conducting experiments related to understanding the biological basis of health disparity in prostate cancer. This involves training in the area of mass spectrometry, data analysis, bioinformatics and functional studies. As a part of her training, Dr. Lloyd is working on uncovering the mechanism associated with changes in polyamine metabolism in AA prostate cancer.

6) Agilent Foundation (Sreekumar) 05/01/2014-04/30/2015 (NCE)
Agilent Technologies
Integrative Analysis of Gene Expression and Metabolomic Data for Cancer Progression
Goals: To generate matched gene expression data for AA and EA PCa tissues for which matched metabolic data has been generated. The study will then integrate the matched datasets using Gene Spring software and identify pathways and compare them with those identified using NETGSA.

7) HELIS Foundation (McGuire) 07/01/2013-06/30/2016
The Landscape of Genomic Rearrangements in Prostate Cancer as Drivers of Altered Cellular Energy Metabolism – An Integrative Genomic and Metabolomics Screen for Novel Therapeutic Targets.
Goals: To develop methods to integrate OMICS data in prostate cancer cell lines specifically gene expression, metabolomics, microRNA and proteomics and to functionally verify the identified pathways.

8) R21 CA173150-01A1 (Kaipparettu) 09/17/2013 – 08/31/2015
NIH
Mitochondria specific metabolomics signature in triple negative breast cancer metastasis
Goals: To define and characterize metabolic alterations associated with triple negative breast cancer cybrids. These are generated by transplanting the mitochondria from triple negative cell lines into benign breast cell lines. The objective is to understand the molecular and metabolic changes that are associate with the aggressive phenotype in these cybrids.

9) R21-CA185516-01 (Sreekumar) 04/01/2104 – 03/31/2016
NIH
High Kynurenine in aggressive triple negative African American breast cancer
Goal: To define the tumor promoting function of Kynurenine and its crosstalk with AHR and RAS signaling.

10) CASIS (Dacso) 04/01/2014-03/31/2017
CASIS
Goal: To develop methods to detect metabolites in biofluids with the longer term goal of applying this to studies in the International Space station.

11) U01CA179674-01A1 (Sreekumar) 07/01/2104 – 06/30/2019
NIH
Delineating racially distinct metabolic pathways in triple negative breast cancer
Goals: To validate elevated levels of unsaturated fatty acids and lipids, functionally characterize the pathways leading to accumulation of 2-OHG and arachidonic acid using in vitro and in vivo models and measure the serum levels of metabolites in tryptophan, unsaturated fatty acids (including arachidonic acid) and 2-HG pathway in AA TN BCa.

Pending Award

12) National Cancer Institute (Frigo) 04/01/2014 – 03/31/2019

NIH
Genetic and metabolic dissection of the camkk alpha signaling axis in prostate cancer
Goals: To study the metabolic pathways regulated by camkk alpha signaling axis in prostate cancer.

OTHER SUPPORT

Andrew J. Morris, Ph.D.

Active

BX001984-01 (Morris) 11/05/12-11/04/16
VA BLR&D Merit Review $150,000 total direct costs current year
Association of a Common Variant of the PPAP2B gene with cardiovascular disease
The major goals of this application are to test specific hypotheses about the mechanistic basis for the strong association of a common polymorphism in the PPAP2B gene with cardiovascular disease. Role: PI

5I01BX001014-03 (Smyth) 10/1/2010-9/30/2019
VA BLR&D Merit Review $150,000 total direct costs current year
Regulation of adipose cells by autotaxin / lysophosphatidic acid signaling
The goal of this project is to test the hypothesis that signaling pathways involved in the synthesis and metabolism of lysophosphatidic acid contribute to diet-induced thermogeneis and regulate the development of obesity. Role Co-PI.

2P20GM103527-06 (Cassis) 09/08/08-07/31/2018
NIH/NIGMS $75,000 total direct costs current year
Center of Research in Obesity and Cardiovascular Disease: Analytical Core
I direct an analytical core of this center grant and serve as a mentor to junior faculty investigators supported by this award. Role: Core director, mentor.

5P42ES007380-16 (Hennig) 04/07/97-03/31/19
NIEHS $250,000 total direct costs current year (requested)
Superfund Basic Research Program: Research Support Core
I direct this core which provides Bioanalytical and Bioinformatics support to investigators of the University of Kentucky Superfund basic research program. Role: Core director.

5P42ES007380-16 (Hennig) 04/07/97-03/31/19
NIEHS: $250,000 total direct costs current year (requested)
Superfund Chemicals, Nutrition, and Endothelial Cell Dysfunction
The goal of this study is to identify mechanisms by which environmental pollutants impair vascular endothelial cell function to promote cardiovascular disease. Role: Co-PI.

ULRF 12-1403 / 1R01HL112788-01A1 (Rataczjak) 06/01/2013-03/31/2014
University of Louisville (NIH flow through) $53,035
Bioactive lipids in stem cell mobilization and homing
The goal of this proposal is to test the hypothesis that the bioactive lipids sphinogsine 1 phosphate and ceramide 1 phosphate regulate bone marrow mobilization and homing of hematopoietic stem cells.
Role: Co-I.

1R01ES023470-01 (Zhou) 09/26/2013 – 06/30/2018
Endocrine disruptor mediated activation of PXR causes dyslipidemia
NIH $221,680
The goal of this study is to define the role of environmental toxins that serve as ligands for PXR as regulators of pathological hyperlipidemia and cardiovascular disease. I will make mass spectrometry based measurements of lipids and environmental toxins. Role: Co-PI.

R56HL124266-01 (Abdel-Latif) 09/01/2014-08/31/2015
Role of Bioactive Lipids in Stem Cell Mobilization and Homing in Cardiac Ischemia
NIH $250,000
The goal of this study is to identify a role for bioactive sphingophopholipids as regulators of bone marrow derived stem cell mobilization and homing to the heart.
Role: Co-I

Pending

1R01HL120507-01 (Morris, Smyth, MPI) 01/01/2015 – 12/30/2019
Lipid phosphate phosphatase 3 as a novel atherosclerosis suppressor
NIH $365,000 (requested, year 1)
The goal of this study is to use pre-clinical models to identify the cell types and mechanisms involved in protective effects of lipid phosphate phosphatase 3 against cardiovascular disease.
Note, this received a priority score of 1.2 and a percentile ranking of 1% so we anticipate it will be approved or funding.
Role: Co-I

1R01 DK103542-01 (Kern) 07/01/2014-06/30/2019
NIH $475,309
Cold Induced Changes in Human Subcutaneous White Adipose
The goal of this study is to define the mechanisms and physiological consequences of cold-induced “browning” of while adipose tissue.
Role: Co-I

Kathleen O'Connor, Ph.D.

D. Research Support

Ongoing

R01 CA109136 (PI: O'Connor, KL) 07/01/04-03/31/17
NIH
"Novel Mechanisms of Carcinoma Cell Migration"
Goals: To define the macromolecular complex that couples β1 integrins to PKA activation thereby limiting RhoA activity at the leading edge; to determine how integrin α6β4 leads to the activation of RhoA; and to elucidate how RhoA function is altered to promote lamellae formation.
Role: PI

T32 CA165990 (PI: Rangnekar, V) 04/01/13-03/31/16
NCI
"Interdisciplinary Research Training in Cancer Biology"
Goals: To develop a cadre of future scientists who can become leaders in integrative team approaches to understand the complex issue of cancer as it relates to potential prevention and treatment strategies.
Role: Co-PI

T32 CA160003 (PI: Evers, BM) 07/01/11-06/30/16
NIH
"Oncology Research Training for Surgeon Scientists"
Goals: To provide intensive and interdisciplinary basic science research training for a minimal two-year period for qualified individuals who are pursuing a career in academic oncologic surgery.
Role: Co-PI

Program Director/Principal Investigator (Last, First, Middle):

D. Research Support

Ongoing Research Support

R01ES022191-01 T.W-M. Fan (PI) 09/01/2012-08/30/2017
Technology Development to Enable Large Scale Metabolomics Analyses (R01)
This project designs and synthesizes chemoselective reagents for targeting metabolites bearing specific functional groups, incorporating a permanent positive change and a stable isotope for increasing sensitivity and identification and quantification of classes of metabolites by mass spectrometry and NMR.
Role: co-I

5R01ES022191-04 T.W-M. Fan (PD) 04/26/2014-08/30/2017
Integrated Chemoselective and Informatic Platform for Large-Scale Metabolomics. Administrative supplement from Targeted Opportunity
This is a joint project with NCI on pre-clinical characterization of metabolic abnormalities in tumors by stable isotope resolved metabolomics for novel target identification. This involved SIRM studies on cells, tissue slices and animal models as well as hyperpolarization NMR in prostate and renal carcinomas driven by germline mutations.
Role: multiple PI

P01CA163223-01A1 A.N. Lane (PD) 3/1/13-2/28/18
Systems Biochemistry in Lung Cancer: Toward a Mechanistic Understanding of NSCLC
The program comprises three project areas utilizing stable isotope resolved metabolomics to gain a mechanistic understanding of NSCLC in situ. The projects combine cell culture, animal models and human subjects to define the influence of the tumor microenvironment on cancer progression.
Role: PD

1 U24 DK097215-01A1 R.M.Higashi (PD) 9/11/13-8/31/18
Resource Center for Stable Isotope-Resolved Metabolomics
This regional center for metabolomics was established at UK to develop and support stable isotope resolved metabolomics.
Role: multiple PI, analytical core director

3U24DK097215-02S1 R.M.Higashi (PD) 9/1/14-3/31/15
Resource Center for Stable Isotope-Resolved Metabolomics
Administrative Supplement for a standards Ring Trial
Role: multiple PI, analytical core director

SBCR Pty Ltd. T.W-M. Fan (PD) 4/1/14-11/30/14
Plasma lipid biomarker(s) for breast cancer diagnosis.
The contract is to valdidate lipid biomarkers in plasma from women with early stage breast cancer
Role: multiple PI

2 R01 DK054921-15 subcontract from U.Minnesota (J. Albrecht, PI) 5/1/14-4/30/16
Cyclin D1/CDK4 Complex in Hepatocyte Proliferation
This project will use SIRM methods to determine the metabolic changes controlled by Cyclin D1 dependent cell cycling in hepatocytes.
Role: co-I

Completed Research Support (last 3 years)

J. G. Brown Chair in Structural Biology A.N. Lane (PI) 01/05/2002-2013
Kentucky's Research Challenge Trust Fund and The James Graham Brown Foundation.
Support for research and activities in any area related to cancer biology.
Role: PI.

Program Director/Principal Investigator (Last, First, Middle):

American Cancer Society RSG-13-139-01-CNE B.F. Clem (PI) 7/1/13-6/30/17
(Role on this grant completed prior to leaving University of Louisville and affiliating with University of Kentucky 09/09/2013.) "Control of Glucose and Glutamine Metabolism by the Retinoblastoma Protein"
Goals: My role in this proposal is to provide NMR-based stable isotope resolved metabolomics to the metabolism of cells controlled by Rb.
Role: Co-I

NCI 1R01 CA166327-01A B.F. Clem (PI) 6/1/13-5/31/18
(Role on this grant completed prior to leaving University of Louisville and affiliating with University of Kentucky 09/09/2013.)"Regulation of Tumor Metabolism by Retinoblastoma Protein"
Goals: My role in this proposal is to supply stable isotope tracing analysis of cells with Rb knockouts, to determine the metabolic regulatory functions of Rb.

1R01CA140991-02 S. Telang (PI) 07/01/2009-04/30/2014
"Targetting Glucose Metabolism in Cancer"
The major goal of this project is to establish the role of PFKFB4 in the invasiveness of transformed cells. My role is to provide NMR support.
Role: Investigator

KLCRP T.W-M. Fan (PI) 12/01/2010-11/30/2013
Stable isotope-resolved metabolomics to elucidate the mechanism of a tumor-associated cytochrome in lung cancer growth and metabolism
The goal is to use metabolomics to characterize exosomal lipids and Pgrmc1 levels in blood from lung cancer patients as biomarkers of therapeutic response.
Role: Co-I

1R01CA118434-03 T.W-M. Fan (PI) 09/01/2008-08/30/2013
Biochemical Mechanisms of Se Anticancer Activity in Lung
This proposal examines the biochemical and cell biological effects of different selenium compounds on lung cancer cells both in vitro and in mouse xenograft models.
Role: Co-I

American Cancer Society S. Telang (PI) 07/01/2009–06/30/2013
Role 6- Phosphofructo-2- Kinase Isoform 4 in NSCLC
My role is to provide NMR-based metabolomics support.
Role: Investigator

5P20RR018733-08 D.M. Miller (PI) 08/01/2003-06/30/2013
Center of Biomedical Research in Molecular Targets
Core Director, NMR Facility. My role is to provide support for protein expression and NMR in projects supported by the grant.
Role: Investigator

1R21CA133688-02 A.N. Lane (PI) 07/31/2009-05/31/2012
"Stable isotopomer analysis of anabolic metabolic pathways in breast cancer"
This project aims to relate effects of nutrient supply and hypoxia on the metabolism of breast cancer cells in culture to the metabolism of the same cells in a mouse xenograft model in discovering the influence of the tumor microenvironment
Role: PI

5RC2GM092729-02 R. Kaddurah-Daouk (PI) 09/01//2009-08/31/2011
Metabolomics Network for Drug Response Phenotype
This grant was concerned with metabolomics readout of drug response in several diseases. My role is to consult on the metabolic biochemistry.
Role: Consultant

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

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

Prefix:	Dr.
First Name*:	Fredrick
Middle Name:	O
Last Name*:	Onono
Suffix:	Ph.D

2. Human Subjects

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

3. Permission Statement*

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

● Yes ○ No

4. Program Income*

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

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

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

Funding Opportunity Number: PAR-12-050. Received Date: 2014-10-13T16:35:51.000-04:00

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

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

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

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

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ❍ Yes ❍ No

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

Previously Reported*: ❍ Yes ❍ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Career Development Award Supplemental Form

OMB Number: 0925-0001

Introduction (if applicable)	
1. Introduction to Application (for RESUBMISSION applications only)	
Candidate Information	
2. Candidate's Background	1253-K01_Onono_Background.pdf
3. Career Goals and Objectives	1254-K01_Onono_CAREER GOALS AND OBJECTIVES.pdf
4. Career Development/Training Activities During Award Period	1255-K01_Onono_CAREER DEVELOPMENT ACTIVITIES.pdf
5. Training in the Responsible Conduct of Research	1256-K01_Onono_TRAINING IN THE RESPONSIBLE CONDUCT OF RESEARCH.pdf
6.Candidate's Plan to Provide Mentoring (as applicable)	
Statements of Support	
7. Plans and Statements of Mentor and Co-Mentor(s)	1257-Sponsor statements rv.pdf
8. Letters of Support from Collaborators, Contributors, and Consultants	1258-consultants statementsR.pdf
Environment and Institutional Commitment to Candidate	
9. Description of Institutional Environment	1259-DESCRIPTION OF INSTITUTIONAL ENVIRONMENT.pdf
10. Institutional Commitment to Candidate's Research Career Development	1260-Evers letter 1 page.pdf
Research Plan	
11. Specific Aims	1261-Specific aims.pdf
12. Research Strategy*	1262-Research plan.pdf
13. Progress Report Publication List (for RENEWAL applications only)	
Human Subject Sections	
14. Protection of Human Subjects	
15. Inclusion of Women and Minorities	
16. Inclusion of Children	
Other Research Plan Sections	
17. Vertebrate Animals	1263-K01_Onono_Vertebrate Animals.pdf
18. Select Agent Research	
19. Consortium/Contractual Arrangements	
20. Resource Sharing Plan(s)	
Appendix (if applicable)	
21. Appendix	

Citizenship*:

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

CANDIDATE'S BACKGROUND:

I received my undergraduate degree with honors and a Master of Science (MSc) degree in biochemistry from top universities in Kenya. During this training I was supported by merit grants from the Kenya government and bilateral development partners. I wrote my MSc dissertation while concurrently employed at the reputable International Livestock Research Institute in Kenya thereby gaining valuable practical research experience and co-authored publications in the journals *PNAS* and *Infection and Immunity.* In these articles, we reported the identification and characterization of candidate vaccine antigens and epitopes that could provide protection against East Coast fever, a devastating parasitic disease of cattle. The antigens were from the schizont stage of *Theileria parva*, the tick-borne parasite that transforms infected lymphocytes to a unique cancer-like phenotype. My specific contributions in these studies were transfection of immortalized skin fibroblasts with pools of random schizont cDNA and screening them with *T. parva*-specific cytotoxic T lymphocytes we isolated from infected bovine blood. I acquired unique skills in cellular immunology, flow cytometry and molecular biology. As a result of my educational background and successful research endeavors, I won a competitive international scholarship to undertake doctoral training in Molecular Medicine at Hannover Medical School (HMS) in Germany.

At HMS, my studies focused on targeting protein posttranslational modification to treat hematological malignancies using myeloid leukemia as a model. The goal of my doctoral research was to map isoprenylated (isoprene covalent lipid modified) proteins in cancer cells that might provide cancer-specific targets for therapeutic interventions. Natural lipid substrates for this modification are biosynthesized as intermediates of the mevalonate pathway. Using xenobiotics, we developed a novel cell-based method that accelerates the identification of modified proteins that are therapeutically relevant for cellular resistance to specific treatment strategies. Utilizing unnatural substrates we rapidly and selectively detected the dynamics of isoprenylated proteins following exposure to different classes of targeted pharmacological agents. We also demonstrated that combination of chemically distinct inhibitors targeting prenyltranferase (enzymes that catalyze isoprenylation) can induce synergistic inhibition of myeloid leukemia cells through a mechanism that partially involves disruption of K-Ras oncogene activity. This doctoral training led to publication of four manuscripts - 2 first author articles in *Molecular and Cellular Proteomics* and *Journal of Molecular Medicine*. I also presented at national and international scientific conferences and received several travel awards. During my doctoral training we collaborated with Dr. H. Peter Spielmann using chemical probes his laboratory had developed to complete my studies. Recognizing the power of these approaches for unbiased identification of isoprenylated proteins I was excited to join the group of Dr. Spielmann for postdoctoral training to expand and augment my prior training with new expertise in chemical biology and associated analytical approaches.

The research I conducted in Dr. Spielmann's lab focused on mechanism of exogenous isoprenol substrate utilization in cancer cells. We discovered that many cancer cells in culture substantially convert exogenous isoprenols to their diphosphate derivatives which are efficient substrates for isoprenylation. Our findings also demonstrated that this is an alternative pathway for the influx/supply or recycling of isoprenoid substrates regulated independently of the classical mevalonate pathway. Genome-wide expression analysis have identified the mevalonate pathway to be significantly upregulated by mutant p53 and promoting tumor invasiveness in breast cancer cells. Interestingly, we found that mutant p53 depletion or pharmacological inhibition of the mevalonate pathway using statins (HMG-CoA reductase inhibitors) enhances the use of exogenous isoprenols, supporting our findings that regulation of this alternative pathway is independent of the mevalonate pathway. The discovery opens up opportunities for design of cell-directed therapies and provides insights into mechanisms underlying pleiotropic therapeutic benefits and unwanted side effects of mevalonate pathway inhibition. These studies, performed in collaboration with Dr. Andrew Morris, enabled me acquire expertise in tandem mass spectrometry. The research was supported, in part, by a prestigious postdoctoral fellowship award from the American Heart Association and formed the basis of a first author publication in the *Journal of Biological Chemistry* and co-authorship in 2 other articles.

Currently, I am a T32 trainee with Dr. Morris learning advanced mass spectrometry approaches to monitor lipid metabolism. I am also receiving training on new skills in animal model and human studies. My research focuses on testing the hypothesis that bioactive lysophospholipids generated from intestinal exposure to phosphatidylcholine in food and bile contribute to obesity-associated disease risk. My past achievements as well as training in **biochemical and molecular techniques together with developing skills in the use of animal and human subjects** illustrate that I have the enthusiasm, capacity and potential to effectively execute the Aims of this K01 application. The training, mentoring, educational resources, interaction with high caliber research scientists and state-of-the-art technology at my disposal will prepare me for a productive independent research career.

CAREER GOALS AND OBJECTIVES:

As a young student growing in rural Africa, I saw firsthand, diseases ravage vulnerable humans and livestock. My dream was to become a research scientist working on interventions for human and animal disease. This burning desire motivated me to seek scientific research training initially in Africa but eventually in Europe and up to U.S.A. focusing on biochemistry, immunology, molecular biology, and currently cancer metabolism. It is this desire to conquer disease and improve human health through responsible clinical and translational research that motivates my application for this K01 grant. My immediate career goal is to complete a program of training that will prepare me to become an independent scientist and investigator in the field of cancer metabolism. In order to achieve this goal I require additional supervised research and career development using mass spectrometry to test a hypothesis on how diet alters cancer outcomes. This will build on my research experience and ultimately begin an independent long-term career focused on the role of diet and obesity in cancer metabolism. Obesity is on a rapid rise worldwide in recent years, and together with body-mass index (BMI) are important predictors of many of the most common site-specific cancers. Positive associations have been reported between BMI and liver, colon, ovarian and postmenopausal breast cancers. These epidemiological observations and the heterogeneity in BMI effects suggest that different mechanisms are associated with different cancer sites and different patient subgroups. There is a critical need to investigate the mechanisms that link obesity with cancer. Although my academic training provides me with a solid research background and my professional experience clearly indicate my potential for directing independent research, I need further training to gain a deeper understanding in cancer biology. I also need mentorship and protected time to acquire advanced analytical research skills to study cancer metabolism and research techniques that facilitate transition and application of basic research findings to translational and clinical advances.

My career in cancer research previously focused on *in vitro* cell-based studies targeting lipid metabolism to treat cancer. Research during my doctoral and early postdoctoral training was focused on prenylation of proteins as an attractive cancer drug target. As my career progressed, I have recognized that effective inhibition of oncogenic small G-proteins, such as K-Ras and RhoA using prenyltransferase inhibitors (PTI) is challenging, which compromises their significance as targets for cancer treatment. This may be attributed, in part, to the incomplete knowledge of the prenylated proteome and unknown identity of the enzyme(s) that phosphorylate isoprenols (alternative prenylation substrates) in humans. I performed RNAi-based and candidate screening to identify genes encoding the “isoprenol kinase” in human cell lines but these studies did not yield the expected outcome. Because of these reasons and due to my desire to apply my basic research background in lipid-dependent G-protein signaling to successful translational and clinical relevance, I became interested in investigating the role of bioactive lipids. I am particularly interested in lysophosphatidic acids (LPA) which also signal through cell surface G-protein coupled receptors to regulate many diseases including cancer, inflammatory and cardiovascular diseases (CVD). Research in my primary mentor’s lab focuses on the role of these lipids but largely on CVD. I would like to take advantage of the available resources in his laboratory focusing my research on the metabolic roles of LPA as a dietary link between obesity and cancer. My research capabilities using mass spectrometry and longstanding interests in lipid metabolism have set the stage for a seamless transition to translational research on diet-dependent obesity as a cancer risk.

This proposal is designed to provide me with additional supervised research and career development to build on my academic training. My primary mentor, Dr. Andrew Morris, has a longstanding research program on lipid metabolism. My co-mentors are internationally recognized experts in cancer research. I have assembled a mentoring committee of well-established investigators with the necessary diversity and excellent pedigree; and importantly will have a regular interaction with them. In addition to their close mentoring, I will have at my disposal necessary resources available, including state-of-the-art equipment, as I focus on my interests in cancer metabolism. I will also use the opportunity to expand my networking and collaboration with internationally acclaimed scientists. It is my expectation that this would increase my chances of succeeding to attain an independent position and also have a profound impact in the field and over the course of completion of this award. The research I will be pursuing is a special area carved out for my work to develop into a R01 project hence I will have developed a niche for myself and would not be a competitor to my mentor. I have had a chance to be a principal investigator in one study at a very early stage through the American Heart Association postdoctoral fellowship but this K01 offers a unique opportunity to accomplish more evidence-based scientific research and generate data that will support and build my authority as a cancer metabolism researcher. To accomplish the goals of this proposal, I will receive continued training in state-of-the-art methodology and courses carefully chosen to establish myself as an expert in the field. These will culminate in generating enough preliminary data to compete for an NIH R03, R21, R01 and foundation grants.

CAREER DEVELOPMENT/TRAINING ACTIVITIES DURING AWARD PERIOD

My academic/research career development will be facilitated by the skills I have honed thus far, along with the expertise that I will be acquiring during this training. To ensure that my training progresses accordingly, I have assembled a training committee that includes mentors(s), collaborators and consultants (see attached letters). The advisory panel includes UK faculty (who I can meet on a regular basis) and external collaborators and consultants who can support my training/ research by providing the required resources. These include:

1. Dr. Andrew J. Morris (UK, mentor) - Prof. Morris is the Director of Small Molecule Mass Spectrometry Core Laboratory. He is an internationally recognized expert in lipid metabolism and signaling. His current areas of interest include studies of bioactive lysophospholipid meditators and investigations into mechanisms linking diet induced obesity and exposure to environmental pollutants to human disease. I have asked Dr. Morris to serve as my primary mentor and career advisor not only because of his expertise in lipid metabolism but also because of his successful training of other young-faculty. We have also published together [Onono et al. 2013, Subramanian et al. 2013 and Subramanian et al. 2014].
2. Dr. Kathleen O'Connor (UK, co-mentor) – Prof. O'Connor is Associate Director of Cancer Education, Markey Cancer Center and Co-Director of the Breast Translational Group, a multidisciplinary group of scientists, clinicians and bioinformaticists dedicated to translational cancer research in breast cancer. Dr. O'Connor's knowledge and expertise in the fields of breast cancer; signal transduction, tumor cell dissemination, and transcriptional control of invasive phenotype will be an asset to the proposed study. She supervised my *in vivo* preliminary metastasis studies and will be my co-mentor and advisor on tumor animal model studies.
3. Dr. Andrew Lane (UK, Co-mentor) - Prof Lane is the Co-Director of the Resource Center for Stable Isotope Resolved Metabolomics at UK Cancer Center. He is the PI of an NCI funded program project grant with the broad goal of understanding how metabolic reprogramming contributes to the development and progression of lung cancer. He has more than 30 years' experience in the field of metabolomics with a particular focus on the development and application of state of the art NMR and mass spectrometry based approaches. He will provide cancer-focused advice, expertise and oversight for the proposed research training plan.
4. Dr. Richard Charnigo (UK, consultant) – Prof. Charnigo is an expert in Statistics and serves as the primary statistical consultant for an NIH-funded COBRE in Obesity and Cardiovascular Disease which supports an institutional career development program for early stage investigators at the University of Kentucky. He worked with my primary mentor on a published study of the effects of the LPA generating enzyme autotaxin on cell migration, particularly breast cancer cells. He will provide advice, assistance and mentoring in statistics
5. Dr. Arun Sreekumar (Baylor College of Medicine, external consultant). Prof. Sreekumar is the Director of Metabolomics for Alkek Center for Molecular Discovery. He is an internationally recognized expert in metabolomics and bioinformatics focused in the area of prostate and breast cancer. I will be consulting him on metabolic profiling and identification of tumor biomarkers.
6. Dr. Marta Torroella-Kouri (University of Miami, collaborator and external consultant). Prof. Torroella-Kouri has extensive experience in various areas of cancer research using *in vitro/in vivo* models of cancer. She will provide me with guidance in mice diet studies and to analyze effects of diet on breast cancer.
7. Dr. Laundette P. Jones (University of Maryland, collaborator and external consultant). Prof Jones is an expert in mammary carcinogenesis and mechanisms of breast tumor development in preclinical mouse models of breast cancer. She will provide intellectual contributions to the design of the mouse model studies and the analysis of the data and interpretations of the findings to the field of breast cancer.

The advisory committee will meet formally (collaborators via teleconference) once every 6 months to evaluate the progress on my training and assist in addressing any issues that arise throughout the duration of the training. They will also serve to guide and critique data analysis, data interpretation, manuscript preparation, identification of grant opportunities, and address any issues that may occur within all training activities during this training period. Individual meetings and visit to the labs of external consultants/collaborators will occur as needed. If this K01 is funded, I will commit >80% of my effort to the K01 mentored training program detailed in my research plan (Table 1). My goal is to publish at least 3 manuscripts from this study in high impact peer-reviewed journals. I will also dedicate ~16% effort to writing a R21, R01 grant in year 3- 4. The proposed career development/training plan consists of didactic coursework, seminars, and workshops offered at UK, as well as a variety of mentored research experiences to enhance my research skills and knowledge base.

Enhance Research Skills: The proposed training program is designed to upgrade and sharpen my preexisting skills and teach me new research tools that I will be using as an independent investigator. I have deficient knowledge in certain key research tools, for e.g., in the utilization of 1) lipidomics for unbiased screening/ discovery of lipid mediators as potential tumor markers and 2) experimental animal models.

Enhance preexisting skills*:* During my graduate studies and subsequent postdoctoral training, I have been

exposed to a number of research tools and methodologies that are crucial to the proposed research strategy. This includes molecular biology techniques and mass spectrometry. Although I have gained considerable research experience, I am not an expert in all of these techniques. Dr. Morris and Dr. Lane, are experts in mass spectrometry, will supervise my training in advanced mass spectrometry to identify and characterize various molecular metabolites. Learn new skills: This includes comprehensive training in animal model and tumor biology. Dr. O'Connor and Dr. Lane are experts in cancer research. They will help me set up experiments aimed at inducing and monitoring tumor and metastasis. In addition, I will have plenty of opportunities to learn these techniques from my colleagues in our laboratory and division.

Enhance Knowledge Base: The courses include: (1) PHA 616 Biology and Therapy of Cancer - to learn cancer biology at the molecular, cellular, and organismic level with an emphasis on aspects of cellular signaling, apoptosis, and the cell cycle which are unique to cancer cells; (2) CPH 613 Molecular Epidemiology, Cancer Prevention and Control – to learn how biomarkers are developed and used for risk assessment; (3) NS 604 Lipid Metabolism - to improve my understanding of factors influencing the absorption of fats and fatty acids, distribution and incorporation of fatty acids into body tissues, the biosynthesis of and catabolism of fatty acids, as well as cholesterol, bioactive eicosanoid production and the involvement of fats in the disease process; (4) STA 580 Biostatistics I- to learn descriptive statistics, hypothesis testing, paired and unpaired tests, ANOVA, contingency tables, log rank test, and regression with biostatistics applications. I will also attend the annual Resource Center for Stable Isotope-Resolved Metabolomics workshop at UK to gain deeper understanding of stable-isotope-enabled studies of metabolism and how to integrate and experimentally design these studies. Other seminars I plan to participate in include, the weekly Markey Cancer Center Research Seminar Series, bi-weekly research presentation as a part of the Metastasis Group, monthly meeting of the BBSRB Cancer Signaling group (research and administration meetings), monthly meetings of the Cancer Cell Signaling Program, the monthly Cancer Center Journal Club and Lab Meetings (Dr. Morris, and O'Connor). I also would like to improve my knowledge in the clinical translational sciences. The CCTS at UK offers a number of didactic courses including a Seminar in Clinical and Translational Science (BSC 733/CPH 671) during fall/spring, which I plan to attend. Further, I will attend the annual MCC Day and BBDOC research day.

Table 1. K01 Yearly Distribution of Percent Effort (>80%)

Activities	Year				
	1	2	3	4	5
Research projects	50	55	50	50	40
Coursework, Workshops, Seminars, & Conferences	15	15	10	10	10
Mentoring consultation	10	5	5	5	5
Manuscript and grant writing	10	10	20	20	30

Enhance Presentation Skills: In order to obtain and succeed in an academic research setting as a faculty member, superb presentation and lecturing skills in and out of the classroom are an absolute necessity. To this end, I plan to present my current research at a number of national conferences, including the American Association for Cancer Research, Annual San Antonio Breast Cancer Symposium and Specialized Keystone Conferences, Furthermore, I plan to present at the local meetings and seminar series at the Markey Cancer Center which will improve my presentation skills.

Manuscript Preparation: Since my first publication in 2006**,** I have gained valuable experience in the writing and preparation of scientific manuscripts for publication. However, since English is not my native language, I feel my written communication skills can be further improved. Through a collaborative process of my writing and subsequent revisions by my mentors and advisory panel, I will receive extensive training in the effective communication and writing of scientific results.

Grantsmanship: As an independent faculty member, I will be expected to prepare and maintain extramural grant funding. Adhering to this expectation will require superb grant writing and management skills. To this end, I will participate in seminars/ workshops sponsored by the UK College of Medicine, Program Development office, and the CCTS. Topics covered have ranged from identifying funding opportunities to proper budget management. In addition, my regular interactions with my mentors will provide guidance in proper grant writing skills and grant management. Further, the issue of grantsmanship will be discussed during formal meetings of the advisory panel. I intend on submitting an R21 application in three and R01 in 4 years.

Student Mentoring: Because my career goal involves working in an academic environment, student mentoring will be a major part of my success as an independent faculty member. I will take two courses (PGY 615 - Seminar in Teaching Medical Sciences and PGY 616 - Practicum in Teaching Medical Sciences) to obtain specific instruction in teaching. I will also participate in the T.E.A.M. program (Training, Education And Mentoring) offered by UK CCTS. The objective of this program (meeting monthly) is to prepare future faculty members for excellence in teaching, research and service and to provide career development opportunities.

TRAINING IN THE RESPONSIBLE CONDUCT OF RESEARCH

Beginning in 2001, I have almost 13 years of laboratory experience conducting research. As a Master of Science student my training involved performing experiments in animal models (cattle). During my doctoral training at Hannover Medical School, I worked closely with my advisor Prof. Christoph Reuter on experimental design, appropriate ethical conduct, appropriate data management and analysis practices, authorship, appropriate peer review and publication practices, and data interpretation and presentation. In addition, I attended a mandatory 1-credit course in Scholarly Integrity and Research Ethics as a part of my PhD program. At the University of Kentucky I completed a number of online training courses offered by AALAS learning library (Animal care and Use in Research and Education). These included courses in Anesthesia and Analgesia, Aseptic Technique for Rodent Survival Surgery, Septic Technique and Surgical Support and Anesthesia, AVMA Guidelines for the Euthanasia of Animals: 2014 Edition and Working with the IACUC: non-VA version. I have also completed Collaborative Institutional Training Initiative (CITI) program coursework for VA researchers. These include VA Human Subjects Protection and Good Clinical Practices, Post-Procedural Care of Rodents, Working with the VA IACUC, Working with Mice in Research Settings, Working with Rats in Research Settings and Avoiding Financial Conflicts of Interest in VA Research.

As a T32 trainee at the University of Kentucky, I am currently taking a mandatory formal course in Fundamentals of BioEthics (PHS 760-010). The course will help me gain an understanding of fundamental ethical principles guiding the conduct and reporting of my research. This course coordinated by UK faculty Dr. Linda P. Dwoskin meets once per week for 2 hours for 13 weeks. Seminars are divided into a 1-hr lecture followed by a 1-hr small-group discussion. The course objectives include; gaining an in-depth understanding of the key issues in the ethical and responsible conduct of research, developing an appreciation for the gravity of research misconduct and how to handle suspected or documented misconduct, understanding current animal and human subject protection standards, understanding the importance of inclusion and diversity of persons from underrepresented population groups, those with disabilities, and individuals with economically disadvantaged backgrounds as investigators, designing and prepare an IACUC protocol application in their area of interest and generating critiques of IACUC protocol applications. Topics covered include BioEthics in the Use of Vertebrate Animals, Management of Research Data, Ethical Issues in Data Interpretation, Responsible Authorship, Research Misconduct, Ethical Issues in Research Administration: Emphasis on Financial Issues, Ethics of Using Human Subjects and MOCK IACUC review, Intellectual Property: Rights, Responsibilities, Ethical Dilemmas, Ethical Issues Requiring Legal Intervention, Conflict of Interest, Ethics Regarding Collaborative Research with Industry, Ethical Issues in Human Resources Management, The Diversity Factor and MOCK IACUC review and Mentor Mentee Relationships. Core faculty associated with the NIH-funded training grants at UK lead these small-group discussions. Furthermore, my mentors will be responsible for continued training in the ethical conduct of research. Through collaborative efforts with my mentors in data collection, analysis, presentation and publishing, I will receive critical feedback that will strengthen training in conducting ethically and methodologically sound research.

Primary Sponsor: Andrew J. Morris, Ph.D.

Qualifications. I am an endowed professor of cardiovascular research at the University of Kentucky. My personal research program concerns lipid metabolism and signaling in cardiovascular and metabolic diseases. Current areas of interest include studies of integral membrane lipid phosphatases and bioactive lysophospholipid mediators and investigations into mechanisms linking diet induced obesity and exposure to environmental pollutants to human disease. We are also invested in the development and application of mass spectrometry-based methods for structural analysis and quantitation of lipids and other biologically important molecules. I direct a core laboratory that provides mass spectrometry services to institutional investigators. I have been an independent investigator since 1993. I have authored or co-authored more than 240 publications in the broad areas of lipid metabolism and signaling and their roles in cardiovascular and metabolic disease and these have been cited more than 12,700 times. This includes 4 publications that have been cited more than 500 times and 28 publications that have been cited more than 100 times. I have contributed to research published in many of the most selective journals including Science, Nature, Cell, Cell Metabolism, Developmental Cell and Proceedings of the National Academy of Sciences. I am currently and have been in the past an appointed editor of scientific journals in my field of study including the Journal of Biological Chemistry and Biochemical Journal. I am currently or have been in the past an invited member of review panels for research programs that are funded by the National Institutes of Health, Department of Defense and National Institute of Environmental Health Sciences. I am a long standing professional member of the American Heart Association and serve as a reviewer for their regional and national research programs. I have been an organizer or co-organizer of several national and international research conferences in my field including FASEB and Gordon Research Conferences. Although I have published papers in the area of bioactive lipids and cancer and have also had personal research funding from NCI this is not the primary research focus of my lab. To provide cancer focused mentoring, Kathleen O'Connor, a very well established cancer cell biologist will serve as co-mentor for this application as detailed in her co-sponsor's letter of support. Andrew Lane, an accomplished cancer researcher who directs NIH/NCI funded programs in stable isotope resolved metabolomics and lung cancer research will also participate in the training program as a co-mentor.

Training record. Specific recent and selected past examples of trainees in my group (some of whom were mentored together with my colleague Susan Smyth) who have competed successfully for significant training awards and/or gone onto success as independent investigators include:

Name	Position	Source of support	Current position
Scott M Hammond	Ph.D. student, 1994-1998	NIH, Pharm. Mfrs Assoc.	Assoc. Prof., Cell Biol., UNC Chapel Hill
Zachary Fulkerson	MD, Ph.D. student (Susan Smyth)	NIH, individual F32	Resident, Internal Medicine Indiana University
Abdel Salous	MD, Ph.D. student 2008-2013	AHA fellowship	Resident, Surgery, George Washington University
Hongmei Ren	Post. Doc. 2006-2011	AHA fellowship, AHA Scientist Dev.	Asst. Prof., Cardiovascular Medicine, University of Kentucky
Tao Wu	Post. Doc. 2004-2012	AHA fellowship	Laboratory Head, Caridian BCT, Denver Co.
Manikandan Panchatcharam	Post Doc, Res. Asst Prof (Susan Smyth)	AHA fellowship, AHA Scientist Dev.	Asst. Prof., Physiology, Louisiana State University
Prabha Nagareddy	Post Doc (Susan Smyth) 2012-	NIH K99/R00	Post-Doc/Asst.Prof. , University of Kentucky

Financial support for the proposed research. My research has been continuously supported by Federal awards for more than 20 years. As detailed in my other support section, I am currently PI or Co-PI of several awards from the NIH (NHLBI and NIEHS) and Department of Veterans Affairs that broadly support studies of lipid metabolism and signaling in human disease processes. I am particularly interested in understanding the link between obesity and human disease so many aspects of the research Fredrick proposes are of mutual interest and direct relevance to my funded work. Accordingly I have more than adequate resources to support the studies Fredrick proposes to conduct examining the role of dietary lipids as a source of circulating bioactive lipid mediators and indeed this is work that will be pursued collaboratively with him. As Fredrick's training and transition to independence advance my expectation is that he will seek individual support for aspects of the work relating to breast cancer that are not within the purview of my active awards.

Evaluation of the candidate. After completing his Ph.D. studies in Germany, Fredrick came to our university to undertake post-doctoral training with my colleague Pete Spielmann. At the time, we were collaborating with Pete on studies of integral membrane lipid phosphatases that could dephosphorylate isoprenoid diphosphates. Pete had developed chemical probes (aniline substituted isoprenols) that could be incorporated into proteins, presumably through conversion to isoprenoid diphosphate intermediates. Isoprenoid diphosphates proved challenging to measure by HPLC/tandem mass spectrometry because they will only ionize in negative mode, which is suppressed by the acidic conditions necessary for reverse phase chromatography of these polar compounds. An added complication is that these compounds will then only form charged phosphate or diphosphate product ions after collisional dissociation. Fredrick recognized that the aniline substituent would make isoprenoid phosphates ionize strongly in positive mode. Accordingly, he embarked on a project to use Pete's aniline substituted isoprenols as tracers to investigate isoprenoid phosphate metabolism and protein isoprenylation in live cells. This was a very ambitious undertaking. To accomplish the goals of the study,

Fredrick had to become proficient in techniques of tandem mass spectrometry (primarily using triple quadrupole instruments) and he also participated in the synthesis and characterization of stable isotope labeled compounds (isoprenoid phosphates and isoprenol cysteine thioethers) that were used as internal standards. With these tools and reagents in hand, he showed that cultured mammalian cells could, in some cases with quite high efficiency, convert exogenous isoprenols to their isoprenoid diphosphate derivatives and then use these as substrates for protein isoprenylation. This demonstration that mammalian cells can recycle or bypass the mevalonate pathway is of particular relevance to efforts to target these pathways for cancer therapy. One of the outcomes of Fredrick's work was the finding that while mutational activation of the p53 tumor suppressor results in activation of the mevalonate pathway in breast cancer cells the process he identified persisted when p53 function was inhibited. This study was published in the *Journal of Biological Chemistry* with Fredrick as a well-deserved first author. After completing this study, we explored the idea of developing RNAi based "synthetic lethality" screens to identify the genes encoding the enzymes responsible for phosphorylation of isoprenols in mammalian cells. This and a candidate gene screening approach has not yet proved to be fruitful. Mindful of the need to remain productive and wanting to maintain an interest in lipid metabolism, Fredrick began a parallel series of studies to investigate the link between diet and circulating levels of the bioactive phospholipid lysophosphatidic acid (LPA). These studies provided a vehicle for Fredrick to extend his training in mass spectrometry to encompass measurements of different lipid species using other types of instruments. They also provided an entry to conducting studies with clinical subjects and animal models which are both areas where Fredrick needs more training. As shown in his proposal, Fredrick made the important observation that plasma levels of LPA are acutely sensitive to fasting and high fat feeding in both humans and mice. In mice, he used mass spectrometry and lipid tracers with unnatural fatty acids to generate evidence that plasma LPA can be made directly from intestinal phosphatidylcholine. These are important and novel findings that could link diet and in particular hyperlipidemia associated with diet dependent obesity with human disease risk. Although the focus of my personal research is on cardiovascular disease, as explained in his proposal, obesity has far greater effects on cancer risk and prognosis, particularly in women, than it has on cardiovascular disease. As with cardiovascular disease, a wealth of genetic and pharmacological evidence identifies a role for LPA as a regulator of cancer initiation and progression. We are currently supporting Fredrick using an institutional NIH/NHLBI T32 grant (directed by my colleague and collaborator Susan Smyth) with the expectation that he will publish a paper with us reporting these effects of diet and feeding on plasma LPA levels and then use this data as a springboard to launch an independently funded research project to investigate the possibility that LPA is a link between diet dependent obesity and cancer risk. Since we have no interest in pursuing cancer research ourselves, this situation provides a perfect opportunity for Fredrick to benefit from resources and ongoing studies in our laboratory while developing a completely independent research project. As a native of Kenya with the life and research experiences outlined in his personal statements, Fredrick is a unique individual. He is very driven to succeed and not shy of taking on complicated and challenging projects. He is also extremely likable which I think has enabled him to pursue these complicated projects by enlisting the help and support of others in a way that encourages people to work together in an altruistic manner. Along with his obvious promise as a researcher, this combination of qualities bodes extremely well for Fredrick's transition to becoming an independent leader of his own research program. Fredrick is, however not the finished article. Although I consider the work he conducted in collaboration with myself and his former mentor to be of high quality he clearly needs to publish more. Indeed, since he started working under my sole direction I have made publication of his work on diet and plasma lysophospholipids our top priority. While he has a solid educational background there are aspects of modern biology (genetics, "omics", systems biology being examples) where he would benefit from more up to date education and training. Fredrick also needs guidance with scientific writing although since his oral research presentations are generally strong and he has taken a lead in writing some papers and this proposal I see this as a feasible goal. The training plan elaborated elsewhere is an effort to build on these strengths while complementing the areas of weakness that will need to be addressed as Fredrick moves towards an independent position.

Training plan. As detailed in his proposal, the research project Fredrick describes will be the primary vehicle for his training in research. Accordingly, as the project develops he will take advantage of complementary opportunities for formal and informal instruction. In addition to the laboratory meetings and personal interactions detailed below, we have seminar series in Obesity, Cardiovascular Disease and Cancer that are organized by the respective research centers that he will be able to attend. We have annual research days for Obesity and the Cancer Center at which Fredrick will be expected to present his work. Our Center for Clinical and Translational Sciences incorporates an institutional KL2 program to support early stage investigators and Fredrick will be expected to participate in this. We have Workshops on Biomedical Mass Spectrometry (organized by me as part of the training component of our NIEHS supported Superfund Center) and our Center for Stable Isotope Resolved Metabolomics offers an annual workshop with lectures and practical instruction about the use of these techniques in basic, translational and clinical research. As the work progresses Fredrick will be expected to present his work at National or International Meetings, for example Gordon or FASEB conferences. As a trainee on our institutional NIH T32 award, Fredrick is already required to participate in formal education in the responsible conduct of research provided through collaborative teaching arrangements as detailed separately in this proposal. He proposes to take courses in Biology and Therapy of Cancer and in drug action and drug metabolism. He will participate in seminars and workshops to provide training in grantsmanship and laboratory management that are organized by the office of Faculty Development in the College of Medicine.

Nature and extent of supervision and commitment to the candidate. Fredrick will continue to be an integrated member of the broader research group that I share with my colleague and collaborator, Susan Smyth. This group comprises approximately 15 individuals, the majority of which are professional staff. Our group also includes pre and post-doctoral fellows as well as clinical fellows in cardiology undertaking research training. Importantly, we have three individuals in transitional appointments who are supported by Scientist Development Grants from the American Heart Association, an NIH K99/R00 award and a Centers of Biomedical Research Excellence Award (see below). We work closely with two other NIH funded early stage investigators who are focused on platelet biology and the role of bioactive lipids in bone marrow derived stem cell trafficking. Accordingly, Fredrick will interact with multiple individuals who are successfully negotiating the career path he now seeks to follow. Our group has weekly lab meetings and journal clubs that Fredrick will participate in. I expect that Fredrick will continue to take the lead in preparing publications arising from his studies. Indeed, as detailed below, research productivity as determined by publications is an area for improvement. Accordingly, a major focus will be on publication of a report detailing the effects of fasting and feeding on circulating bioactive lipids in mice and humans based on the preliminary studies shown in the proposal. As the work progresses, I would expect Fredrick to begin to publish as the corresponding author, particularly since (as detailed below) I have no intention of pursuing research into the link between bioactive lipid metabolism and cancer myself. I work in the laboratory myself. I have no teaching or administrative responsibilities other than directing a Mass Spectrometry Core laboratory and indeed almost all of my effort is supported by the Department of Veterans Affairs or the NIH. Consequently, if I am not out of town I am always available in the laboratory or office to meet with any members of our research group. In Fredrick's case, we can therefore continue to meet informally as often as possible daily or even more frequently if needed to ensure that he receives all supervision necessary to ensure success of his research training plan.

Relationship of candidate's research to ongoing work in the mentor's laboratory. I see this as a perfect opportunity for Fredrick to develop research of his own while benefitting from substantial research infrastructure developed by myself and my collaborators to investigate cardiovascular complications of obesity. Although I have been engaged collaboratively in efforts to define the role of lysophosphatidic acid in cancer this is not an area of active investigation in our group and we have no interest or intention in working in this field. Consequently, Fredrick can conduct his basic studies of the relationship between diet, obesity and hyperlipidemia and lysophosphatidic acid metabolism in mouse models and clinical subjects with the full support of my laboratory and research funding with the expectation that this will lead to a series of collaborative publications during the primary mentored phase of his training. At the same time, he will be encouraged to begin to extrapolate this work to the cancer models described in the proposal which would then be an area that he can publish on and pursue independently. My hope and expectation would be that his transition to cancer research could be funded through pilot grant support that is available for work in this area through our COBRE program and the Markey Cancer Center. Eventually, Fredrick would be free to use any or all of the data generated through his proposed research project as the basis for personal independent research funding applications to support him as an independent investigator.

Plan for transition to independent status. In my opinion, the University of Kentucky is a perfect environment to enable Fredrick's transition to independence. Over the past ~10 years, the research enterprise at the University of Kentucky has grown considerably in both scale and impact. Key accomplishments include an NIH Clinical and Translational Sciences Award, NCI designation of the Markey Cancer Center and a series of other large programmatic research awards to support research in Aging, Nanotechnology and Environmental Disease. Recruitment of the best early stage investigators to the institution has also been a priority. At the same time, we have refined a very well developed strategy for mentoring "home grown" investigators to independence that I propose could be used to facilitate Fredrick's transition to independent investigator status. To a large extent, our efforts in this area have been supported by the NIH "Centers for Biomedical Research Excellence" (COBRE) program which is an institutional career development award available to institutions in qualifying states to develop the careers of early stage investigators. I am an active participant in a program of this type that focusses on the link between obesity and cardiovascular disease but I am also now involved in development of COBRE in Cancer Metabolism. I have been personally involved in our funded COBRE for more than 7 years (including its original award and competitive renewal). I mentor or interact extensively with multiple early career stage individuals associated with this program, who are now NIH funded investigators (currently 7 individuals with another trainee from my group currently being supported). I believe that the program of research and training outlined in this application would prepare Fredrick for support by a program of this type. Indeed, the above mentioned COBRE in Cancer Metabolism would, if funded, be a perfect fit for Fredrick. Furthermore, support and career development of faculty in underrepresented groups must be made a priority for our institution. Indeed of more than 200 basic sciences faculty as far as I am aware we only have one African American colleague. This is inconsistent with the composition of the population of the state that we serve and also at odds with the public face of the university (as presented by our very successful athletics programs). Accordingly this is an area where we must do better. Indeed, I see this K01 program as an equal opportunity for both Fredrick's personal career development but also for the institution to increase the diversity of its biomedical research workforce and, eventually its faculty. If this application is successful, I will work as hard as needed to ensure that as much as possible is done to secure institutional resources to support Fredrick's transition from a mentored position to eventually become an independent funded investigator.

Summary. In summary, while Fredrick Onono is not currently ready to become an independent investigator, I believe that the promise he has shown so far and his personal qualities make it very likely that, with

appropriate training and support, this is a realistic goal. For the reasons outlined above, I also believe that my laboratory and the broader support available for Fredrick at the University of Kentucky for research at the interface of metabolic disease and cancer can provide a strong environment to develop Fredrick's career in a direction that will position him to address important and unanswered research questions and eventually transition to become an independent investigator. Overall, in my opinion support from this award would be an effective use of NIH/NCI funds to support a promising young scientist who I believe will go onto bring credit to the K01 program. I support this application without reservation. I will do whatever I can to make this a success.

Co-Sponsor: Kathleen O'Connor, Ph.D.

I will serve as a co-mentor for Fredrick in the program of research and training outlined in this application.

Qualifications. I am a Professor in the Department of Molecular and Cellular Biochemistry and the Markey Cancer Center and Associate Director of Cancer Education in the Markey Cancer Center to oversee our graduate and post-graduate educational mission. My research concerns integrin signaling and how it controls cAMP metabolism and the Rho family of small GTPases to promote breast and colon carcinoma invasion. Of relevance to Fredrick's project, we found that autotaxin is regulated by integrin α6β4 and contributes to breast cancer invasion. Indeed, autotaxin is up regulated in the cancer cells of one-third of all clinical breast tumor specimens possibly through recruitment of immune infiltrating cells (unpublished). I also serve as the co-director of the Markey Breast Translational Group and organize our annual Breast Cancer Research Symposium each year. My knowledge and expertise in the fields of cancer signal transduction, cancer biology, transcriptional control of an invasive phenotype and breast cancer makes me particularly qualified to mentor Fredrick in these areas.

Training record. Individuals working under my direction have been supported by research training awards from the NIH and private foundations as detailed in the table below. I am also Associate Program Director for a NIH/NCI funded T32 program "Oncology Research Training for Surgeon-Scientists" that functions through the Markey Cancer Center and Department of Surgery for the training of surgeons as clinician-scientists. I am also the Associate Director for a NIH/NCI funded T32 "Interdisciplinary Research Training in Cancer Biology," which supports funding for three post-docs and one pre-doc trainee.

Trainee Name	Predoc/ Postdoc	Prev. Degree Institution	Training Period	Title of Research Project (funding)	Current Position
Carpenter, B	Predoc	MS, Murray State BS, Georgetown	2010-present	Integrin **α6β4** in DNA Repair Mediated Gene	O'Connor Lab
Cruz-Monserrate,	Predoc	BS, U. Puerto Rico Humacao	2003-08	Integrin α6β4 in pancreatic cancer	Instructor, MD Anderson Cancer
Tallman, M. (UTMB)	Predoc	BS, Arizona State University	2006-08	Effect of cyto-architecture on	Physician, Emergency
Chen, M (UTMB)	Predoc	MD, Anhui Medical U Inst. of Epidemiology	2008-11	Regulation of S100A4/metastatin	Asst. Prof, Univ. of Kentucky
Stewart, R	Post-doc	BS, University of California, Berkeley	2013-present	Clinical impact of integrin α6β4	O'Connor Lab
Yang, J	Post-doc	U of Louisiana	2009	sFRP1 in pancreatic cancer biology	Post-Doc Scholar, Mass General
Zhou, W. (UTMB)	Post-doc	UTMB, PhD	2002	PKA signaling during carcinoma migration	Dermatologist, Long Island Jewish

As detailed below, I am also very invested in mentoring of early stage investigators which has been a particular focus of our Cancer Cell biology program. Individuals of this type that I have in the past or currently mentor with success in competing for individual research support include:

Investigator	Grant awarded	Funding source	Position
M. Falzon	R01CA083940	NIH, NCI	Established investigator
L. Elferink	R01CA119075	NIH, NCI	Established investigator
S. Sastry	R01CA118405	NIH, NCI	New investigator
BP Zhou	R01CA125454	NIH, NCI	New investigator
J. Xie	R01CA094160	NIH, NCI	Established investigator
J. Jia	R01GM079684	NIH, NIGMS	New investigator
R. Plattner	R01CA166499	NIH, NCI	Established investigator
Q. She	R01CA175105	NIH, NCI	New investigator
Q. She	CTSA career Development Award	UK CCTS	New investigator
C. Huang	RSG-13-184-01-CSM	ACS, RSG	New investigator
Y. Wu	RSG-13-187-01-CSM	ACS	New investigator
R. Stewart	CTSA pilot award	UK CCTS	Post-doc
M. Chen	ACS-Institutional research grant	ACS	New investigator

Financial support for the proposed research. My research has been continually funded by NIH for the last 10 years. While the funds that support the research in my lab are not directly necessary for the research Fredrick proposes because of our common interests he will benefit from resources supported by my active awards.

Evaluation of the candidate. Fredrick came to UK to work with Dr. Peter Spielmann in my department on protein isoprenylation. This is where I first became acquainted with Fredrick. I have been collaborating with

Pete for the past several years on the impact of artificial isoprenoid derivatives on signaling through the Rho family of small GTPases and how these compounds impact breast cancer invasion. Fredrick was working on the mevalonate pathway and its impact on cardiovascular disease, a project he was funded through the American Heart Association to pursue. Through our collaborative efforts, Fredrick became interested in how cancer cells utilize these artificial isoprenoids, which is critical to achieve our eventual goal to use these compounds to target protein isoprenylation for therapeutic intervention. His collaboration with and mentorship from Andrew lead to his recent JBC paper. Funding shortages in Dr. Spielmann's lab made it difficult for Fredrick to complete and publish all of his postdoctoral work. Andrew is committed to increasing this rate of publication, as he has not only the ability and drive to do so, but also the resources in the lab to facilitate and this is currently a major area of emphasis in Fredrick's training. Fredrick is a hard working dedicated individual with an outstanding technical portfolio. His expertise to date makes him an outstandingly promising researcher with unique capabilities to define the mechanisms that link obesity, metabolic disease and cancer, which drives my interest to be an active participant in his career development. Fredrick has great potential, but still requires additional mentorship and training to enable his transition to independence. Andrew Morris is a highly dedicated researcher with outstanding expertise and technical prowess in the field of lipid metabolism where he is recognized worldwide for his expertise in this field. In my experience, Dr. Morris' dedication to mentorship is of the highest quality, which will be instrumental to the final stages of career development needed for Fredrick to transition to a successful independent investigator. Andrew's mentorship coupled with my expertise and resources in cancer research (see below), I expect Fredrick to fulfill his potential as an independent investigator.

Training plan and commitment to the candidate. As a leader in the Markey NCI-designated Cancer Center, I am familiar with all of the resources available to Fredrick and will ensure he has the needed resources available to him. These resources include pilot funding mechanisms, core resources, collaborators, seminars and networking opportunities. As the Associate Director of Cancer Education, I coordinate and participate in the mentorship of our junior faculty. We have a robust mentorship program and dedication to the development of our junior faculty. We do this as a coordinated program with the faculty member's home department. Each faculty is given a mentoring committee comprised of 4-5 mentors hand-picked to give solid feedback on the various aspects of the investigator's research interests. This committee works diligently with the junior faculty member to critique their projects and proposal until they become federally funded with an R01-equivalent grant. Over the last several years, this program has yielded great success with our junior faculty being awarded 4 American Cancer Society RSGs, 2 American Heart Associate awards, three K08s, and multiple R01s and one R01 awarded on the first submission. I believe our dedication to the development of our junior faculty is top notch and expect that Fredrick will greatly benefit from this dedicated mentorship program and achieve his goal to become an independent, funded cancer researcher and professor. I am also the Co-Director of the Breast Translational Group, for which Fredrick is a part. The Breast Translational Group is a multidisciplinary group of basic scientists, clinicians, bioinformaticists, statisticians and trainees who collaborate to drive the translational aspects of breast cancer research at Markey. Furthermore, this group develops shared resources to facilitate research. This group recently completed a 347 patient breast cancer tissue array that is fully annotated through the Kentucky Cancer Registry that is available to Markey Cancer Center researchers.

Summary. Development of Fredrick's career through this K01 award will dramatically enhance not only Fredrick's training and career development, but also the development of an important project that helps bridge our understanding of how obesity links not only heart disease (as studied by Dr. Morris) but also breast cancer. I am convinced that awarding Fredrick this K01 will be money well spent toward his career development and uncovering a critical link between obesity, circulating fatty acids and breast cancer aggressiveness.

Co-Sponsor: Andrew N. Lane, Ph.D.

I will serve as a co-mentor for the program of research and training outlined in this application, in particular on the use of stable isotope based tracer methods for discovery and determination of diet-dependent production of bioactive lipid mediators and lipid metabolites that may contribute to obesity associated cancer risk. As there is increasing interest in the links between diet, obesity and various cancers, this research is timely, addresses an important question in the field and fits very well with my own interests in understanding how metabolic reprogramming promotes the development and progression of cancer.

Qualifications. I am an endowed professor of cancer research at the University of Kentucky with a long-standing interest in the development and application of state of the art technologies studying cancer metabolism. My research makes extensive use of stable isotope tracers to follow the fate of individual atoms from source molecules such as glucose and glutamine to intermediates and products of metabolic transformations. This relies heavily on the two primary analytical platforms, namely high resolution NMR and mass spectrometry, and applying the necessary informatics tools for reducing the data into biochemically interpretable forms. I direct an NCI Program Project Grant Systems Biochemistry on non-small cell lung cancer, which uses the stable isotope tracing method in cell culture, animal models and human subjects to learn about the tumor microenvironment. I am also a multiple PI and Director of the Analytical Core of a Commons Funds U24 regional metabolomics center, whose remit is to promote the use of metabolomics in human health research. Our Center focuses on stable isotope tracer technologies. With my colleagues Drs. Teresa Fan and Richard Higashi, I have developed a graduate level Systems Biochemistry course, and we recently edited a Handbook on Metabolomics that focuses on isotope tracing methods.

Training record. I have directly supervised 7 graduate students and a further 15 as a committee member. I

have also supervised 16 undergraduate students 11 postdoctoral fellows and I have mentored two faculty members.

Financial support for the proposed research. Our Center, supports pilot awards for collaborative research in the broad area of stable isotope resolved metabolomics. As his project develops, I would see this as a very feasible avenue to support Fredrick's efforts to apply these technologies to his research. The Center has annual calls for U24 Center pilot projects, of which typically 5 are funded. In addition, the U24 Center partners with the CTSA and Markey cancer center to award pilot grants to UKY faculty. Three were awarded in 2014. These awards provide access to advice in experimenatal design, Core facilities and provide recharge costs for experiments. These grants are awarded with the proviso that the PI attends the annual workshop for hands on training in sample preparation, experimental design, and data analysis. **Evaluation of the candidate.** I joined the faculty at the University of Kentucky in 2013 have interacted with Fredrick through my involvement in collaborative research with his mentor, Andrew Morris which is funded by a Pilot grant from our U24 stable isotope resolved metabolomics center, the Markey Cancer Center and the Center for Clinical and Translational Sciences and concerns the use of stable isotope tracers to investigate how changes in expression of a lipid metabolizing enzyme impacts on monocyte responses to stimulation with oxidized low density lipoprotein. From these interactions it is clear that Fredrick has a solid background in biochemistry and significant experience in biomedical mass spectrometry using multistage instruments. He has recently completed a substantial and significant project using these approaches to investigate isoprenoid phosphate metabolism and is now in a strong position to apply these skills to studies of dietary phospholipid metabolism. I therefore believe that he has the background necessary to benefit from the training I can provide in the application of advanced analytical and computational approaches for metabolite identification and flux analysis. Indeed, given the importance of diet and obesity as risk factors for cancer and their association with poorer clinical outcomes the research that Fredrick proposes represents a very exciting application for these technologies. With expertise in these approaches, Fredrick will acquire a unique set of skills that should position him well for transition to an independent career track.

Training plan. As detailed in his training plan, Fredrick is investigating the possibility that dietary consumption of phospholipids and/or intestinal exposure to bile derived phospholipids as a result of a high fat diet contributes to cancer risk and cancer progression by promoting the formation of bioactive lipids. His proposal focuses on lysophosphatidic acid because, as shown in his preliminary data, this lipid can be formed from dietary precursors and has effects on cancer cells in vitro that are consistent with a role in metastasis. Although Fredrick's preliminary data support the idea that these observations can also be translated to preclinical models it is possible if not likely that lysophosphatidic acid is not the only bioactive metabolite that can be formed from dietary phospholipids. Accordingly the approaches I can provide assistance with will be applied for unbiased identification of diet dependent changes in the plasma lipidome with a focus on using stable isotope tracers and high resolution mass spectrometry for these analyses. This will be the primary area of my involvement in Fredrick's training. I will also be able to advise on preparing proposals for the pilot projects in terms of experimental design and budgeting for the stable isotopes and mass spectrometry approaches.

Nature and extent of supervision and commitment to the candidate. I will interact with Fredrick as often as needed to ensure success of his research and training plan. As his work progresses, Fredrick can participate in our lab meetings and journal clubs in the area of cancer metabolism and in our weekly "omics integration" forum that includes presentations from local and regional speakers. Our resource center for stable isotope metabolomics offers a ten day workshop in conjunction with a daylong symposium. Frederick will attend this workshop which includes lectures and hands on laboratory classes covering the theory and application of stable isotope techniques in combination with advanced analytical approaches (NMR, LC MS, GC MS, direct infusion MS) as well as instruction in data analysis and interpretation.

Relationship of candidate's research to ongoing work in the mentor's laboratory. As explained above, the research Frederick proposes is clearly within the broad scope of my research interests but at the same time does not represent an area that I intend to focus on. Accordingly Fredrick would be free to use any data I help him obtain to assist him with his efforts to develop a personal independent research program.

Plan for transition to independent status. I am committed to the career development of early stage investigators and believe that the University of Kentucky can provide a very strong environment to support Fredrick's transition to independence. The University has benefitted tremendously from support provided by the NIH Centers for Biomedical Research Excellence (COBRE) program. I am currently participating (with Fredrick's mentors and others) in preparation of a proposal for a COBRE in Cancer Metabolism. The primary purpose of this award is to provide support for early career investigators to enable them to compete successfully for independent NIH support. If the research training plan we propose for Fredrick comes to fruition, support from this COBRE grant could be an obvious next step.

Summary. I am highly supportive of Fredrick's application for this award. His focus on diet, obesity and cancer risk fits very well with the research priorities of the Markey Cancer Center and represents an area where both the institution and the NIH have made substantial and significant investments in research infrastructure at the University of Kentucky. Fredrick's background in biochemistry and mass spectrometry that should position him well to benefit from the training I can provide. At the end of this training, he will have acquired a unique set of skills and capabilities that will position him for an effective transition to becoming an independent cancer researcher.

UNIVERSITY OF KENTUCKY

09 October 2014

Re: NIH Mentored Career Development Award for Underrepresented Minorities

Dear Colleagues,

I am writing to detail my willingness to provide advice, assistance, and mentoring in statistics to Fredrick Onono as he works on the research training plan detailed in his application for an NIH Mentored Career Development Award for Underrepresented Minorities. I am a Professor of Statistics and Biostatistics at the University of Kentucky, and I have been involved in cardiovascular research for about 10 years. In fact, I collaborate with Dr Onono's mentor Andrew Morris and his colleague Susan Smyth. The statistical support I provide often entails standard approaches to data analysis, but I also maintain active research programs in mixture modeling and nonparametric regression, upon which I can draw if needed. As noted in my biosketch, I have multiple collaborative publications with Dr. Morris and/or Dr. Smyth including, of particular relevance to Dr Onono's project, a study of the effects of the LPA generating enzyme autotaxin on cell migration that was published in FASEB Journal last year. I am also the primary statistical consultant for the NIH funded Center of Biomedical Research Excellence in Obesity and Cardiovascular Disease which supports an institutional career development program for early stage investigators. Accordingly, I feel well qualified to serve in this role.

Sincerely.

Richard Charnigo, Ph.D.
Professor of Statistics
Professor of Biostatistics
University of Kentucky
RJCharn2@aol.com

MILLER
SCHOOL OF MEDICINE
UNIVERSITY OF MIAMI

October 1st 2014.

Fredrick O. Onono Ph.D.
Cardiovascular Research Center,
741 South Limestone Street
B252 Biomedical Biosciences Research Building,
University of Kentucky
Lexington, Kentucky 40536-0509.

Dear Fredrick,

I will be very happy to collaborate with you in your exciting project “Intestinal Phosphatidylcholine exposure and breast cancer risk”. I will provide you with assistance in your mice diet studies to analyze inflammation, obesity, high fat diets and breast cancer. My laboratory has undertaken a variety of experiments using mouse models, as well as related *ex vivo* and *in vitro* cellular work, to investigate the impact of body weight change and dietary interventions on the development and progression of mammary tumors. We are particularly interested in the role of obesity or of consumption of high fat diets in the absence of obesity, in local breast adipose tissue inflammation and breast cancer progression. We have also performed studies in human minority populations affected by breast cancer, such as African-American and Latinas, as compared with Caucasians, investigating whether ethnicity is associated with breast adipose tissue inflammation and survival.

Particularly relevant to your application, our laboratory has recently completed a preliminary mass spec lipidomic characterization of breast adipose tissue in mice in collaboration with Dr. Sanjoy Bhattacharya from our university. We examined the lipid signature of breast adipose tissue in obese and lean mammary tumor-bearing and normal mice. Our results showed that among the four main phospholipid classes, PC, PE, PI and PS, PCs turned out to be the most frequent phospholipids in the mammary adipose tissue of obese tumor-bearers followed by lean tumor bearers. Our data suggest that PC in the mammary fat is associated with the presence of mammary tumors.

I look forward to this opportunity to continuing our work together integrating our areas of interest and expertise.

With best regards,

Department of Microbiology and Immunology • Leonard M. Miller School of Medicine
Post Office Box 016960 (R-138) •Miami, Florida 33101
Location: 1600 NW 10th Avenue, 3045A RMSB •Miami, Florida 33136
305-243-6655 • Fax: 305-243-5522 • http://chroma.med.miami.edu/micro

Marta

Marta Torroella-Kouri, Ph.D.

Associate Professor of Microbiology and Immunology and Epidemiology and Public Health

Department of Microbiology and Immunology

University of Miami Miller School of Medicine

1600 NW 10th Ave, Rosentiel Medical School Building room 3123A,

Miami, FL, 33136.

Ph: (305) 243-6260

E-mail: mtorroella@med.miami.edu

Fax: (305) 243-5522

UNIVERSITY of MARYLAND
SCHOOL OF MEDICINE

Department of Pharmacology
655 West Baltimore Street, BRB 4-002
Baltimore, MD 21201
410-706-7331 / 410-706-0032 fax

October 2, 2014

Fredrick O. Onono Ph.D.
Cardiovascular Research Center
741 South Limestone Street
B252 Biomedical Biosciences Research Building,
Lexington, Kentucky 40536-0509.

Dear Fredrick,

I am writing to affirm my willingness to serve as a collaborator on your K01 application entitled "*Intestinal Phosphatidylcholine Exposure and Breast Cancer Risk*". I believe that this project will generate fundamental data regarding how certain dietary constituent contributes to breast cancer metastasis in humans, and may have important implications for developing strategies for breast cancer prevention.

Experiments in our research program work together towards a common goal: To translate our mechanistic basic research to the clinic by identifying early biomarkers which are associated with the development of breast cancer that could yield new targets for prevention of this disease. I have over 14+ years of experience in mammary carcinogenesis and studying the mechanisms of breast tumor development, using *in vitro* cell culture and preclinical mouse models of breast cancer. Our lab has also made significant contributions towards understanding how exposures to endogenous and exogenous factors impact the ultimate fate of whether a mammary epithelial cell develops normally or turns cancerous. I will be happy to provide intellectual contributions to the design of the mouse model studies and the analysis of the data and interpretations of the findings to the field of breast cancer.

Wishing you success on your upcoming submission to NIH.

With Best Regards,

Laundette Jones, PhD
Assistant Professor
Department of Pharmacology
University of Maryland School of Medicine

October 2, 2014

Fredrick Onono, Ph.D
Post-Doctoral Trainee
University of Kentucky

Arun Sreekumar
Associate Professor
Department of Molecular & Cellular Biology
Director Metabolomics
Alkek Center for Molecular Discovery
Baylor College of Medicine
One Baylor Plaza
Margaret Alkek Biomedical Building for Research
Mail Stop BCM-130
Houston, TX 77030
Phone: 713-798-3305
Fax: 713-798-8711
E-mail: sreekuma@bcm.edu

Sub: Letter of support for your K01 entitled, “Intestinal Phosphatidylcholine exposure and breast cancer risk.”

Dear Dr. Onono,

I would like to express my strong support for your K01 entitled, “Intestinal Phosphatidylcholine exposure and breast cancer risk.”

I would be delighted to serve as a consultant on your proposed research project as it builds on my own research on metabolic alterations associated with subtypes of breast cancer. I will be able to offer my expertise in metabolomics especially hypothesis-verifying metabolic profiling of metabolites and data analyses. I hope this opportunity to ask questions and discuss protocols will provide you with the opportunity to launch independent investigations of metabolism in cancer.

Your proposal is unique and new, showing strong innovation and I have no doubt it will be a success and will shed much needed light into the mechanisms of obesity associated cancer risk. As an established research scientist and an experienced reviewer of research proposals, I believe this research project is important, feasible, and consistent with the goals of the National Institutes of Health. I am hopeful that this proposal will be a success.

Sincerely,

Arun Sreekumar, Ph.D.
Associate Professor
Department of Molecular & Cellular Biology
Director Metabolomics
Alkek Center for Molecular Discovery
Baylor College of Medicine

Andrew J. Morris
Professor, University of Kentucky College of Medicine
Director, Biomedical Mass Spectrometry Core Laboratory
Research Investigator, Veterans Affairs Medical Center
Lexington, KY

August 21, 2014

Re: Mouse diets with defined phosphatidylcholine composition

Dear Andrew,

I am writing to document my willingness to work with you to provide mouse diets with defined phosphatidylcholine composition. I understand that you are proposing to use these diets as part of studies described in your VA Clinical Sciences Research and Development Merit Award Proposal "Mechanisms linking intestinal phosphatidylcholine exposure to human cardiovascular disease risk". The studies you propose address a pressing public heath need. The focus of your project on association of intestinal phosphatidylcholine exposure and cardiovascular disease risk is of particular interest to me given the position of Harlan Laboratories as a leader in providing rodent diets for preclinical research.

As you know, we have already provided you with multiple constituents of our diets which you have analyzed for phosphatidylcholine composition using HPLC tandem mass spectrometry. Although levels of this lipid were relatively low in the plant, milk and animal fat products included in our diets we were interested to learn that phosphatidylcholine is highly abundant in casein which the major source of protein in atherogenic promoting rodent diets. Data provided by your laboratory has shown that ethanol extraction reduces the phosphatidylcholine composition of our standard casein preparation by ~75%. Accordingly we will work with you to prepare mouse diets with low phosphatidylcholine levels that can be used for your proposed studies to investigate dietary phosphatidylcholine absorption, distribution and metabolism using stable isotope tracers and eventually study the impact of manipulating dietary phosphatidylcholine composition on atherosclerosis in mouse models.

We can work together to continue to measure phosphatidylcholine levels in mouse diets and their constituents to devise methods to generate diets that are low in this lipid. At that point we will then be able to sell you the diets needed for your research at prices that will be determined at the time the diets become available.

Best of luck with your proposal,

Tina Herfel, Ph.D.
Nutritionist, Technical Services
Teklad Diets Research Models and Services

Harlan Laboratories, Inc.
2826 Latham Drive, Dock A
Madison, WI 53713
Tel: (608)230-2213
therfel@harlan.com

DESCRIPTION OF INSTITUTIONAL ENVIRONMENT

Overview: The University of Kentucky is the largest and highest ranked Research University in the state. The common location of our Colleges of Medicine, Public Health, Pharmacy, Agriculture, Engineering and Arts and Sciences on the Campus of the University in Lexington Kentucky provides unique opportunities for collaborative and cross disciplinary research. In the past 5 years, the University has gained a number of awards that mark the best institutions, most notably the selection of the Markey Cancer Center as an NCI designated Cancer Center and receipt of a Clinical and Translational Science Award from the NIH. Recognizing that state of the art instrumentation and technical expertise are essential for a competitive research program, significant effort and resources have been devoted to building the infrastructure necessary to sustain and continue to grow our research enterprise. Accordingly all necessary resources and facilities are available to the candidate to enable success of the proposed research.

Shared resources and facilities: The candidate will have access to the following shared resource facilities that are operated by the vice president for research, the Markey Cancer Center or the College of Medicine and are essential for completion of the research described in his proposal.

Division of laboratory animal resources. This is a fully accredited facility that supports the biomedical research community by providing high quality veterinary services and humane care and treatment of animals used in biomedical research. As a resource for technical expertise, this facility can provide advice and practical assistance as needed with studies using mouse models that are described in the proposal.

Center for Clinical and Translational Sciences. This center was established in 2006 with the goal of accelerating the translation of basic science discoveries to tangible improvements in public health, is supported by NIH/NCTRACS and integrates support for clinical and translational research and career development. Of relevance to this proposal it supports a clinical research laboratory that can be used for human clinical studies.

***Small molecule mass spectrometry core laboratory*.** This facility is supported by the College of Medicine and provides a broad range of mass spectrometry services using a variety of HPLC and GC coupled multistage mass spectrometers. This facility was central in generating preliminary data shown in the proposal.

Resource center for stable isotope resolved metabolomics. This is an NIH "Roadmap" supported center that provides state of the art capabilities in high resolution mass spectrometry and nuclear magnetic resonance spectroscopy to researchers using stable isotope based methods to monitor metabolic pathways in vitro and in vivo including human clinical studies.

Intellectual environment: The intellectual environment at the University of Kentucky is uniquely rich for studies at the interface of obesity, metabolic disease and cancer which are a particular focus of faculty associated with the following centers and programs.

Markey Cancer Center. This NCI designated cancer center houses research programs in Cancer Cell Biology and Signaling, Cancer Prevention and Control, Drug Discovery, Delivery and Translational Therapeutics and Redox Injury and Repair. In addition to sponsoring seminar series and a research day it supports cancer specific focus groups and sustains shared resources that include redox biology and biospecimens that will be of particular relevance to the proposed research.

Barnstable Brown Kentucky Diabetes and Obesity Center. This center functions to integrate diabetes and obesity research at the University with a particular focus on defining mechanisms linking obesity to disease. In addition to sponsoring a seminar series and research day it can also provide access to shared instrumentation for pathology and mouse metabolic phenotyping.

Saha Cardiovascular Research Center. This is an internationally recognized center for cardiovascular disease research with a broad focus on basic, preclinical (animal) and clinical research. The center provides particular expertise in mouse models of hyperlipidemia and cardiovascular disease and houses faculty with a strong interest in understanding how obesity predicts cardiovascular disease risk.

Resource Center for Stable Isotope Resolved Metabolomics. This is an NIH supported center that develops and provides an integrated resource for NMR and mass spectrometry based technologies to monitor metabolic pathways using stable isotope based technologies.

Coursework, journal clubs, seminars and presentations: The Morris/Smyth and O'Connor labs have weekly lab meetings and journal clubs at which the applicant will be able to present his research and participate. Weekly seminars featuring local and invited external speakers in areas of relevance to the research training plan are organized by the Markey Cancer Center, Saha Cardiovascular Research Center and Barnstable Brown Diabetes and Obesity Center. These centers also have annual research days with opportunities for oral and poster presentations. We also have an interest group in metabolomics.

Key faculty: All of the faculty identified in the proposal (Morris, O'Connor, Lane) have active well-funded productive personal research programs in the area of the proposed research with ample time to devote to training and mentoring the candidate.

UK HealthCare
Markey Cancer Center

NCI·CC
A Cancer Center Designated by the National Cancer Institute

University of Kentucky
Markey Cancer Center
800 Rose Street
CC140 Ben F. Roach Building
Lexington, KY 40536-0093
859/323-6556
859/323-2074 Fax

B. Mark Evers, M.D.
Director, Markey Cancer Center
Vice Dean for Academic Affairs, College of Medicine
Professor and Vice-Chair for Research, Department of Surgery
Markey Cancer Foundation Endowed Chair
Physician-in-chief, Oncology Service Line
mark.evers@uky.edu

August 19, 2014

Dear Colleagues,

I am writing in my capacity as Vice Dean for Academic Development and Director of the University Of Kentucky Markey Cancer Center to detail our strong institutional support for Fredrick Onono's application for an NCI Mentored Research Scientist Development Award to Promote Diversity (K01). Dr. Onono is a native of Kenya who holds U.S. permanent residency and is eligible for this award as an African American. I am also excited about the area of research that Fredrick will pursue. Obesity increases the risk of some cancers and results in worse clinical outcomes in cancer patients, particularly women. Recognizing the importance of the interplay between metabolism and cancer progression, the NCI-designated Markey Cancer Center has made significant investments in the recruitment and support of research at the interface of metabolism and cancer. This includes our NIH-supported Resource Center for Stable Isotope Resolved Metabolomics, which houses faculty with interests and expertise in the integrated use of nuclear magnetic resonance spectroscopy, mass spectrometry and informatics. Interactions with these investigators and Fredrick's ongoing involvement in mass spectrometry based studies of lipid metabolism and signaling will provide him with unique training opportunities that will prepare him for a career as an independent investigator. I can also attest to the opportunities for formal and informal training in research and career development provided by the Markey Cancer Center. These include bi-weekly meetings of our Breast Cancer Translational Research Interest group, in which Fredrick can participate and present his findings, weekly cancer research seminars featuring invited speakers from outside our institution, and our annual Cancer Research Day with opportunities for poster and oral research presentations. I am also writing to document our ongoing and unconditional commitment to Fredrick's career development during the proposed transitional phase of his research career. These opportunities will be provided irrespective of the outcome of this K01 application. Fredrick has been supported by an individual Fellowship from the American Heart Association and is now supported by a position on an instructional NIH/NHLBI fellowship award, "Scholars in Translational Cardiovascular Sciences," directed by his co-mentor Susan Smyth. His position in this program will be evaluated annually with continuing support dependent on research progress, publications and availability of funds. We are fully committed to providing Fredrick with the support resources and time needed to develop his career as an independent research scientist. This includes access to the mentoring opportunities outlined in this application, access to research space, access to shared resources and facilities including our exceptional core facilities for mass spectrometry, metabolomics, animal and clinical research.

Fredrick is currently a post-doctoral scholar, so he has essentially all of his time devoted to research and professional development with no teaching or administrative responsibilities. His research space and immediate research needs are accommodated through use of facilities in the Morris and Smyth laboratories, which are part of the Cardiovascular Research Center but adjacent to the laboratories of his co-mentors. While these mentors are clearly highly qualified to provide guidance to Fredrick, the work he proposes is entirely his own and he will be free to pursue it in the longer term as an independent investigator. Accordingly, the work proposed represents an outstanding opportunity for Fredrick to take advantage of substantial research resources while developing a research niche for himself that will be unique at our institution. As Fredrick's career develops, contingent on his research progress and the needs of his developing personal research program, an appointment in a research track faculty position might become appropriate. As detailed by his mentors, the College of Medicine has two active Centers of Biomedical Research Excellence (COBRE) awards. These are institutional awards from NIH/NIGMS that support both pilot grants and larger R01-level research grants to enable the transition of young researchers to compete for independent research funding. Fredrick will be encouraged to obtain pilot support from one of these COBRE grants, for example through our initiative in supporting research at the interface of cancer and metabolic diseases instigated in collaboration with the COBRE-supported Center for Research In Obesity and Cardiovascular Disease. Further support as a principal investigator of a COBRE-supported project has enabled a number of "home grown" investigators to attain independence and R01-supported personal research programs. As his career develops, COBRE support might be a suitable way for Fredrick to continue his transition to independence.

In summary, Fredrick Onono is a promising young scientist who, with appropriate mentoring, has the potential to develop into a successful independent researcher. Fredrick's success is important to our college, and we hope that the support and commitments outlined above in combination with the support provided by this K01 mechanism will enable him to achieve this goal. If I can provide any further assistance, please let me know.

Sincerely,

B. Mark Evers, MD

SPECIFIC AIMS

Diet-dependent obesity is a risk factor for many cancers including postmenopausal breast cancer[1]. Obesity is also associated with more aggressive breast cancer subtypes and poorer clinical outcomes in breast cancer patients[2]. The increasing prevalence of overweight and obese postmenopausal breast cancer patients makes it critical to identify the mechanisms that underlie this link between obesity and breast cancer risk and prognosis. A better understanding of the link between diet, obesity and breast cancer risk might then lead to improvements in healthful nutrition for obese or overweight patients or identify markers for breast cancer risk assessment or targets for pharmacological intervention to mitigate breast cancer risk associated with obesity.

The incidence of obesity or being overweight are both increasing in the United States mainly due to access to food high in calories and the sedentary lifestyle of modern society[3]. The aim of this K01 application is to support mentored training in advanced mass spectrometry/metabolomics and preclinical models that will enable me to become an independent investigator working at the interface of obesity and cancer research. Several hypotheses have been proposed to explain the link between obesity and disease risk. One idea is that consumption of a high fat diet and/or increased synthesis and storage of fats is associated with the production of bioactive lipids or lipid-derived molecules that themselves promote disease[4]. I propose to investigate the possibility that intestinal exposure to the phospholipid phosphatidylcholine (PC) constitutes a link between diet and breast cancer risk by promoting the synthesis of a PC derived bioactive lipid, lysophosphatidic acid (LPA). Breast cancer cells are acutely responsive to LPA which stimulates their migration, growth and survival *in vitro*. Genetic and pharmacological targeting of LPA synthesis and signaling attenuate breast cancer tumor growth and metastasis in mouse models[5]. PC is the major phospholipid in plants and animals and therefore highly abundant in the diet. Bile secreted into the intestine after a fatty meal also contains significant quantities of PC. The major intestinal metabolite of PC, lysoPC (LPC) is an important precursor for synthesis of LPA in the blood. Our preliminary data show that plasma levels of LPA in humans and mice are acutely sensitive to fasting and high fat feeding and that, in mice, dietary PC is directly converted to LPA in the blood. The enzyme responsible for making LPA from LPC, autotaxin (ATX), is a secreted phospholipase D. ATX is strongly expressed in adipose tissue so synthesis of circulating LPA from dietary lipids could be further enhanced in obese individuals. In preliminary studies, pharmacological inhibition of ATX significantly decreases breast cancer metastasis to bone in a mouse model. Together, these observations support our overarching hypothesis that increased intestinal exposure to PC from diet and possibly bile leads to LPA synthesis in blood plasma to promote breast cancer metastasis. We will test this hypothesis in two specific aims:

Aim 1. To determine the contributions of dietary and bile derived PC to diet-dependent post-prandial increases in plasma LPA levels in mice. The major pathway for intestinal absorption of PC involves hydrolysis by secreted phospholipase A_2 (PLA_2) and uptake of the lysophospholipid (LPC) and free fatty acid products of this reaction by enterocytes that package them into lipoprotein particles (primarily chylomicrons but also LDL) for delivery into the circulation via the lymphatic system. We hypothesize that this process contributes to diet dependent post-prandial LPA synthesis in the plasma. We will test this hypothesis in mice using diets with defined PC composition, stable isotope and/or unnatural acyl chain containing metabolic tracers and mass spectrometry and then explore the impact of manipulations of dietary PC content on plasma LPA levels in mice fed normal or high fat diets.

Aim 2. To define interactions between dietary phosphatidylcholine consumption and the secreted lysophospholipase D autotaxin as mediators of metastasis in mouse models. We will use a potent orally bioavailable ATX inhibitor to test the hypothesis that pharmacological inhibition of ATX attenuates breast cancer metastasis in mouse models, and exploit information gained from the studies described in Aim 1 to investigate the impact of dietary manipulations that increase intestinal PC levels to promote synthesis of circulating LPA on this process.

Completion of these studies will provide important new information about how a specific dietary constituent could contribute to breast cancer metastasis in humans. Accordingly the value of the information we expect to generate for efforts to promote healthful nutrition as a protective strategy against breast cancer risk would be wide ranging and the work we propose might also provide an impetus for future translational studies exploring the possibility that pharmacological inhibition of LPA synthesis and signaling could be a viable strategy to mitigate human breast cancer risk, particularly in obese subjects. Whatever the outcome, the project will provide an excellent vehicle for advanced training in research so that I can acquire skills, credibility and recognition and generate exploratory data that will enable me to successfully compete for an NIH R01 award as an independent investigator focusing on understanding mechanisms linking obesity to cancer risk.

IMPACT AND SIGNIFICANCE

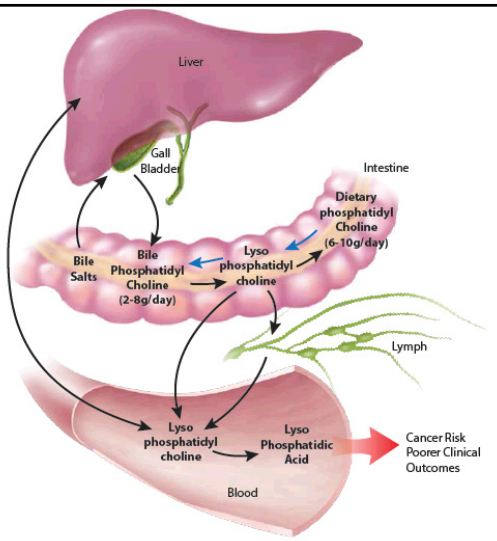

Fig. 1. Mechanisms linking intestinal phosphatidylcholine exposure, lysophosphatidic acid production and cancer risk.

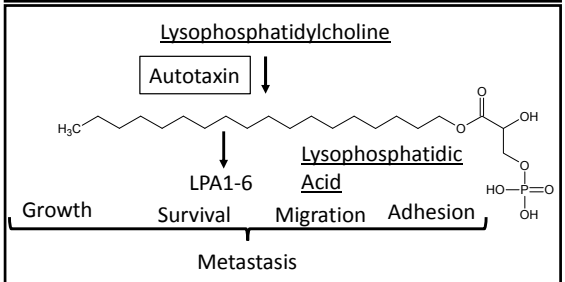

Fig 2. LPA metabolism and Signaling

Obesity is a risk factor for postmenopausal breast cancer. More than 64% of American adult women aged 20 and older are either overweight or obese (BMI of 25 or higher)[3]. Obesity is associated with increased risk for many cancers including postmenopausal breast cancer[1; 6]. Obesity is also associated with poorer clinical outcomes in breast cancer patients[2]. This epidemiological link between obesity and cancer risk is well established but the underlying molecular mechanisms are generally less clear. One of several mechanisms that have been suggested to link obesity to disease risk is that increased consumption, synthesis and storage of lipids and fatty acids drives the formation of biologically active lipids or lipid metabolites that themselves promote disease processes[4]. For example, some recent reports indicate that diet-dependent production of cholesterol metabolites called oxysterols may contribute to growth of estrogen receptor positive breast cancers[7; 8]. We propose to investigate a conceptually similar idea that dietary phospholipids and fats promote synthesis of a bioactive lysophospholipid called lysophosphatidic acid that is implicated as a regulator of breast cancer progression and metastasis and increased in the blood after a high fat meal (**Fig 1**).

Lysophosphatidic acid formed by phospholipase D catalyzed hydrolysis of lysophosphatidylcholine is a mediator of tumor growth and metastasis. Lysophosphatidic acid (LPA) denotes a family of radyl substituted derivatives of glycerol-3-phosphate that function as receptor directed bioactive mediators[9]. Although LPA is an obligatory intracellular intermediate in the *de novo* synthesis of phospholipids and triglycerides, this lipid is also found extracellularly where it can be formed by hydrolysis of lysoglycerophospholipids, notably lysophosphatidylcholine (LPC) by the secreted lysophospholipase D, autotaxin (ATX) encoded by the ENPP2 gene (**Fig.2**)[10]. LPA is present at physiologically relevant levels in blood plasma and other biological fluids, notably malignant effusions associated with several cancers[11]. LPA is a potent signaling molecule that induces cell proliferation, survival, motility, cytoskeletal rearrangement, and differentiation[11; 12]. Most signaling actions of LPA are mediated through a series of six high-affinity cell-surface G protein-coupled receptors designated LPA1-6. LPA1-3 belong to the Endothelial Differentiation Gene subfamily while LPA4-6 form a subfamily closely related to purinergic receptors. Increased expression of LPA1-3 has been observed in human breast cancers[13; 14]. These receptors couple to downstream signaling pathways that include activation of phospholipases C and D, MAP kinases, Rho family GTPases and the AKT protein kinase[15]. LPA and its receptors promote breast cancer metastasis by stimulating tumor cell adhesion, motility, invasion and migration[16; 14; 17-19]. ATX is the major enzyme catalyzing LPA production from LPC[15]. It was originally discovered as a secreted protein that promotes cancer cell motility[20]. ATX is strongly expressed in differentiated adipocytes and both RNA and protein are elevated in visceral and subcutaneous fat in obese mice and humans[21]. Knockout of ATX in mice is embryonic lethal whereas heterozygous ATX-knockout appear normal but have reduced plasma LPA concentrations compared with wild type littermates[22]. ATX is also among the most up-regulated genes in highly metastatic cancers[23]. ATX expression correlates with invasive capacity of breast cancer cell lines *in vitro*[24]. Transgenic overexpression of ATX and LPA1-3 in mammary epithelium induces high frequency invasive metastatic cancer in mice[25]. Small molecule ATX inhibitors decrease primary tumor growth in mouse breast cancer models[26-28] while inhibition of both ATX and LPA receptors has been reported to reduce bone, liver and lung metastasis in preclinical mouse models of breast cancer[29-36].

Dietary phospholipids are precursors for synthesis of circulating lysophosphatidic acid. As explained above, LPA is abundant in plasma and produced by ATX, a secreted phospholipase D. The substrates for this

enzyme are lysophospholipids which are also abundant in plasma, particularly LPC which is present at high (0.1-0.2 mM) levels. Several lines of evidence implicate dietary phosphatidylcholine (PC) as a precursor for plasma LPC. As the major membrane phospholipid in most living organisms PC is abundant in many foods, particularly meats, eggs and grains. Dietary PC consumption in adult humans is therefore in the range of 6-10g/day[37]. PC is also the most abundant phospholipid in bile which is secreted into the intestine through the bile duct. PC and bile salts are essential for solubilization of dietary fats, oils and lipids to enable digestion by secreted phospholipase A_2 (PLA_2) and subsequent absorption by enterocytes[38]. Bile secretion is diet dependent and can result in delivery of as much as 2-8 g/day of PC into the human intestine. Bile salts are efficiently absorbed by intestinal enterocytes and returned to the liver via the hepatic portal vein. The major mechanism for absorption of both dietary and bile derived PC involves hydrolysis by PLA_2 to generate LPC and a free fatty acid both of which are actively assimilated by enterocytes (**Fig.1**)[39]. Studies using radioisotope labeled PC and LPC indicate that, while some further degradation and metabolism (primarily reacylation to generate PC) of enterocyte associated LPC can be detected, the majority of LPC enters the peripheral circulation[40; 38]. Our preliminary studies using mass spectrometry and unnatural odd carbon chain length substituted PC demonstrate an acute effect of fasting and high fat feeding on human and mouse plasma LPA levels that is consistent with a contribution of intestinal PC to production of circulating LPA. While plasma LPA levels could be a primary determinant of breast cancer metastasis, these same pathways deliver dietary lipids and fats to tissues including mammary adipose tissue so it is possible that they also contribute to localized LPA synthesis and signaling in the immediate tumor environment. Measurement of plasma LPA pools might therefore report changes in local LPA levels that are necessarily much harder to monitor experimentally.

Summary. Obesity is a risk factor and poor prognostic factor for breast cancer but the mechanisms involved are not well understood so explaining this link is a critical barrier to progress in the field. We propose to test the concept that synthesis of a bioactive lipid, LPA, from a dietary and intestinal source, PC, links obesity to breast cancer risk and poorer clinical outcomes. Although this idea has not yet been tested directly, elevated plasma lipid levels have been reported in pre- and postmenopausal breast cancer patients and elevated plasma LDL levels have been associated with more aggressive tumor progression in breast cancer patients[41; 42] and imaging mass spectrometry shows that PC is significantly increased in the cancer microenvironments of six types of cancer including breast cancer[43; 44]. Completion of the research might enable new strategies for the diagnosis and clinical management of the disease and could provide a basis for nutrition-based approaches to reduce breast cancer disease risk.

INNOVATION

The research we propose is conceptually and technically innovative because:

1. We propose the novel concept that the bioactive lipid LPA is a link between diet, obesity and cancer risk.
2. We will employ state of the art mass spectrometry-based methods to monitor uptake and metabolism of dietary lipids which will include studies using stable isotope tracers. Although the primary focus of our study is on a particular lipid (LPA), these studies could be extended to encompass unbiased identification of diet dependent lipid metabolites that could contribute to obesity associated cancer risk.
3. We will use innovative mouse models to modulate plasma LPA synthesis by manipulation of dietary phospholipid content.
4. We will employ an orally bioavailable inhibitor of ATX with nM potency that was generated by Pfizer Inc. in research that my mentor, Dr Morris was associated with to evaluate the impact of blocking LPA synthesis and signaling on breast cancer metastasis in mouse models.

APPROACH

Overview. We have two aims that are partially interdependent but can also be pursued in parallel. The first aim is to establish the relationship between dietary fat and phospholipid consumption and plasma LPA levels in mice using acute fasting and feeding studies and dietary manipulations of animals with free access to food. We hope that these experiments will enable strategies for manipulation of circulating LPA levels in mice through manipulations of dietary PC and or fat consumption. The second aim will be to determine the effect of pharmacological inhibition of the LPA generating enzyme ATX on breast cancer metastasis in a mouse model. Information gained from the first aim of the study will be used to determine the effects of dietary manipulations that alter plasma LPA levels on breast cancer metastasis alone or in combination with ATX inhibition.

Statistical analysis. Results will be reported as mean ± SD or as median with interquartile range, if the data are nonparametric. For parametric data, student's *t*-test will be used to compare two groups defined by one factor (genotype). Two-way ANOVA will be used to compare groups defined by two factors, and repeated-measures ANOVA to compare two groups assessed repeatedly over time. SigmaStat version 11 will be used

to analyze the data. Statistical significance will be defined by $P < 0.05$. Data analysis of tumor growth based on volume and bioluminescence imaging will involve descriptive statistics and linear mixed models to compare kinetics of tumor growth among different diet groups. Data analysis for other endpoints such as number of metastases and biomarker assessments from tissue samples will be analyzed using general linear models.

Aim 1. To determine the contribution of dietary and bile derived phosphatidylcholine to post-prandial increases in plasma lysophosphatidic acid levels in mice.

Rationale: The major pathway for intestinal absorption of PC involves hydrolysis by intestinal PLA_2 and uptake of LPC and free fatty acid by enterocytes. Post-prandial increases in plasma levels of LPC and its metabolite LPA could therefore result from increased intestinal absorption of dietary and bile derived PC. Our preliminary data show that mice LPA levels are acutely sensitive to fasting and re-feeding and that dietary PC contribute to the pool of circulating LPA. We hypothesize that dietary sources of plasma LPC contribute to LPA synthesis to promote metastasis in a process that is exacerbated by dietary phospholipid and fat consumption.

Strategy. We will initially investigate the effect of fasting and feeding mice using oral gavage with triglycerides with and without PC containing appropriate stable isotope or unnatural radyl chain substituent tracers and mass spectrometry based analytical approaches to monitor the absorption and metabolism of lipids derived from intestinal PC. These studies will eventually be extended to mice fed *ad libitum* with diets formulated to contain defined levels of PC to set the stage for studies in which the impact of dietary PC and pharmacological inhibition of LPA generation on breast cancer metastasis will be investigated.

Preliminary studies: LPA levels and are acutely sensitive to fasting and re-feeding in humans.

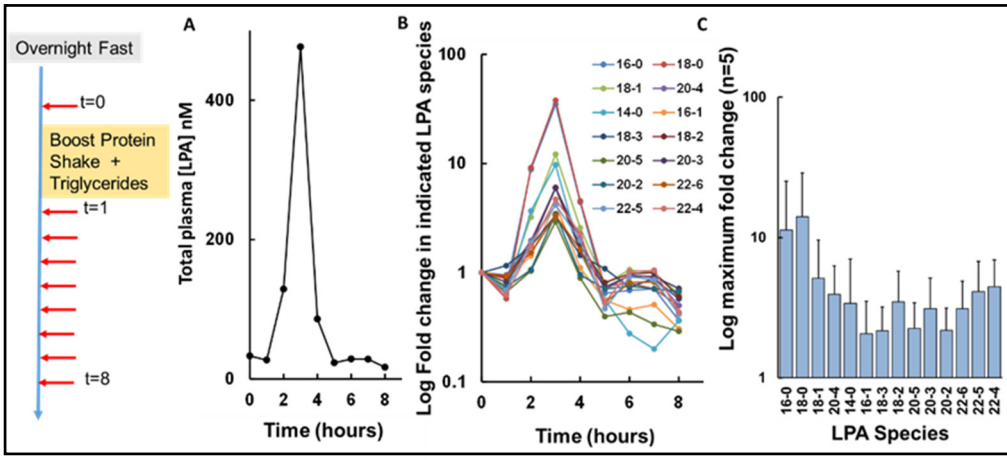

Fig 3. Changes in plasma LPA levels following fasting and re-feeding in obese human volunteers. A. Study design. **B.** Absolute total (nM) and **C.** Fold relative to baseline molecular species changes in plasma LPA in a representative individual after fasting and high fat feeding. **C.** Summarized maximal fold changes in LPA species following high fat feeding (n=5, mean ±SD).

To establish the validity and clinical relevance of our central hypothesis we conducted a preliminary human study to investigate the impact of fasting and feeding a high fat meal on plasma LPA levels in 5 human subjects. After overnight fasting, blood was drawn to obtain baseline LPA levels. Volunteers were given a drink (Boost energy drink supplemented with corn oil and cream to provide ~50% of daily fat intake) and blood drawn every hour. Lipids were extracted from plasma using acidified organic solvents with inclusion of appropriate internal standards to monitor analyte recovery. Using HPLC ESI tandem mass spectrometry based methods we measured plasma levels of 15 abundant LPA species. Quantitation was achieved by stable isotope dilution and by reference to offline calibration curves[45]. Total plasma LPA levels in a representative individual were substantially elevated 3 hours after feeding and declined subsequently. We observed variable and in some cases substantial (>10-fold) increases in multiple LPA molecular species. Of particular interest the two most sensitive plasma LPA species to re-feeding were the saturated 16:0 and 18:0 species. In this preliminary experiment, the high fat meal was replete with triglycerides but did not contain PC. So a reasonable hypothesis is that the increase in LPA is a consequence of an increase in circulating levels of bile-derived LPC serving as an ATX substrate. Indeed, we observed parallel increases in plasma LPC following high fat feeding (not shown). While there was some inter-individual variability in this relatively small study group, increases in post feeding plasma levels of all LPA species were observed in all of the participants (**Fig. 3**). Multiple investigators have explored the possibility that plasma LPA levels may be a biomarker for human disease risk but these studies have been confounded by considerable inter-individual variability in levels and molecular species profile of this class of lipid[46]. Our data demonstrate that a likely reason for this variability is that circulating levels of these lipids are acutely sensitive to diet.

Metabolism of intestinal PC generates plasma LPC and LPA in mice. We determined if mouse plasma LPA levels were also sensitive to fasting and high fat feeding and investigated the possibility that intestinal PC

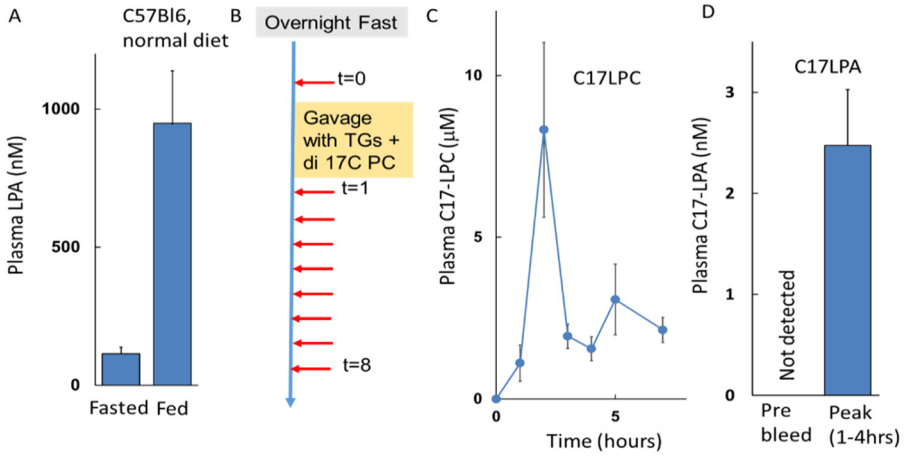

Fig 4. Changes in plasma LPA levels following fasting and re-feeding in mice. A. Total plasma LPA levels in C57Bl6 mice after fasting for 12 hrs. or with free access to normal chow. **B.** Study design. **C.** Increases in plasma C17 LPC levels after gavage with diC17PC/Triglycerides (n=4, mean ±SD). D. Peak increase in plasma C17 LPA after gavage with diC17PC/Triglycerides (n=4, mean ±SD).

could be directly converted to LPC and LPA in plasma. Plasma LPA levels were high in C57Bl6 mice (n=4) that had free access to food (standard chow diet which is rich in PC, see below) at night when measured first thing in the morning but declined by ~80% after 12 hours without access to food **Fig. 4A.** Mice were fasted overnight and gavaged with olive oil, in some cases combined with a synthetic PC containing the unnatural 17 carbon (C17) fatty acid (**Fig. 4B**). We observed time-dependent increases in C17 LPC in plasma from animals that received the diC17 PC (**Fig. 4C**). While no significant levels of C17 LPA were observed in the pre bleed plasma samples, formation of his lipid was observed at times between 1 and 4 hours (**Fig. 4D**). These observations are certainly consistent with our central hypothesis but more work will be needed to establish the relationship between dietary PC and plasma LPA levels. LPA may turnover rapidly in plasma which could explain why plasma C17 LPA are low in comparison to the large accumulation of C17 LPC.

Component	PC (pmol/ 10mg)
Anhydrous milk fat	2.96
Lard	10.32
Tocopherol stripped lard	2.67
Beef tallow	5.85
Coco Butter	2.05
Coconut oil	18.90
Palm oil	0.75
Corn oil	5.99
Tocopherol stripped corn oil	2.17
Casein	591.08
Ethanol extracted Casein	160.88

Table 1. PC in mouse diets

Phosphatidylcholine levels in mouse diets. The PC content of mouse diets is not reported in the literature or provided by the manufacturers. We worked with Harlan Laboratories (Teklad Diets) to quantitate PC in separate components used to formulate these diets. PC was not detected in any carbohydrate preparations and was relatively low in multiple animal and vegetable sources of mouse diet fat but surprisingly present at high levels of ~ 0.1% w/w in whey protein (casein) (**Table 1**). Casein is the primary source of protein in standard laboratory mouse diets from multiple manufacturers including the standard chow diet we use as noted above. We presume this reflects the abundance (~0.2 mM levels) of PC in milk. Diets containing ~20% casein w/w would (assuming an upper limit of 5 g food intake/day) provide 10 mgs of PC. Consumption of a PC rich diet could contribute to diet dependent increases in LPA observed in mice that were fasted in comparison to mice with free access to this diet (**Fig. 4**).

1.1 Testing the effect of fasting and feeding with fats and phospholipids on post prandial LPA levels in mice. Because we propose to use BALB/c mice for our tumor metastasis model in aim 2 we propose to use this strain for our studies. Six week old female BALB/c mice will be purchased and maintained on 12 hour light/dark cycle. The mice will be fed water and standard rodent chow diet (2014 Teklad Harlan Rodent Diet) *ad libitum* before and during the experiment. At 8-10 wk of age, mice will be fasted overnight and randomly assigned to two groups (n=5 per group). One group will be orally gavaged with 10 μl/g body weight of irradiated olive oil. The second group will be gavaged with olive oil combined with mass labeled or unnatural fatty acid containing tracers which will be stable isotope labeled (^{2}H, ^{13}C and ^{15}N) and unnatural odd carbon chain length substituted PC that are available from commercial sources. Blood will be collected from these mice for measurements of plasma lipids at baseline and then at 4 times up to 8 hours after feeding. Plasma will be prepared immediately and stored frozen for measurements of lipids (PC, LPC and LPA), ATX and other markers as needed.

1.2 Lipid extraction and measurement: Lipids and their isotopologs will be extracted from plasma using acidified organic solvents with inclusion of appropriate internal standards to monitor analyte recovery. Using HPLC ESI tandem mass spectrometry based approaches with triple quadrupole or quadrupole time of flight instruments we will measure plasma levels of the abundant PC, LPC and LPA species. Quantitation will be achieved by stable isotope dilution and by reference to offline calibration curves. A particular strength of this

approach is that by using mass spectrometry and metabolic tracers, studies using a common protocol are effectively multiplexed so data on both endogenous and mass labeled lipids can be obtained from a single individual. Given the sensitivity of these analytical methods (limits of quantitation of~1 fmol), the volumes of orally administered lipid preparations and the volumes of plasma we can collect, the work we propose is feasible and should provide definitive information about fasted and post-prandial levels of these lipids. While the volumes of blood and plasma that can be obtained from live mice are very low, the sensitivity of our mass spectrometry methods is adequate to make the measurements we need in as little as 5 microliters of plasma. Assuming a mouse blood volume of 1.5 ml if we draw 50 μl of plasma for analysis we would need to administer as little as ~10 μg of the tracer lipid to enable detection (assuming it is efficiently transported into plasma). Since it is very likely that the tracer lipid will accumulate in other tissues we propose to initially dose mice with 10 mg of the tracer lipid as we did in the preliminary experiments to ensure detection in blood. Experiments will be designed with all necessary controls (blanks, calibration standards, external and internal quality controls) to ensure technical robustness of the data.

1.3 Effect of feeding mice *ad libitum* with diet containing defined PC levels on plasma LPA levels. Female BALB/c mice (n=5/group) will be fed *ad libitum* with water and standard rodent chow or diets formulated to contain defined levels of PC. The overwhelming source of PC in mouse diets is casein but phospholipids can be removed from casein by ethanol extraction (~75% reduction/ single extraction, **Table 1**). We will generate diets with low PC using repeatedly extracted casein which will be supplemented with PC (egg yolk lecithin) to produce diets with defined PC composition. We have an agreement from Harlan laboratories (see letter) to formulate a diet using PC-stripped casein that can be manipulated to achieve defined PC levels. We will use Teklad TD.02018 diet or derivatives of this diet with defined PC composition (low PC or high PC). Blood will be drawn from each mouse in the morning and evening once a week for 5 weeks. Plasma will be prepared immediately and stored frozen for measurements of lipids (PC, LPC and LPA) and ATX. Lipid measurement and quantitation will be performed as described above.

1.4 Effect of LDL receptor deficiency induced hyperlipidemia on plasma LPA levels in mice. Although based on our preliminary studies in C57Bl6 mice we expect to observe effects of fasting, feeding and dietary PC on plasma LPA levels in wild type BALB/c mice we will also explore the possibility that these effects will be exacerbated in settings where plasma liproprotein clearance is attenuated. We will do this using a recently described approach in which mice will be given recombinant adeno-associated viral vectors which direct expression of a gain of function alleles of protein convertase substilisin/kexin type 9 (PCSK9) to induce prolonged hyperlipidemia by inducing LDL receptor degradation[47]. These recombinant viruses (constructed using the transfer vector rAAV8-D377Y-murine PCSK9) will be purchased from the Viral Vector Core at the University of Pennsylvania. Animals will be administered with a single ~0.1 ml tail vein injection of ~5.9 x 10^{11} viral genomes in saline. Elevations in plasma LDL levels are achieved in these animals ~21 days post administration of the virus. We will verify induction of hyperlipidemia by measurement of plasma cholesterol and triglyceride levels. We will investigate the impact of fasting and re-feeding with diets containing fats, PC and metabolic tracers on plasma LPA levels in mice with induced hyperlipidemia. We will also feed these mice *ad libitum* with standard chow or diets formulated to contain defined levels of PC and measure levels of lipids in their plasma.

1.5 To investigate the contribution of bile derived PC to plasma LPC and LPA levels. Although the sterol and bile acid composition of human and mouse bile has been examined extensively, despite the high abundance of PC in bile there have been surprisingly few detailed studies of the PC molecular species composition of bile. In agreement with one report, our unpublished preliminary data indicate that PC species with saturated fatty acid chains are more abundant in bile than in plasma. If these PC species are a source of LPC for LPA generation then it would be reasonable to speculate that their levels would be increased by oral administration of triglycerides alone to mice and humans. We will profile PC species in mouse bile. We anticipate that feeding a high fat meal will promote secretion and absorption of bile derived PC that can be detected in the blood. Increases in 16:0 and 18:0 LPA in humans following high fat feeding (**Fig. 3**) are consistent with this possibility.

1.6 Effect of diet on plasma ATX levels. Because the LPA generating enzyme ATX may be increased in the plasma of hyperlidemic mice we will extend our study to compare fasted and post-prandial levels of ATX in hyperlipidemic and wild type mice. Plasma ATX levels will be determined by western blotting and measurements of plasma lysoPLD activity or by proteomic assays for ATX that quantitate ATX-derived peptides from a tryptic digest of plasma proteins using mass labeled peptides as internal standards.

Expected outcomes. This study will provide important new information about the sources and levels of LPC and LPA in the circulation. We will determine how levels of these lipids are impacted by diet and hyperlipidemia. In particular, by using mass labeled tracers we will determine if LPC and LPA are generated directly from dietary lipids. Based on our preliminary data and older studies of phospholipid absorption in humans using radioisotope tracers we anticipate observing a strong association between dietary PC consumption and circulating levels of LPC and LPA that can be explained, at least in part, by the actions of ATX on LPC delivered to the plasma from intestinal sources (either bile or diet). Although not within the scope of this proposal, we are concurrently exploring how LPC and LPA are distributed among plasma lipoprotein components and suspect that LDL is an important source of both lipids. With this in mind it will be particularly interesting to see if down regulation of the LDL receptor increases plasma levels of LPA in freely fed mice and/or results in larger post prandial increases in plasma LPA levels. An important goal of this aim will be to determine if manipulation of dietary PC levels alone or in combination with functional deficiency of the LDL receptor impacts on plasma LPA levels. This information will be important for the studies proposed in the second aim.

Problems and alternative approaches. This aim is dependent on established robust analytical methods with adequate sensitivity. If interference from endogenous metabolites confounds our measurements we have access to higher resolution instruments in our NIH-supported Stable Isotope Resolved Metabolomics resource center directed by Dr. Teresa Fan and Dr. Andrew Lane (University of Kentucky Markey Cancer Center). Although we consider it unlikely, it is possible that our study will fail to demonstrate a link between diet and plasma LPC and LPA levels or that, if we do observe this link, the pathway we favor (direct absorption of intestinal LPC) is not the mechanism involved. As an alternative approach, we could use the high resolution mass spectrometry capabilities offered by this resource to profile lipid metabolites that have incorporated components of PC from diet by monitoring incorporation of mass labeled atoms from dietary PC into isotopologs of these metabolites. We could then determine the levels of these metabolites, how they are regulated and their potential impact on disease progression. Another potentially confounding issue is that intravenously administered LPA is rapidly eliminated from the circulation[45] which might indicate rapid turnover of circulating LPA. The studies proposed here could be extended to include “pulse-chase” methodologies using stable isotope tracers to determine the half-life of endogenously generated circulating LPA.

Aim 2. To define interactions between dietary phosphatidylcholine consumption and the secreted lysophospholipase D autotaxin as mediators of metastasis in mouse models.

Rationale. Pharmacological antagonism or genetic deficiency of certain LPA responsive G-protein coupled receptors ameliorates breast cancer metastasis in mouse models. Similarly, up regulation of LPA signaling by LPA administration or genetic deficiency of the LPA inactivating enzyme LPP3 accelerates metastasis in these models. ATX is the major (probably sole) source of circulating LPA and we hypothesize that dietary PC is an important precursor for LPC substrates for generation of pro-metastatic LPA by ATX.

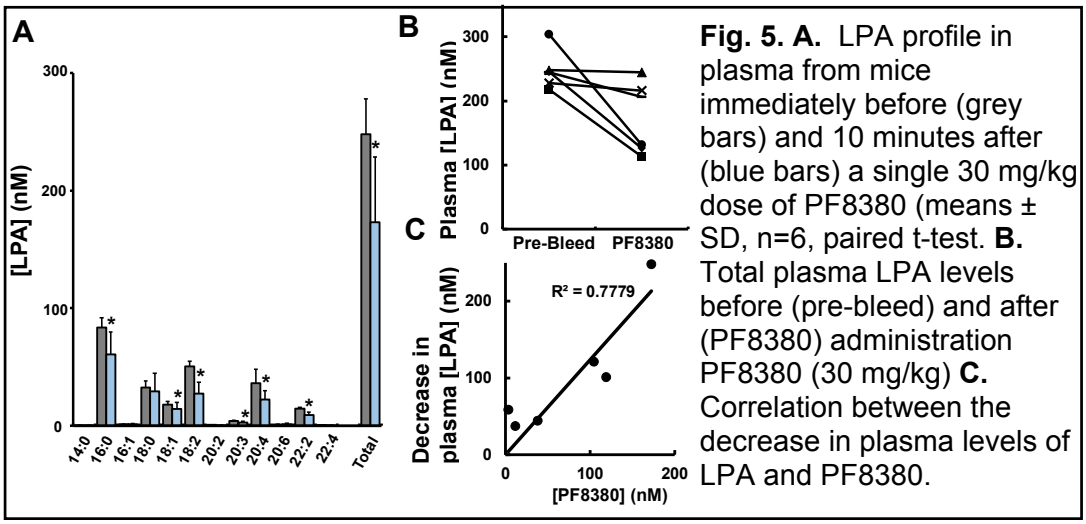

Fig. 5. A. LPA profile in plasma from mice immediately before (grey bars) and 10 minutes after (blue bars) a single 30 mg/kg dose of PF8380 (means ± SD, n=6, paired t-test. **B.** Total plasma LPA levels before (pre-bleed) and after (PF8380) administration PF8380 (30 mg/kg) **C.** Correlation between the decrease in plasma levels of LPA and PF8380.

Strategy. We will use mouse models of breast cancer and information gained from studies described in the first aim to determine the impact of dietary PC alone or possibly in combination with hyperlipidemia induced by LDL receptor deficiency on murine tumor growth and metastasis. We will determine if pharmacological inhibition of the LPA generating enzyme ATX also attenuates tumor growth and metastasis in these models and determine if ATX inhibition further accentuates the postulated protective effect of PC-deficient diets or the exacerbating effect of LDL receptor deficiency induced hyperlipidemia on tumor growth and metastasis.

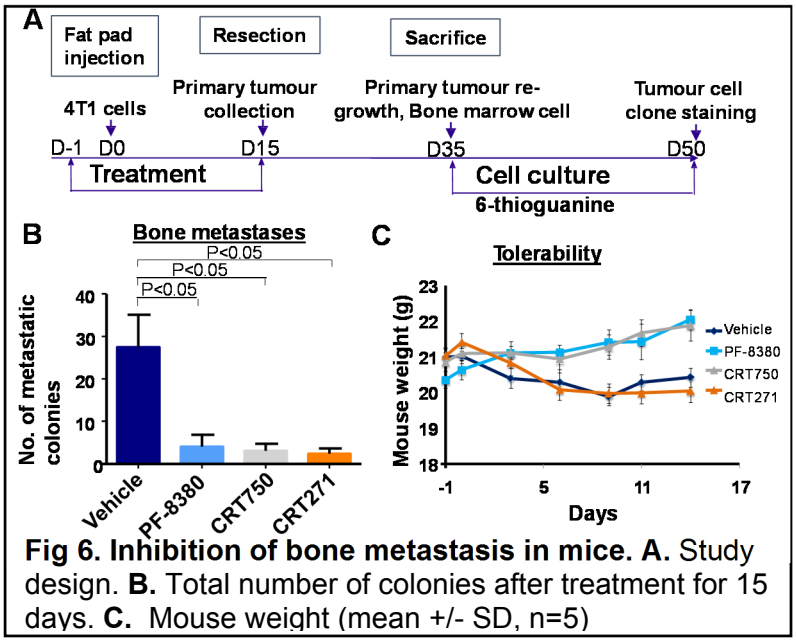

Fig 6. Inhibition of bone metastasis in mice. A. Study design. **B.** Total number of colonies after treatment for 15 days. **C.** Mouse weight (mean +/- SD, n=5)

Preliminary studies: Inhibition of autotaxin decreases metastasis in breast cancer mouse model. My mentor, Dr Morris, has been engaged in collaborative studies to identify and characterize small molecule inhibitors of ATX[48; 49]. Some of this work involves researchers at Cancer Research Technology in the UK[50]. As part of these studies, Dr Morris compared the potency and efficacy with which an orally bioavailable ATX inhibitor with nM potency termed PF-8380 that was identified by Pfizer Inc.[51] decreased plasma LPA levels in mice (**Fig 6**). Having established that all three compounds effectively inhibited ATX in vivo these studies were extended to explore the effect of ATX inhibition on breast cancer metastasis using the 4T1 cell syngeneic mouse model with orthotopically injected tumor cells[52] **Fig. 6A**. This model has the advantage of bypassing the immunologic host-verses-graft reaction and allows investigating tumor progression in an environment of intact immune system. Ten-week old female BALB/c mice (n=5) were treated once daily from day one with vehicle control or 100 mg/kg of PF-8380 or two proprietary compounds (CRT750 or CRT271) that are also nM potency ATX inhibitors after injection with 4T1 cells (20,000 cells). Primary tumors were measured twice weekly until resection, which occurred on day 15. Mice were sacrificed at day 35, bone marrow cells harvested by flushing from both hind limbs of each animal and cultured in media supplemented with 6-thioguanine (10µg/mL). Cells from each mouse were seeded on one well of a P6 culture plate and incubated for 15 days. Out of all the cells flushed from the bone marrow, only 4T1 cells should be resistant to 6-thioguanine treatment and proliferate to form colonies which we stained and counted. Low numbers of colonies were detected in cultures from mice treated with ATX inhibitors indicating significant decrease in bone metastases ($p<0.05$) (**Fig. 6B)**. However weights of the animals were unaltered by PF8380 treatment suggesting that this compound is well tolerated at 100 mg/kg dosing (**Fig. 6C)**.

2.1 Does dietary PC contribute to tumor growth and metastasis in mice? We will use syngeneic mouse model with orthotopically injected tumor cells to study the contribution of intestinal PC to tumor progression. Female BALB/c mice 8 – 10 weeks old will be maintained at standard 12-h light-dark cycle. To evaluate the contribution of dietary PC to tumor growth and metastasis, mice (n=10 per group) will be given free access to water and either a standard rodent chow or a PC depleted diet *ad libitum*. Spontaneous metastasis will be induced, after anesthesia, by orthotopically injecting 20,000 double labeled 4T1-luc2-GFP cells into the mammary fat pads. 4T1-luc2-GFP cells are particularly useful since they are suitable for monitoring tumor development *in vivo* with luciferase after injection of luciferin intraperitoneally and imaging using noninvasive bioluminescence[53]. GFP expression in tumors can also be observed *ex vivo* by fluorescence microscopy. After 4-5 days, the rate of primary tumor growth will be measured twice weekly using calipers to determine tumor volumes, until resection. Mice will be anesthetized with isoflurane followed by collection of blood into tubes containing anticoagulants and EDTA. Plasma will be prepared from blood by centrifugation, and frozen for analysis of plasma lipids, cytokines and other inflammatory markers. Metastatic outgrowth will be monitored by luminescent imaging. After 5-6 weeks, the animals will be euthanized and bone marrow, all lymph nodes, lungs, livers, and any other organ suspected of harboring a metastasis collected for histological analysis. Metastatic outgrowth will also be evaluated in bone marrow cells by culturing the cells in medium containing 6-thioguanine followed by staining.

2.2 Does hyperlipidemia affect dietary PC contribution to tumor development? LDL receptor deficiency will be induced as described in Aim 1.3. Animals with LDL receptor deficiency exhibit hyperlipidemia because they can't clear plasma lipoproteins. After confirmation of hyperlipidemia, mice will be assigned (n = 10 per group) to receive a standard chow or a PC depleted diet. Tumor growth will be initiated as described above. Measurements of lipids, cytokines and inflammatory markers will be done. Tumor growth and metastasis will be evaluated in these mice using the methods outlined above.

2.3 Does pharmacological inhibition of ATX alter tumor growth and metastasis in mice? PF8380 is available to us from CRT[50]. This compound is soluble in water at 0.15 mg/ml. A 20g mouse drinks ~5ml of water/day so it may be possible to administer the drug in drinking water. However, we have concerns about stability of the compound in solution so dosing by "voluntary oral administration" will proceed empirically. As an alternative, the drug can be administered by oral gavage which was done in the original report by dosing ~0.1 ml of a 0.5 mg/ml solution in 0.5 % methyl cellulose & 0.025% tween-20 in sterile water[51]. We can monitor plasma PF8380 and LPA levels and plasma ATX activity to determine the efficiency of dosing. In rats a single oral dose of PF8380 caused inhibition of plasma ATX that was persistent for 24 hours so it is possible that relatively infrequent dosing may be sufficient to achieve sustained ATX inhibition in these studies. Mice will be placed on diets as described above and the effect of ATX inhibition by PF8380 on tumor growth and metastasis monitored as above. During the course of tumor development and drug treatment, mice will be monitored for signs of toxicity daily, and body weights will be determined at least three times per week.

2.4 Does postprandial plasma LPA promote breast cancer cell migration and motility *in vitro*? Our preliminary data suggest that plasma LPA levels are sensitive to fasting and high fat feeding and we expect to observe that plasma LPA levels in mice are sensitive to dietary PC consumption and perhaps further enhanced by LDL receptor deficiency induced hyperlipidemia. However, because it is likely that the plasma pool of LPA is heterogenous (for example LPA may be associated with serum albumin or with different plasma lipoprotein fractions) which is an issue that, although beyond the scope of this proposal, we are interested in. LPA induces cell migration in breast cancer cell lines[48]. As a preliminary approach to determining if the increases and/or changes in plasma distribution of LPA result in difference in biological activity we will examine the potency with which plasma from mice subjected to our various experimental manipulations promotes LPA-dependent breast cancer cell migration and motility. Serum starved 4T1 cells will be plated into the upper wells of a membrane chamber and incubated at 37°C for 30 min to allow attachment to the filter membrane. Blood plasma will be added to the lower chamber and after incubation for 24 hours, nonmigrated cells will be removed from the membrane while migrated cells will be fixed and stained to quantitate for migration. Depending on the outcome of these studies we may also use a time-lapse video microscopy based single-cell motility assay and cell trajectory analysis according to previously described methods to monitor the speed and directionality of cell migration. Briefly, cells plated at low densities on glass-bottom dishes coated with fibronectin will be cultured for 3 hours. Blood plasma will be added and cell motility measured with a Nikon Biostation IMQ. Movement of individual cells will be analyzed using NIS-Element AR software. Images and movies of migrating cells will also be acquired. Plasma contains other non-lipid factors that could promote cell migration. To determine if the migration and invasion observed is LPA dependent, we will repeat these migration and motility assays in the presence or absence of Ki16425, a commercially available LPA receptor inhibitor that inhibits LPA receptor dependent breast cancer cell migration[33].

Expected outcomes. We anticipate that mice fed PC rich diets will have higher amounts of circulating LPA indicating a substantial flux of bioactive lipids from diet. We also anticipate higher levels of systemic LPA in hyperlipidemic (LDL receptor deficient) mice. We expect that increases in LPA levels will increase the rate of primary tumor growth and enhance tumor metastasis. Based on our previous experiences, published data and patent literature from the Pfizer group that developed PF8380, we anticipate that the proposed inhibition of ATX will be well tolerated in mice. We expect that PF8380 will be effective in producing a sustained ATX inhibition during the duration of our proposed study. The ATX inhibition studies we have proposed will produce definitive data documenting the impact of a specific diet and ATX inhibition on tumor metastasis. Data on LPA production will be a mechanistic link for diet and cancer metastasis.

Problems and alternative approaches. Our preliminary assessments of PF8380 indicate that it is well tolerated in mice. However, if prolonged dosing of PF8380 is not well tolerated we will consider other potent orally bioavailable ATX inhibitors (e.g. ONO-8430506 that has recently been reported in the literature[35]). Should these pharmacological experiments prove problematic my mentor has ongoing breeding program for ATX hypomorphic mice (ENPP2-/+) on a C57Bl/6 background. Published reports indicate that ENPP2-/+ mice are viable and fertile but hypomorphic for ATX. These mice also have reduced circulating levels of LPA. While not suitable for the syngenic breast cancer model described above these animals could be crossed with mice expressing MMTV LTR driven transgenes to determine the impact of genetic ATX deficiency on mammary tumor growth and metastasis. This model would also enable studies of pharmacological inhibition of ATX on tumor growth and metastasis in ENPP2-/+ mice which may offer a more sensitive system to detect effects of ATX inhibition on this process and could also be used to model different subtypes of breast cancer which is not readily possible using the 4T1 cell transplantable tumor model.

Vertebrate Animals.

1. Description of the proposed use of animals: The studies proposed in in this application will be carried out in female BALB/c mice aged 8 – 12 weeks. We intend to use ~ 300 mice. Mice will be used to define a role for dietary and bile derived phosphatidylcholine in regulating circulating lysophosphatidic acid levels. These experiments will involve overnight fasting and oral gavage with a mixture of triglycerides and phospholipids. Blood will be drawn at baseline and subsequent time points for analysis of plasma phospholipids. Mice will also be used to determine the role of dietary phosphatidylcholine and the lysophosphatidic acid generating enzyme autotaxin (ATX) in etiology of breast cancer. These latter experiments will involve administration of a potent orally available inhibitor of the enzymatic activity of ATX. We will also induce hyperlipidemic in some of the mice used for the studies. This will be done using a recently described method that bypasses the need for breeding mice genetically deficient for lipoprotein receptor. It involves injecting mice with recombinant adeno-associated viral vectors which direct expression of a gain of function alleles of protein convertase substilisin/kexin type 9 (PCSK9) to induce prolonged hyperlipidemia by inducing LDL receptor degradation. Mice treated with the ATX inhibitor will be monitored daily for signs of morbidity including lethargy, jaundice, stress, pain and death. All mice used for the study of metastatic disease will be euthanized and organs of interest collected for histological analysis when one group shows signs of morbidity and high tumor burden by imaging.

All mice will be housed in the AAALAC-approved animals resource center at the University of Kentucky. Animals will be allowed at least 1 week to adapt to the *local vivarium* before any experiments will take place. All appropriate animal care (food, water, bedding, cage cleaning etc.) will be performed by the trained staff of the Animal Resource center.

2. Animal justification. Mice were chosen because they are valuable for testing and studying experimental therapeutics that might lead to drugs to treat human diseases. The physiological processes we hope to investigate are similar in mice and humans but doing these studies to monitor absorption and metabolism of dietary phosphatidylcholine can easily be manipulated in mice. The well-defined genetic background of mice also allows us to minimize variation due to the individual. The syngeneic BALB/c mouse model we will use to study the contribution of intestinal PC to tumor progression is well established as an animal model for breast cancer research. This model has the advantage of bypassing the immunologic host-verses-graft reaction and allows investigating tumor progression in an environment of intact immune system. The overall goal of this research is to establish a causative role for dietary and bile derived phosphatidylcholine in the progression of breast cancer which we hypothesize involves conversion to the bioactive lipid mediator lysophosphatidic acid through the actions of ATX. Direct testing of this hypothesis requires the use of a preclinical model in which longer term manipulations of diet and ATX activity can be conducted to explore their effect on cancer growth and metastasis. The numbers of mice used for each experiment will be the minimum required to achieve sufficient statistical power and on published studies using the same models by other investigators. Unless otherwise noted, our studies are powered to detect a minimum of a 20% change in the measured parameter between control and experimental data (i.e 80% power).

Lipid absorption studies

The purpose of these experiments will be to determine if effects of fasting and re-feeding on plasma levels of lipids of interest we observe in human clinical studies also happen in mice as a necessary prelude to using mice as a model to investigate the impact of manipulations of dietary phospholipids and ATX activity on breast cancer. We will use a maximum of 50 mice

Effects of diet (phosphatidylcholine composition of diet)

We will formulate variants of the standard or high fat mouse diets containing defined phosphatidylcholine (PC) levels (ie. PC deficient or PC rich diets). Mice will be fed these diets, with or without administration of ATX inhibitors and effects on plasma levels of interest determined in comparison to mice fed the standard rodent chow or high fat diet. Maximum estimated total = 100

Breast cancer metastasis studies.

The purpose of these studies will be to evaluate the contribution of dietary phosphatidylcholine to diet-dependent development of breast cancer in standard mouse models. These studies will be extended to include experiments to determine effects of inhibition of autotaxin. We anticipate needing a maximum of 150 mice.

3. Procedures and Facilities to Assure Adequate Maintenance and Veterinary Care of Animals:

University of Kentucky's Division of Laboratory Animal Resources (DLAR) encompasses a total of 88,446 square feet in multiple buildings across the campus. The DLAR has maintained AAALAC (Association for the Assessment and Accreditation of Laboratory and Care International) accreditation since 1966. Animal care is provided by 30 animal technicians, three animal care supervisors, a facilities and operations manager, three clinical veterinary technicians, two surgery technicians, a surgery supervisor, and four research analysts. Pathology, clinical laboratory, surgical, and medical services are under the direction of five veterinarians, two are board certified laboratory animal specialists, two have advanced post-graduate degrees and one is a veterinary pathologist experienced in laboratory animal medicine. In addition, DLAR has a full-time training supervisor who trains both research staff and DLAR staff in animal care and use procedures.
DLAR veterinarians and veterinary technicians are available for the management of medical and/or research related problems daily, as well as evenings and holidays. In addition, the DLAR emphasizes preventative medicine that is exemplified by a rodent screening serology program and health monitoring programs for other species. All protocols involving animals are performed under the supervision of the University of Kentucky Institutional Animal Care and Use Committee (IACUC). Animals are housed according to regulations stipulated by the Animal Welfare Act (AWA) and the Guide for the Care and Use of Laboratory Animals. Enrichment programs have been developed to promote species-specific behaviors and appropriate interactions for social species, as appropriate for the research using these species.

The DLAR Experimental Surgery facility has two prep rooms, two surgeon scrub rooms, three operating rooms, one operating room with fluoroscopy and two recovery rooms. The DLAR Experimental Surgery facility has four fully equipped surgical suites that can be utilized for survival surgery. Two suites are maintained in a sterile condition for survival surgery.

All investigators and their personnel working with animals complete DLAR training requirements before they are approved to work with animals to assure that animals are treated and cared for in a humane way. This training is documented and completion is required for obtaining IACUC approval. Animals are housed according to regulations stipulated by the Animal Welfare Act (AWA) and the Guide for the Care and Use of Laboratory Animals to promote the conduct of scientifically sound research.

4. Procedures to minimize discomfort, distress, pain, and injury.
Where necessary, all procedures are performed under anesthesia (either isoflurane or pentobarbital). Animals are observed for at least 30 min to one hour to insure that they show no ill effects of any potentially harmful procedure. Animals kept postoperatively will be given clean bedding, adequate food and water, and warm quarters. All procedures on animals will be performed by properly qualified personnel. No pain-producing procedures are planned using conscious mice. No procedures will be performed on animals without prior approval of the Institutional Animal Care and Use Committee. Questions regarding care of potential discomfort to mice will be directed to Harold F. Stills, Jr., DVM, DACLAM, Director of the Division of Laboratory Animal Resources.

5. Methods of euthanasia.
Euthanasia will be conducted using the CO_2 chamber and then cervical dislocation, and is consistent with the 2000 Report of the American Veterinary Medical Association (AVMA) panel.