



LAWRENCE
LIVERMORE
NATIONAL
LABORATORY

Annual Progress Report for the Resource for the Development of Biomedical Accelerator Mass Spectrometry

K. Kulp

April 3, 2013

Disclaimer

This document was prepared as an account of work sponsored by an agency of the United States government. Neither the United States government nor Lawrence Livermore National Security, LLC, nor any of their employees makes any warranty, expressed or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States government or Lawrence Livermore National Security, LLC. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States government or Lawrence Livermore National Security, LLC, and shall not be used for advertising or product endorsement purposes.

This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.



Lawrence Livermore National Laboratory

Kenneth W. Turteltaub, Ph.D.
Physical and Life Sciences Directorate
L-452
7000 East Avenue
Livermore CA 94550
Phone: 925-423-8152
Turteltaub2@llnl.gov

Division of Extramural Activities Support, OER
National Institutes of Health
6705 Rockledge Dr, Room 2207, MSC 7987
Bethesda, MD 20817
(301) 594-6584

March 27, 2013

Dear Extramural Activities Support,

Please find enclosed the annual progress report for grant 8 P41GMI03483-14,
"Resource for the Development of Biomedical Accelerator Mass Spectrometry".

Sincerely,

Kenneth W. Turteltaub, Ph.D.
Principal Investigator



Department of Health and Human Services
Public Health Services

Review Group ZRG1BCMBK40	Type 5	Activity P41	Grant Number P41GM10348
-----------------------------	-----------	-----------------	----------------------------

Grant Progress Report

Total Project Period	
From: 9/01/2000	Through: 5/31/2014
Requested Budget Period	
From: 6/01/2013	Through: 5/31/2014

1. TITLE OF PROJECT
Resource for the Development of Biomedical Accelerator Mass Spectrometry (AMS)

2a. PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR
(Name and address, street, city, state, zip code)
Turteltaub, Kenneth W.
Lawrence Livermore National Lab
7000 East Ave, L-452
Livermore CA, 94550

2b. E-MAIL ADDRESS
turteltaub2@llnl.gov

2c. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Biology and Biotechnology Division

2d. MAJOR SUBDIVISION
Physical and Life Sciences Directorate

2e. Tel: 925-423-8152 Fax: 925-422-2282

3a. APPLICANT ORGANIZATION
(Name and address, street, city, state, zip code)
Lawrence Livermore National Laboratory
7000 East Ave
Livermore CA, 94550

3b. Tel: Fax:

3c. DUNS: 827171463

4. ENTITY IDENTIFICATION NUMBER
20-5624386

6. HUMAN SUBJECTS No Yes

6a. Research Exempt <input checked="" type="checkbox"/> No <input type="checkbox"/> Yes	If Exempt ("Yes" in 6a): Exemption No.	If Not Exempt ("No" in 6a): IRB approval date 11/7/2012
--	---	---

5. NAME, TITLE AND ADDRESS OF ADMINISTRATIVE OFFICIAL
Julia Fone - Interagency Business Office
Lawrence Livermore National Lab
7000 East Ave, L-180, Livermore, CA 94550-9234

6b. Federal Wide Assurance No. 00004274

6c. NIH-Defined Phase III Clinical Trial No Yes

Tel: 925-423-4730 Fax: 925-423-4220

E-MAIL: fone1@llnl.gov

7. VERTEBRATE ANIMALS No Yes

7a. If "Yes," IACUC approval Date

7b. Animal Welfare Assurance No. A3184-01

10. PROJECT/PERFORMANCE SITE(S)
Organizational Name: Lawrence Livermore National Laboratory
DUNS: 827171463

8. COSTS REQUESTED FOR NEXT BUDGET PERIOD

8a. DIRECT \$1,024,162 8b. TOTAL \$1,714,245

Street 1: 7000 East Ave

Street 2:

9. INVENTIONS AND PATENTS No Yes

If "Yes," Previously Reported Not Previously Reported

City: Livermore County: Alameda

State: CA Province:

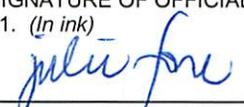
Country: US Zip/Postal Code: 94550-9234

Congressional Districts: 15

11. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (Item 13)
Julia Fone, Interagency Mission Business Office (IBO)

TEL: 925-423-4730 FAX: 925-423-4220 E-MAIL: fone1@llnl.gov

12. Corrections to Page 1 Face Page

13. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.	SIGNATURE OF OFFICIAL NAMED IN 11. (In ink) 	DATE 3/20/13
--	---	-----------------

DETAILED BUDGET FOR NEXT BUDGET PERIOD – DIRECT COSTS ONLY	FROM 6/1/2013	THROUGH 5/31/2014	GRANT NUMBER 8 P41 GMI03483-14
---	-------------------------	-----------------------------	--

List PERSONNEL (*Applicant organization only*)
 Use Cal, Acad, or Summer to Enter Months Devoted to Project
 Enter Dollar Amounts Requested (*omit cents*) for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Turteltaub, Kenneth W	PD/PI	3.6			85,016	51,860	136,875
Bench, Graham	Proj Investig	2.4			38,520	23,497	62,017
Buchholz, Bruce	Proj Investig	2.4			25,064	15,289	40,353
Malfatti, Michael	Proj Investig	1.2			11,338	6,916	16,254
Ognibene, Ted	Proj Investig	2.4			25,064	15,289	40,353
Kulp, Kristen	Proj Investig	2.4			27,665	16,875	44,540
Stewart, Benjamin	Proj Investig	8.4			71,539	43,639	115,179
Haack, Kurt	Technician	12.			78,439	47,848	126,287
→ SUBTOTALS					362,645	221,213	583,858

CONSULTANT COSTS

EQUIPMENT (*Itemize*)

SUPPLIES (*Itemize by category*)

Radiochemicals (\$6,120)	Animal Purchases (\$3,060)
Electrophoresis< HPLC and CE supplies (\$14,535)	Misc Expenditures(\$6,120)
Sample Spect. Interface Supplies (\$9,180)	Graphite prep supplies(\$37,485)

76,500

TRAVEL

Domestic Travel

11,924

INPATIENT CARE COSTS

OUTPATIENT CARE COSTS

ALTERATIONS AND RENOVATIONS (*Itemize by category*)

OTHER EXPENSES (*Itemize by category*)

Publications and Information Management (\$3,060)	BioAMS Facility (\$284,580)
Equip maint, service contacts and other expenses (\$51,000)	

335,580

SUBTOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD

\$ 1,024,162

CONSORTIUM/CONTRACTUAL COSTS

DIRECT COSTS

CONSORTIUM/CONTRACTUAL COSTS

FACILITIES AND ADMINISTRATIVE COSTS

TOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD (*Item 8a, Face Page*)

\$ 1,024,162

DETAILED BUDGET FOR NEXT BUDGET PERIOD – DIRECT COSTS ONLY	FROM 6/1/2013	THROUGH 5/31/2014	GRANT NUMBER 8 P41 GMI03483-14
---	-------------------------	-----------------------------	--

List PERSONNEL (*Applicant organization only*)
 Use Cal, Acad, or Summer to Enter Months Devoted to Project
 Enter Dollar Amounts Requested (*omit cents*) for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Continued from previ	PD/PI						
Christman, Lesa	Admin	1.8			8,224	5,017	13,240
SUBTOTALS →					8,224	5,017	13,240

CONSULTANT COSTS

EQUIPMENT (*Itemize*)

SUPPLIES (*Itemize by category*)

TRAVEL

INPATIENT CARE COSTS

OUTPATIENT CARE COSTS

ALTERATIONS AND RENOVATIONS (*Itemize by category*)

OTHER EXPENSES (*Itemize by category*)

SUBTOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD \$

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
------------------------------	--------------	--

CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
------------------------------	-------------------------------------	--

TOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD (*Item 8a, Face Page*) \$

BUDGET JUSTIFICATION

GRANT NUMBER 8P41GMI03483-14

Provide a detailed budget justification for those line items and amounts that represent a significant change from that previously recommended. Use continuation pages if necessary.

No significant changes from the previous budget are proposed. The personnel, supplies and other expenses are the same as last year.

CURRENT BUDGET PERIOD

FROM 6/1/2012

THROUGH 5/31/2013

Explain any estimated unobligated balance (including prior year carryover) that is greater than 25% of the current year's total budget. We do not expect to have any carryover for this year.

**For New and Renewal Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED
For Non-competing Progress Reports (PHS 2590) – Submit only Active Support for Key Personnel**

PHS 398/2590 OTHER SUPPORT

Provide active support for all key personnel. **Other Support includes all financial resources, whether Federal, non-Federal, commercial or institutional, available in direct support of an individual's research endeavors, including but not limited to research grants, cooperative agreements, contracts, and/or institutional awards.** Training awards, prizes, or gifts do not need to be included.

There is no "form page" for other support. Information on other support should be provided in the *format* shown below, using continuation pages as necessary. **Include the principal investigator's name at the top and number consecutively with the rest of the application.** The sample below is intended to provide guidance regarding the type and extent of information requested.

For instructions and information pertaining to the use of and policy for other support, see Other Support in the PHS 398 Part III, Policies, Assurances, Definitions, and Other Information.

Note effort devoted to projects must now be measured using person months. Indicate calendar, academic, and/or summer months associated with each project.

Format

NAME OF INDIVIDUAL

ACTIVE/PENDING

Project Number (Principal Investigator) Source Title of Project (or Subproject)	Dates of Approved/Proposed Project Annual Direct Costs	Person Months (Cal/Academic/ Summer)
The major goals of this project are...		

OVERLAP (summarized for each individual)

Samples

Bench G

ACTIVE

LLNL Internal Funds (Bench) 10/1/2012– ongoing 8.8 calendar
 LLNI/DOE \$190,000
 CAMS Director and Strategic Mission support

This funding enables Dr. Bench to direct , lead and represent the Center for Accelerator Mass Spectrometry to LLNL and the external community.

PENDING

none

OVERLAP

There is no scientific overlap between the two projects

**For New and Renewal Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED
For Non-competing Progress Reports (PHS 2590) – Submit only Active Support for Key Personnel**

PHS 398/2590 OTHER SUPPORT

Provide active support for all key personnel. **Other Support includes all financial resources, whether Federal, non-Federal, commercial or institutional, available in direct support of an individual's research endeavors, including but not limited to research grants, cooperative agreements, contracts, and/or institutional awards.** Training awards, prizes, or gifts do not need to be included.

There is no "form page" for other support. Information on other support should be provided in the *format* shown below, using continuation pages as necessary. **Include the principal investigator's name at the top and number consecutively with the rest of the application.** The sample below is intended to provide guidance regarding the type and extent of information requested.

For instructions and information pertaining to the use of and policy for other support, see Other Support in the PHS 398 Part III, Policies, Assurances, Definitions, and Other Information.

Note effort devoted to projects must now be measured using person months. Indicate calendar, academic, and/or summer months associated with each project.

Format

NAME OF INDIVIDUAL

ACTIVE/PENDING

Project Number (Principal Investigator) Source Title of Project (or Subproject)	Dates of Approved/Proposed Project Annual Direct Costs	Person Months (Cal/Academic/ Summer)
The major goals of this project are...		

OVERLAP (summarized for each individual)

Buchholz, B.

ACTIVE

1R01HL105532-01 (PIs: Anversa, Kajstura, Buchholz) 2/1/11-1/31/16 2.4 cal. mo.

NIH/NHLBI

Redefining Human Myocardial Biology

These studies will offer a novel perspective of the processes which govern the lifespan of the human heart.

Role: MPI

18IB-0016 (PI: Borowsky) 8/1/12-1/31/14 0.6 cal. mo.

California Breast Cancer Research Program

Our objective is to distinguish mammary and cancer stem cell populations in human breast and determine their turnover using carbon-14 bomb pulse dating with accelerator mass spectrometry.

Role: Co-Investigator

8P41GM103483 (PI: Turteltaub) 9/1/99-5/31/14 2.4 cal. mo.

NIH/NIGMS

Resource for the Development of Biomedical Mass Spectrometry

The goal of this proposal is to expand the use of accelerator mass spectrometry in bioscience for NIH projects.

Role: Co-Investigator

P01 AG043353-01 Anversa (PI) 2/1/13-1/31/18 3.0 cal. mo

NIH/NIA

Aging of the Heart

The goal of the project is to determine how the function and turnover of cells in the heart change with age.

The Accelerator Mass Spectrometry Core (Core B) prepares DNA and measures isotope ratios of natural ¹⁴C/C and tracer ¹⁴C/C or ³H/H. The Core provides expertise in DNA preparation for AMS, high precision measurements, data analysis, data interpretation.

Role: Core Leader of Core B, Accelerator Mass Spectrometry Core

CAMS Institutional Operation Bench (Director) Ongoing 3.6 cal. mo.

Lawrence Livermore National Laboratory

Operation, Improvement and Promotion of AMS in Geoscience, Energy, and Bioscience.

The CAMS Institute operates a multipurpose AMS facility and collaborates with several institutions.

Role: Scientific Staff

OVERLAP
None

**For New and Renewal Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED
For Non-competing Progress Reports (PHS 2590) – Submit only Active Support for Key Personnel**

PHS 398/2590 OTHER SUPPORT

Provide active support for all key personnel. **Other Support includes all financial resources, whether Federal, non-Federal, commercial or institutional, available in direct support of an individual's research endeavors, including but not limited to research grants, cooperative agreements, contracts, and/or institutional awards.** Training awards, prizes, or gifts do not need to be included.

There is no "form page" for other support. Information on other support should be provided in the *format* shown below, using continuation pages as necessary. **Include the principal investigator's name at the top and number consecutively with the rest of the application.** The sample below is intended to provide guidance regarding the type and extent of information requested.

For instructions and information pertaining to the use of and policy for other support, see Other Support in the PHS 398 Part III, Policies, Assurances, Definitions, and Other Information.

Note effort devoted to projects must now be measured using person months. Indicate calendar, academic, and/or summer months associated with each project.

Format

NAME OF INDIVIDUAL

ACTIVE/PENDING

Project Number (Principal Investigator) Source Title of Project (or Subproject)	Dates of Approved/Proposed Project Annual Direct Costs	Person Months (Cal/Academic/ Summer)
The major goals of this project are...		

OVERLAP (summarized for each individual)

Samples

Haack, K.

ACTIVE

P41 013461	09/1/2009-05/31/2014	12 calendar
NIH/NCRR	\$1,600,000	
Resource for Development of Biomedical Mass Spectrometry		
Development of AMS techniques for quantitation of biochemicals		
Role: Technician		

OVERLAP: none

**For New and Renewal Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED
For Non-competing Progress Reports (PHS 2590) – Submit only Active Support for Key Personnel**

PHS 398/2590 OTHER SUPPORT

Provide active support for all key personnel. **Other Support includes all financial resources, whether Federal, non-Federal, commercial or institutional, available in direct support of an individual's research endeavors, including but not limited to research grants, cooperative agreements, contracts, and/or institutional awards.** Training awards, prizes, or gifts do not need to be included.

There is no "form page" for other support. Information on other support should be provided in the *format* shown below, using continuation pages as necessary. **Include the principal investigator's name at the top and number consecutively with the rest of the application.** The sample below is intended to provide guidance regarding the type and extent of information requested.

For instructions and information pertaining to the use of and policy for other support, see Other Support in the PHS 398 Part III, Policies, Assurances, Definitions, and Other Information.

Note effort devoted to projects must now be measured using person months. Indicate calendar, academic, and/or summer months associated with each project.

Format

NAME OF INDIVIDUAL

ACTIVE/PENDING

Project Number (Principal Investigator) Source Title of Project (<i>or Subproject</i>)	Dates of Approved/Proposed Project Annual Direct Costs	Person Months (Cal/Academic/ Summer)
The major goals of this project are...		

OVERLAP (*summarized for each individual*)

Samples

Kulp, Kristen S.

ACTIVE

NCCR 5 P41 RR013461 (Turteltaub)	9/1/2009 – 5/31/2014	2.4 calendar
NIH/NCRR	\$1,600,000	
Resource for the Development of Biomedical Accelerator Mass Spectrometry (AMS)		

The major goals of this project are to expand the present capabilities of BioAMS by developing a fully integrated HPLC/AMS interface, to study biochemical pathways down to the single cell level, and to validate methods for AMS in human translational research.

LDRD 13-ERD-045 (S. Pannu)	10/1/2012 – 9/30/2013	2.4 calendar
LLNL Internal Investment	\$1,200,000	
Bioengineering systems for medical countermeasures		

The major goal of this project is to develop an in-vitro microfluidics platform that will support the long term growth of human neurons. The platform will be used to understand mechanisms of toxicant exposure and develop countermeasures

LLNL other funding		7.2 calendar
--------------------	--	--------------

Funding to perform managerial duties in support of lab programs

OVERLAP: none

**For New and Renewal Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED
For Non-competing Progress Reports (PHS 2590) – Submit only Active Support for Key Personnel**

PHS 398/2590 OTHER SUPPORT

Provide active support for all key personnel. **Other Support includes all financial resources, whether Federal, non-Federal, commercial or institutional, available in direct support of an individual's research endeavors, including but not limited to research grants, cooperative agreements, contracts, and/or institutional awards.** Training awards, prizes, or gifts do not need to be included.

There is no "form page" for other support. Information on other support should be provided in the *format* shown below, using continuation pages as necessary. **Include the principal investigator's name at the top and number consecutively with the rest of the application.** The sample below is intended to provide guidance regarding the type and extent of information requested.

For instructions and information pertaining to the use of and policy for other support, see Other Support in the PHS 398 Part III, Policies, Assurances, Definitions, and Other Information.

Note effort devoted to projects must now be measured using person months. Indicate calendar, academic, and/or summer months associated with each project.

Format

NAME OF INDIVIDUAL

ACTIVE/PENDING

Project Number (Principal Investigator) Source Title of Project (or Subproject)	Dates of Approved/Proposed Project Annual Direct Costs	Person Months (Cal/Academic/ Summer)
The major goals of this project are...		

OVERLAP (summarized for each individual)

Samples

Malfatti, M.A.

ACTIVE

NCRR 5 P41 RR013461

9/1/2009 – 8/31/2014

2.40 calendar

NIH/NCRR

\$987,446

Resource for the Development of Biomedical Accelerator Mass Spectrometry (AMS)

Role: Co-investigator

The major goals of this project are to expand the present capabilities of BioAMS by developing a fully integrated HPLC/AMS interface, to study biochemical pathways down to the single cell level, and to validate methods for AMS in human translational research.

PNNL/284

1/1/2009 – 12/31/2013

4.8 calendar

Battelle Memorial Institute

Cooperative Research and Development Agreement \$357,805

Multi-Scale Toxicology: Building the Next Generation of Tools for Toxicology

Role: Co-Investigator

The major goal of this project is to develop a suite of new capabilities for assessing the safety of materials designed for use in humans or to which humans will be exposed.

PLS-LDRD: 11-ERD-O12:

10/1/10-9/30/13

2.8 calendar

LLNL/DOE

\$400,131

A Rapid Response System for Toxin Removal:

Role: PI

The goal of this project is to develop a novel therapeutic that packages cytochrome P450 (CYP450) proteins into nanolipoprotein particles (NLPs), which when administered intravenously, will enhance the body's ability to metabolize certain chemical/biowarfare agents to inactive compounds.

PLS-LDRD: 13-ERD-042:

10/1/12-9/30/15

2.0 calendar

LLNL/DOE

\$598,000

Optimizing Drug Efficacy

Role: Co-Investigator

The goal of this project is to develop novel methodologies for correlating genetic variation in humans with drug response. Understanding how the genetic make-up of a person influences drug efficacy and toxicity is key to developing customized effective treatments and minimizing harmful side effects.

OVERLAP: none

**For New and Renewal Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED
For Non-competing Progress Reports (PHS 2590) – Submit only Active Support for Key Personnel**

PHS 398/2590 OTHER SUPPORT

Provide active support for all key personnel. **Other Support includes all financial resources, whether Federal, non-Federal, commercial or institutional, available in direct support of an individual's research endeavors, including but not limited to research grants, cooperative agreements, contracts, and/or institutional awards.** Training awards, prizes, or gifts do not need to be included.

There is no "form page" for other support. Information on other support should be provided in the *format* shown below, using continuation pages as necessary. **Include the principal investigator's name at the top and number consecutively with the rest of the application.** The sample below is intended to provide guidance regarding the type and extent of information requested.

For instructions and information pertaining to the use of and policy for other support, see Other Support in the PHS 398 Part III, Policies, Assurances, Definitions, and Other Information.

Note effort devoted to projects must now be measured using person months. Indicate calendar, academic, and/or summer months associated with each project.

Format

NAME OF INDIVIDUAL

ACTIVE/PENDING

Project Number (Principal Investigator) Source Title of Project (or Subproject)	Dates of Approved/Proposed Project Annual Direct Costs	Person Months (Cal/Academic/ Summer)
The major goals of this project are...		

OVERLAP (summarized for each individual)

Samples

Ognibene, T. J.

Active

35142 (Ognibene)	10/12-09/13	3.0 calendar
LLNL	\$330,000	
Spectroscopic Detection Development		
Development of spectroscopic detection methods for 14C		

30990 (Bench)	10/12-09/13	2.4 calendar
LLNL	\$1,500,000	
CAMS Biocarbon Operation		
Operation of 1-MV BioAMS Spectrometer		

Pending Research Support

35142 (Ognibene)	10/13-09/14	3.0 calendar
LLNL	\$300,000	
Spectroscopic Detection Development		
Development of spectroscopic detection methods for 14C		

30990 (Bench)	10/13-9/14	2.4 calendar
LLNL	\$1,500,000	
CAMS Biocarbon Operation		
Operation of 1-MV BioAMS Spectrometer		

Overlap: None

**For New and Renewal Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED
For Non-competing Progress Reports (PHS 2590) – Submit only Active Support for Key Personnel**

PHS 398/2590 OTHER SUPPORT

Provide active support for all key personnel. **Other Support includes all financial resources, whether Federal, non-Federal, commercial or institutional, available in direct support of an individual's research endeavors, including but not limited to research grants, cooperative agreements, contracts, and/or institutional awards.** Training awards, prizes, or gifts do not need to be included.

There is no "form page" for other support. Information on other support should be provided in the *format* shown below, using continuation pages as necessary. **Include the principal investigator's name at the top and number consecutively with the rest of the application.** The sample below is intended to provide guidance regarding the type and extent of information requested.

For instructions and information pertaining to the use of and policy for other support, see Other Support in the PHS 398 Part III, Policies, Assurances, Definitions, and Other Information.

Note effort devoted to projects must now be measured using person months. Indicate calendar, academic, and/or summer months associated with each project.

Format

NAME OF INDIVIDUAL

ACTIVE/PENDING

Project Number (Principal Investigator) Source Title of Project (or Subproject)	Dates of Approved/Proposed Project Annual Direct Costs	Person Months (Cal/Academic/ Summer)
The major goals of this project are...		

OVERLAP (summarized for each individual)

Stewart, B.

Department of Energy

10/01/2012- 04/01/2013

9 calendar

\$3,000,000

Basic and applied science research and development for DOE focus areas

OVERLAP: This project has no overlap with the Bio-AMS research.

**For New and Renewal Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED
For Non-competing Progress Reports (PHS 2590) – Submit only Active Support for Key Personnel**

PHS 398/2590 OTHER SUPPORT

Provide active support for all key personnel. **Other Support includes all financial resources, whether Federal, non-Federal, commercial or institutional, available in direct support of an individual's research endeavors, including but not limited to research grants, cooperative agreements, contracts, and/or institutional awards.** Training awards, prizes, or gifts do not need to be included.

There is no "form page" for other support. Information on other support should be provided in the *format* shown below, using continuation pages as necessary. **Include the principal investigator's name at the top and number consecutively with the rest of the application.** The sample below is intended to provide guidance regarding the type and extent of information requested.

For instructions and information pertaining to the use of and policy for other support, see Other Support in the PHS 398 Part III, Policies, Assurances, Definitions, and Other Information.

Note effort devoted to projects must now be measured using person months. Indicate calendar, academic, and/or summer months associated with each project.

Format

NAME OF INDIVIDUAL

ACTIVE/PENDING

Project Number (Principal Investigator) Source Title of Project (or Subproject) The major goals of this project are...	Dates of Approved/Proposed Project Annual Direct Costs	Person Months (Cal/Academic/ Summer)
<u>OVERLAP (summarized for each individual)</u>		

Samples

Turteltaub, K.W.

ACTIVE

Program Management (Turteltaub) Physical & Life Sciences Directorate LLNL Institutional Support for oversight of Biosciences	Ongoing	4.2 calendar
Program Management (Turteltaub) Global Security Directorate LLNL Institutional Support for oversight of Medical Countermeasures	Ongoing	4.2 calendar

OVERLAP: none

PROGRESS REPORT SUMMARY	GRANT NUMBER 8P41GM103483-14	
	PERIOD COVERED BY THIS REPORT	
PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR Kenneth W. Turteltaub	FROM 4/1/2012	THROUGH 3/31/2013

APPLICANT ORGANIZATION
Lawrence Livermore National Laboratory

TITLE OF PROJECT (Repeat title shown in Item 1 on first page)
Resource for the Development of Biomedical Accelerator Mass Spectrometry

A. Human Subjects (Complete Item 6 on the Face Page)		
Involvement of Human Subjects	<input checked="" type="checkbox"/> No Change Since Previous Submission	<input type="checkbox"/> Change
B. Vertebrate Animals (Complete Item 7 on the Face Page)		
Use of Vertebrate Animals	<input checked="" type="checkbox"/> No Change Since Previous Submission	<input type="checkbox"/> Change
C. Select Agent Research	<input checked="" type="checkbox"/> No Change Since Previous Submission	<input type="checkbox"/> Change
D. Multiple PD/PI Leadership Plan	<input checked="" type="checkbox"/> No Change Since Previous Submission	<input type="checkbox"/> Change
E. Human Embryonic Stem Cell Line(s) Used	<input checked="" type="checkbox"/> No Change Since Previous Submission	<input type="checkbox"/> Change

SEE PHS 2590 INSTRUCTIONS.

WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary, Targeted/Planned Enrollment Format Page.

A.-C.) Specific Aims, Studies and Results, Significance: For information regarding aims, results and significance, please see the Annual Progress Report (APR) for P41 RR013461-2013 which is appended to this document. It has also been submitted electronically to the NCRR.

D.) Plans: No significant changes to the submitted grant proposal are expected for next year.

Item A) There is no change in the human subjects research protocols planned for the coming year. A protection of human subjects assurance identification form (OF310) is included with this report. Copies of approval letters are included in this report. Copies of approved protocols can be provided upon request.

Item B) Approval for Vertebrate Animal Use has been documented in the attached letter from the chairperson of the LLNL IACUC. Copies of Approved protocols are labeled OOU and can be provided upon request.

E) Publications: Citations of publications by the resource staff are listed on the following page as well as in the APR.

F) Project-Generated Resources: The research supported by this grant did not result in any data, research materials, protocols, software or other information to be shared with other investigators.

Update:

Key personnel changes:

We have added two postdocs to the project. Their biosketches are included in the following pages.

Protection of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption (Common Rule)

Policy: Research activities involving human subjects may not be conducted or supported by the Departments and Agencies adopting the Common Rule (56FR28003, June 18, 1991) unless the activities are exempt from or approved in accordance with the Common Rule. See section 101(b) of the Common Rule for exemptions. Institutions submitting applications or proposals for support must submit certification of appropriate Institutional Review Board (IRB) review and approval to the Department or Agency in accordance with the Common Rule.

Institutions must have an assurance of compliance that applies to the research to be conducted and should submit certification of IRB review and approval with each application or proposal unless otherwise advised by the Department or Agency.

1. Request Type <input type="checkbox"/> ORIGINAL <input type="checkbox"/> CONTINUATION <input type="checkbox"/> EXEMPTION	2. Type of Mechanism <input type="checkbox"/> GRANT <input type="checkbox"/> CONTRACT <input type="checkbox"/> FELLOWSHIP <input type="checkbox"/> COOPERATIVE AGREEMENT <input type="checkbox"/> OTHER: _____	3. Name of Federal Department or Agency and, if known, Application or Proposal Identification No.
4. Title of Application or Activity		5. Name of Principal Investigator, Program Director, Fellow, or Other

6. Assurance Status of this Project (*Respond to one of the following*)

- This Assurance, on file with Department of Health and Human Services, covers this activity:
Assurance Identification No. _____, the expiration date _____ IRB Registration No. _____
- This Assurance, on file with (*agency/dept*) _____, covers this activity.
Assurance No. _____, the expiration date _____ IRB Registration/Identification No. _____ (*if applicable*)
- No assurance has been filed for this institution. This institution declares that it will provide an Assurance and Certification of IRB review and approval upon request.
- Exemption Status: Human subjects are involved, but this activity qualifies for exemption under Section 101(b), paragraph _____

7. Certification of IRB Review (Respond to one of the following IF you have an Assurance on file)

- This activity has been reviewed and approved by the IRB in accordance with the Common Rule and any other governing regulations.
by: Full IRB Review on (date of IRB meeting) _____ or Expedited Review on (date) _____
 If less than one year approval, provide expiration date _____
- This activity contains multiple projects, some of which have not been reviewed. The IRB has granted approval on condition that all projects covered by the Common Rule will be reviewed and approved before they are initiated and that appropriate further certification will be submitted.

8. Comments

9. The official signing below certifies that the information provided above is correct and that, as required, future reviews will be performed until study closure and certification will be provided.	10. Name and Address of Institution	
11. Phone No. (<i>with area code</i>) 12. Fax No. (<i>with area code</i>) 13. Email:	15. Title	
14. Name of Official		
16. Signature	17. Date	

Authorized for local Reproduction

Sponsored by HHS

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0990-0263. The time required to complete this information collection is estimated to average 30 minutes per response. If you have comments concerning the accuracy of the time estimate(s) or suggestions for improving this form, please write to: U.S. Department of Health & Human Services, OS/OCIO/PRA, 200 Independence Ave., S.W., Suite 336-E, Washington D.C. 20201, Attention: PRA Reports Clearance Officer.



12 March 2013

RE: NIH/NCRR Grants P41 RR013461, NIGMS P41 GM10348

To Whom It May Concern:

I am writing this letter to document that the Research Resource for Biomedical Accelerator Mass Spectrometry funded by National Institutes of Health (NIH) NCRR Grant P41 RR013461 and NIGMS P416 M10348 are in compliance with the Lawrence Livermore National Laboratory's (LLNL) Institutional Animal Care and Use Committee (IACUC) guidelines covering collaborative and service projects.

The Research Resource requires that all projects to the Resource involving the use of vertebrate animals conform to the appropriate NIH and United States Department of Agriculture (USDA) animal welfare guidelines. The approval of an application to the Research Resource is contingent on the applicant providing documentation of review and approval by the applicant's IACUC. The Resource registers each project with LLNL's IACUC for review of all such documents to ensure that these requirements are met. No studies can begin nor are samples accepted for analysis until this review has been completed and deemed satisfactory.

Our Animal Welfare Assurance Number is A3184-01.

If you have any questions or need further information please feel free to call me at (925) 423-4285

Sincerely,

George W. Anderson, Jr., Ph.D., RBP
IACUC Chair

Lawrence Livermore National Laboratory
P.O. Box 808, L-452
Livermore, California 94551
Telephone: 925-422-1100
Fax: 925-423-5555

RESOURCE PUBLICATIONS

Publications (in Pubmed central):

1. J. S. Chang, P. N. Lara Jr., and C.-X. Pan, "Progress in Personalizing Chemotherapy for Bladder Cancer," *Advances in Urology*, vol. 2012, Article ID 364919, 10 pages, 2012. doi:10.1155/2012/364919 (SPID 0003;SPID 0048)
2. M. A. Dall'Era, L. Cheng and C.-X. Pan, Contemporary management of muscle-invasive bladder cancer, *Expert Review of Anticancer Therapy* July 2012, Vol. 12, No. 7, Pages 941-950 , DOI 10.1586/era.12.60 (doi:10.1586/era.12.60) (SPID 0003)
3. N. Etminan, R. Dreier, B.A. Buchholz, P. Bruckner, H.-J. Steiger, D. Hänggi, R.L. MacDonald. (2013) Exploring the age of intercranial aneurysms using carbon birth dating: preliminary results. *Stroke* in press. Online January 18, 2013, DOI: 10.1161/STROKEAHA.112.673806. (SPID 0066)
4. M.J.S. Falso, B.A. Buchholz. (2013) Bomb pulse biology. *Nucl. Instr. and Meth. B* **294**, 666-670. (SPID 0055)
5. Kajstura, J, Rota, M, Cappetta, D, Ogorek, B, Arranto, C, Bai, YN, Ferreira-Martins, J, Signore, S, Sanada, F, Matsuda, A, Kostyla, J, Caballero, MV, Fiorini, C, D'Alessandro, DA, Michler, RE, del Monte, F, Hosoda, T, Perrella, MA, Leri, A, Buchholz, BA, Loscalzo, J, Anversa, P. Cardiomyogenesis in the Aging and Failing Human Heart. *CIRCULATION* 2012; 126:1869-U238 (SPID 0064)
6. Malfatti, M. A., Palko, H. A., Kuhn, E. A., Turteltaub, K. W. (2012) Determining the pharmacokinetics and long-term biodistribution of SiO₂ nanoparticles *in vivo* using accelerator mass spectrometry. *Nano Letters*, 12, 5532-5538 (SPID 0003)
7. Novotny JA, Fadel JG, Holstege DM, Furr HC, Clifford AJ. This kinetic, bioavailability, and metabolism study of RRR- α -tocopherol in healthy adults suggests lower intake requirements than previous estimates. *J Nutr.* 2012Dec;142(12):2105-11. doi: 10.3945/jn.112.166462. Epub 2012 Oct 17. PubMed
8. N.H. Schebb, B.A. Buchholz, B.D. Hammock, R.H. Rice. Metabolism of the antibacterial triclocarban by human epidermal keratinocytes to yield protein adducts. *J. Biochem. Mol. Tox.* **26** (2012) 230-234. DOI: 10.1002/jbt.21411 (SPID 0038)
9. Stewart BJ, Navid A, Kulp KS, Knaack JL, Bench G. D-Lactate production as a function of glucose metabolism in *Saccharomyces cerevisiae*. *Yeast.* 2013Feb;30(2):81-91. doi: 10.1002/yea.2942. Epub 2013 Jan 30. PubMed (SPID 0002)
10. D.N. Stewart, J. Lango, K.P. Nambiar, M.J.S. Falso, P.G. FitzGerald, B.D. Hammock, B.A. Buchholz. (2013) Carbon turnover in water-soluble protein of the adult human lens. *Molecular Vision* 2013; 19:463-475 <http://www.molvis.org/molvis/v19/463> (SPID

0055;SPID 0038)

11. W. Xiao, J. Luo, T. Jain, J. W. Riggs, H. P Tseng, P. T Henderson, S. R. Cherry, D. Rowland, K. S. Lam Biodistribution and pharmacokinetics of a telodendrimer micellar paclitaxel nanoformulation in a mouse xenograft model of ovarian cancer *Int J Nanomedicine*. 2012; 7: 1587–1597. Published online 2012 March 27. doi: 10.2147/IJN.S29306 **(SPID 0073)**

Publications (not yet deposited in Pubmed central):

12. Sarachine Falso MJ, Buchholz BA, de Vere White R. Characterization of the Populations Isolated Using the Aldefluor Assay in Three Bladder Cancer Cell Lines with Differential Sensitivity to Cisplatin. *Anticancer Research* 32 (3): March 2012. **(SPID 0062)**
13. Zhang H, Aina OH, Lam KS, de Vere White R, Evans C, Henderson P, Lara PN, Wang X, Bassuk JA, Pan CX. Identification of a bladder cancer-specific ligand using a combinatorial chemistry approach. *Urol Oncol*. 2012 Sep;30(5):635-45. doi:10.1016/j.urolonc.2010.06.011. Epub 2010 Oct 2. PubMed
14. Cimino GD, Pan CX, Henderson PT. Personalized medicine for targeted and platinum-based chemotherapy of lung and bladder cancer. *Bioanalysis*. 2013 Feb;5(3):369-91. doi: 10.4155/bio.12.325. PubMed **(SPID 0048)**
15. Jiao, Y., Navid, A., Stewart, B. J., McKinlay, J. B., Thelen, M. P. and Pett-Ridge, J. (2012). Syntrophic metabolism of a co-culture containing *Clostridium cellulolyticum* and *Rhodospseudomonas palustris* for hydrogen production. *International Journal of Hydrogen Energy* **37**, 11719-11726. **(SPID 0065)**
16. Neuwirth, C., Mosesso, P., Pepe, G., Fiore, M., Malfatti, M., Turteltaub, K., Dekant, W., Mally, A, Furan carcinogenicity: DNA binding and genotoxicity of furan in rats in vivo, *MOLECULAR NUTRITION & FOOD RESEARCH* 2012, 56 (9) 1363-1374
17. T. J. Ognibene and G. A. Salazar (2013). "Installation of hybrid ion source on the 1-MV LLNL BioAMS spectrometer." *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* 294(0): 311-314. **(SPID 0001)**
18. G. Salazar and T. Ognibene (2013). "Design of a secondary ionization target for direct production of a C⁻ beam from CO₂ pulses for online AMS." *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* 294(0): 300-306. **(SPID 0001)**
19. C.F. Speller, K.L. Spalding, B.A. Buchholz, D. Hildebrand, J. Moore, R. Mathewes, M.F. Skinner, D.Y. Yang. (2012) Personal identification of cold case remains through combined contribution from anthropological, mtDNA and bomb-pulse dating analyses. *J. Forensic Sci.* **57**, 1354-1360. **(SPID 0054)**

20. A.T. Thomas, B. J. Stewart, T. J. Ognibene, K. W. Turteltaub and G. Bench (2012). " Directly coupled HPLC-AMS measurement of chemically-modified protein and peptides" *Analytical Chemistry*, in press. **(SPID 0001)**

Book Chapter Published:

1. Buchholz, B. A., Sarachine Falso, M. J., Stewart, B. J., Haack, K. W., Ognibene, T. J., Bench, G., Salazar Quintero, G. A., Malfatti, M. A., Kulp, K. S., Turteltaub, K. W. and Lyubimov, A. V. (2012). Bioanalytics for Human Microdosing, *Encyclopedia of Drug Metabolism and Interactions*, John Wiley & Sons, Inc.
2. Stewart, B. J., Bench, G., Buchholz, B. A., Haack, K. W., Malfatti, M. A., Ognibene, T. J. and Turteltaub, K. W. (2012). Accelerator Mass Spectrometry in Pharmaceutical Development, *Mass Spectrometry Handbook*, John Wiley & Sons, Inc., pp. 259-269.

Abstracts Published:

1. Ricciardi, M, Pesapane, A, Polverino, Arranto, C, Arranto, Palano, G, Lam, H, Castano, A, Matsuda, A, Rota, M, Leri, A, Rosas, I, Perrella, M, Buchholz, BA, Kajstura, J, Loscalzo, J, Anversa, P. Birth Dating of Human Lung Cells by Accelerator Mass Spectrometry, *CIRCULATION RESEARCH* 2012; 111:E382.
2. OgOrek, B, Hosoda, T, Rondon, C, Gurusamy, N, Gatti, A, Bardelli, S, Quaini, F, Bussani, R, Silvestri, F, Daniela, C, Beltrami, AP, del Monte, F, Rota, M, Urbanek, K, Buchholz, BA, Leri, A, Beltrami, CA, Anversa, P, Kajstura, J. Myocyte Turnover in the Aging Human Heart. *CIRCULATION RESEARCH* 2012; 111(4) Supplement: S Meeting Abstract: 19
3. R. Green & J. Miller. New insights into cobalamin absorption and metabolism using accelerator mass spectrometry, *A12, Clin Chem Lab Med* 2012;50(2):A1–A69.

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 Enright, Heather Ann eRA COMMONS USER NAME (credential, e.g., agency login)		POSITION TITLE Postdoctoral Fellow, Biosciences and Biotechnology Division	
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 Hartford	B.S.	2005	Chemistry-Biology
University of California, Davis	Ph.D.	2011	Chemistry
Battelle Memorial Institute, Lawrence Livermore National Laboratory	Postdoctoral	09/12	Pharmacology/Toxicology
Lawrence Livermore National Lab	Postdoctoral	10/12-present	Pharmacology/Toxicology

A. Personal Statement

I am currently a postdoctoral researcher within the BioAMS group using the resource for *in vivo* pharmacology and toxicology studies. My research utilizes accelerator mass spectrometry (AMS) for three projects: pharmacokinetics and biodistribution of iron oxide nanoparticles, long term detection of doxorubicin and its metabolites in cardiac tissue and placental transfer of an endocrine disruptor, triclocarban.

B. Positions and Honors

Positions and Employment

2003-2005 Research Assistant, Department of Chemistry, University of Hartford
2005-2011 Graduate Student Researcher, Department of Chemistry
University of California, Davis, Davis, CA
04/2011-09/2012 Gordon Battelle Postdoctoral Fellow, Battelle Memorial Institute, Biosciences and Biotechnology Division, Lawrence Livermore National Lab, Livermore, CA
10/2012-present Postdoctoral Fellow, Lawrence Livermore National Lab, Livermore, CA

Awards, honors and committees

2011-2012 – Lawrence Livermore National Laboratory Biosciences and Biotechnology Division Seminar Series, coordinator
2010 - Travel Award, Society of Biological Inorganic Chemists, Metals in Medicine Gordon Conference

C. Selected peer-reviewed publications

Malfatti M, **Palko HA**, Kuhn EA, Turteltaub KW. Determining the Pharmacokinetics and Long-Term Biodistribution of SiO₂ Nanoparticles In Vivo Using Accelerator Mass Spectrometry. *Nanoletters* (2012) 12(11): 5532-5538.

Tu C, Ng TSC, Sohi HK, **Palko HA**, House A, Jacobs RE, Louie AY. Receptor-targeted Iron Oxide Nanoparticles for Molecular MR Imaging of Inflamed Atherosclerotic Plaques. *Biomaterials* (2011) 32(29): 7209-7216.

Palko H, Fung J, Louie AY. Positron Emission Tomography: A novel technique for investigating the bio-distribution and transport of nanoparticles. *Inh Tox* (2010) 22(8): 621-626.

Lee B, Seshadri V, **Palko H**, Sotzing GA. Ring-sulfonated poly(thienothiophene). *Advanced Materials* (2005), 17(14): 1792-1795.

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 McNerney, Margaret Windy	POSITION TITLE Postdoctoral, Biosciences and Biotechnology Division		
eRA COMMONS USER NAME (credential, e.g., agency login) mcnerney1			
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 California, Davis	B.S.	06/03	Psychobiology
University of Notre Dame	M.A.	06/06	Psychology
University of Notre Dame	Ph.D.	05/12	Psychology

A. Personal Statement

The goal of the proposed research is to utilize accelerator mass spectrometry for the sensitive quantitation of radiolabeled material. I have the expertise and experience necessary to implement *in vitro* systems for the proposed work. I have developed culture protocols for frog and rat primary neurons at the University of Notre Dame and LLNL. I have also performed several assays on these cultures, including markers for viability, fluorescence microscopy, histochemistry, and immunocytochemistry. At LLNL, I have also cultured human placental barrier BeWo and HUVEC cells on transwell plates, and have conducted research assessing the passage of carcinogens through these models. I have also created primary cultures of mouse and rat dorsal root ganglion cells for assay and electrophysiology research. If funded, I will combine my knowledge of neurobiology and endothelial barrier culture models in my roll to complete the goals of this project.

B. Positions and Honors

Positions and Employment

2001-2003 Research Assistant, Psychology Department, University of California, Davis
2004-2006 Research Assistant, Cognitive Neuroscience Group, University of Notre Dame
2007-2009 Research Associate, Army Aeroflight Dynamic Directorate, NASA Ames Research Center
2007-2012 Research Assistant, Memory Group, University of Notre Dame
2008-2012 Research Assistant, Neuroendocrinology Group, University of Notre Dame
2008-2012 Research Assistant, Bohn Analytical Chemistry Group, University of Notre Dame
2012-present Postdoctoral Researcher, Bioscience and Biotechnology, Lawrence Livermore National Labs

Other Experience and Professional Memberships

2004- Cognitive Neuroscience Society
2010- Society for Neurosciences

Honors

2008 Graduate School Professional Development Grant, University of Notre Dame
2011 Minor in Quantitative Analysis, University of Notre Dame

C. Selected Peer-reviewed Publications

Most relevant to the current application

Additional recent publications of importance to the field (in chronological order)

1. **McNerney MW**, West RL. (2007). An imperfect functional relationship between prospective memory and the prospective interference effect. *Memory and Cognition*, 35(2), 275-126. PMID: 17645168.
2. West RL, **McNerney MW** (2007). Impaired strategic monitoring as the locust of a focal prospective memory deficit. *Memory and Cognition*, 13(2), 115-126. PMID: 17566943.
3. West RL, **McNerney MW**, Travers S (2007). Gone but not forgotten: the effects of cancelled intentions on the neural correlates of prospective memory. *International Journal of Psychophysiology* 64(3), 215-225. PMID: 17107728.
4. **McNerney MW**, Goodwin K, Radvansky GA (2011). A novel study: a situation model analysis of reading times. *Discourse Processes* 48, 453-474.
5. Radvansky GA, Gibson B, **McNerney MW** (2011). Synesthesia and memory: color congruence, von Restorff, and false memory effects. *Journal of Experimental Psychology: Learning, Memory, and Cognition* 37(1), 219-229. PMID:21244115.
6. Magliano J, Kopp K, **McNerney MW**, Radvansky GA, Zacks J (2012). Aging and perceived event structure as a function of modality. *Neuropsychology, Development, and Cognition. Section B, Aging, Neuropsychology, and Cognition* 19(1-2), 264-282. PMID: 22182344.
7. Gibson BS, Radvansky GA, Johnson AC, **McNerney MW** (2012). Grapheme color synesthesia can enhance immediate memory without disrupting the encoding of relation cues. *Psychonomic Bulletin and Review*. 19(6), 1172-1177. PMID: 22836925.

Program Director/Principal Investigator (Last, first, middle): Turteltaub, Kenneth W

GRANT NUMBER
8P41GMI03483-14

CHECKLIST

1. PROGRAM INCOME (See instructions.)

All applications must indicate whether program income is anticipated during the period(s) for which grant support is requested. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)
6/1/2013-5/31/2014	0.00	

2. ASSURANCES/CERTIFICATIONS (See instructions.)

In signing the application Face Page, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the application instructions when applicable. Descriptions of individual assurances/certifications are provided in Part III of the [PHS 398](#), and listed in Part I, 4.1 under Item 14. If unable to certify compliance, where applicable, provide an explanation and place it after the Progress Report (Form Page 5).

3. FACILITIES AND ADMINISTRATIVE (F&A) COSTS

Indicate the applicant organization's most recent F&A cost rate established with the appropriate DHHS Regional Office, or, in the case of for-profit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office.

F&A costs will **not** be paid on construction grants, grants to Federal organizations, grants to individuals, and conference grants. Follow any additional instructions provided for Research Career Awards, Institutional National Research Service Awards, Small Business Innovation Research/Small Business Technology Transfer Grants, foreign grants, and specialized grant applications.

DHHS Agreement dated: _____ No Facilities and Administrative Costs Requested.

X No DHHS Agreement, but rate established with DOE/NNSA-Approval Date January, 2012

CALCULATION*

Entire proposed budget period: Amount of base \$ 1,054,176 x Rate applied 62.76782 % = F&A costs \$ 661,684

Add to total direct costs from Form Page 2 and enter new total on Face Page, Item 8b.

*Check appropriate box(es):

Salary and wages base Modified total direct cost base Other base (Explain)

X Off-site, other special rate, or more than one rate involved (Explain)

Explanation (Attach separate sheet, if necessary.):

LLNL applies a multiple rate structure per DOE/NNSA approval.

ALL PERSONNEL REPORT

GRANT NUMBER

8P41GM103483-14

Place this form at the end of the signed original copy of the application. Do not duplicate.

Always list the PD/PI(s). In addition, list all other personnel who participated in the project during the current budget period for at least one person month or more, regardless of the source of compensation (a person month equals approximately 160 hours or 8.3% of annualized effort). Use the following abbreviated categories for describing Role on Project:

- PD/PI
- Co-Investigator
- Faculty Collaborator
- Staff Scientist (doctoral level)
- Postdoc (Postdoctoral Scholar, Fellow, or Other Postdoctoral Position)
- Grad Rsch Asst (Graduate Research Assistant)
- Undergrad Rsch Asst (Undergraduate Research Assistant)
- Rsch Asst (Research Assistant/Coordinator)
- Technician
- Consultant
- Biostatistician
- Other (Specify)

If personnel are supported by a Reentry or Diversity Supplement or American Recovery and Reinvestment Act (ARRA) funding, please indicate such after the Role on Project, using the following abbreviations: RS - Reentry Supplement; DS - Diversity Supplement; AF - General ARRA Supplement; ASE - ARRA Summer Experience funding.

Use Cal (calendar), Acad, or Summer to enter months devoted to project.

Commons ID	Name	Degree(s)	SSN (last 4 digits)	Role on Project	DoB (MM /YY)	Cal	Acad	Summer
Kenneth_T urteltaub	Kenneth Turteltaub	PhD		PI	1/51	3.6		
	Graham Bench	PhD		co-PI	9/64	3.6		
	Ted Ognibene	PhD		Co-Investigator	8/65	2.4		
Michael_Ma lfatti	Michael Malfatti	PhD		Co- Investigator	6/65	3.6		
Bruce_Buc hholz	Bruce Buchholz	PhD		Co- Investigator		2.4		
Kristen_Kul p	Kristen Kulp	PhD		Co- Investigator	7/61	3		
	Kurt Haack	B.S.		Rsch Asst	6/58	12		
	M. Windy McNerney	PhD		Postdoc		3		
	Heather Enright Palko	PhD		Postdoc		12		
	Benjamin Stewart	PhD		Postdoc	3/75	6		
	Paul Daley	PhD		Faculty Collaborator		2.4		
	Ed Kuhn	BS		Rsch Asst	9/54	6		
	Avi Thomas	BS		Grad Rsch Asst		12		
	Victoria Lau	BS		Technician		12		

ALL PERSONNEL REPORT

GRANT NUMBER
8P41GM103483-14

Place this form at the end of the signed original copy of the application. Do not duplicate.

Always list the PD/PI(s). In addition, list all other personnel who participated in the project during the current budget period for at least one person month or more, regardless of the source of compensation (a person month equals approximately 160 hours or 8.3% of annualized effort). Use the following abbreviated categories for describing Role on Project:

- PD/PI
- Co-Investigator
- Faculty Collaborator
- Staff Scientist (doctoral level)
- Postdoc (Postdoctoral Scholar, Fellow, or Other Postdoctoral Position)
- Grad Rsch Asst (Graduate Research Assistant)
- Undergrad Rsch Asst (Undergraduate Research Assistant)
- Rsch Asst (Research Assistant/Coordinator)
- Technician
- Consultant
- Biostatistician
- Other (Specify)

If personnel are supported by a Reentry or Diversity Supplement or American Recovery and Reinvestment Act (ARRA) funding, please indicate such after the Role on Project, using the following abbreviations: RS - Reentry Supplement; DS - Diversity Supplement; AF - General ARRA Supplement; ASE - ARRA Summer Experience funding.

Use Cal (calendar), Acad, or Summer to enter months devoted to project.

Commons ID	Name	Degree(s)	SSN (last 4 digits)	Role on Project	DoB (MM /YY)	Cal	Acad	Summer
	Daniel McCartt	BS		Grad Rsch Asst		12		
	Lesa Christman	BS		Other - administration		6		

PROGRESS REPORT SUMMARY	GRANT NUMBER	
	P41RR013461-14	
PERIOD COVERED BY THIS REPORT		
PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR	FROM	THROUGH
KENNETH W TURTELTAUB, PHD	04/01/2012	03/31/2013
APPLICANT ORGANIZATION		
LAWRENCE LIVERMORE NATIONAL LABORATORY		
TITLE OF PROJECT (Repeat title shown in Item 1 on first page)		
RESOURCE FOR THE DEVELOPMENT OF BIOMEDICAL ACCELERATOR MASS SPECTROMETRY		

A. Human Subjects (Complete Item 6 on the Face Page)

Involvement of Human Subjects No Change Since Previous Submission Change

B. Vertebrate Animals (Complete Item 7 on the Face Page)

Use of Vertebrate Animals No Change Since Previous Submission Change

C. Select Agent Research No Change Since Previous Submission Change

D. Multiple PD/PI Leadership Plan No Change Since Previous Submission Change

E. Human Embryonic Stem Cell Line(s) Used No Change Since Previous Submission Change

SEE PHS 2590 INSTRUCTIONS.

WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary, Targeted/Planned Enrollment Format Page.

Protection Against Research Risks

- Yes Will human subjects or human data be used over the next year?
- Yes Is IRB approval current for all participating research sites?
- N/A Has human subjects training been completed by all key personnel?
- Yes Has a data and safety monitoring plan been put in place?
- N/A Have the gender/minority requirements for recruitment/retention been met
- Yes Will vertebrate animals be used over the next year?
- Yes Have IACUC approvals been obtained for all participating research sites?

Personnel Roster

Name, Degree	Department, Non-Host Institution Information
Ahn, Kichang	Ptrl West Ca Usa
Alkass, K, BS	Dept Of Forensic Medicine Karolinska Institute Sweden
Allen, Lindsay, PHD	Usda Whnrc Ca Usa
Bacha, Tula, PHD	Rutgers University Nj Usa
Baird, William, PHD	Environmental And Molecular To Oregon State University Or Usa
Bench, Graham, PHD	Center For Ams
Bergmann, Olaf I, MD	Cellular & Molecular Biology Karolinska Institute Sweden
Bova, G. Steven, MD	Johns Hopkins Md Usa
Brown, Karen, PHD	Cancer Biomarkers & Prevention U Of Leicester Uk
Bruckner, Peter, PHD	Institute Of Physiological Chemistry Germany
Buchholz, Bruce A, PHD	Center For Ams
Buckpitt, Alan, PHD	Veterinary Medicine, Molecular Univ Of Ca, Davis Ca Usa
Clifford, Andrew J, PHD	Nutrition Uc Davis Ca Usa
Cooke, Daniel, MD	Radiology/Biomedical Imaging Uc San Francisco Ca Usa
Corley, Richard, PHD	Biological Sciences Pacific Northwest National Laboratory Wa Usa
Daley, Paul, PHD	Environmental Restoration
Dekant, Wolfgang, PHD	Dept Of Toxicology University Of Wurtzburg Germany
Dennison, Michael, PHD	Envisonmental Toxicology Univ California, Davis Ca Usa

Personnel Roster

Name, Degree	Department, Non-Host Institution Information
Devere White, Ralph Dr, BCH, MB, OTH	Urology Dept; Ucd Cancer Cente Univ. Of Ca, Davis Ca Usa
Doerner, Thomas	Humboldt University Berlin Germany
Dogru, Ozgar, PHD	Physics Rutgers University Nj Usa
Dreier, Rita, PHD	Institute Of Physiological Chemistry Germany
Druid, Henrik, PHD, MD	Dept Of Forensic Medicine Karolinska Institute Sweden
Dueker, Stephen R, PHD	Vitalea Science Ca Usa
Dyer, Martin, BCH, DPHL, MB	Cancer Studies/Molecular Med. University Of Leicester Uk
Edge, Albert, PHD	Harvard Medical School Ma Usa
Elder, Alison Cp, PHD	Environmental Medicine University Of Rochester Ny Usa
Enright, Heather, PHD	Biosciences And Biotechnology
Etminan, Nima, PHD	Department Of Neurosurgery University Of Duesseldorf Germany
Farmer, Peter, DPHL	Cancer Biomarkers & Prevention Univ Of Leicester Uk
Fitzgerald, Paul, PHD	Cell Biology & Human Anatomy University Of California Davis Ca Usa
Frisen, Jonas, PHD, MD	Cell And Molecular Biology Karolinska Institute Sweden
Gandara, David, MD	University Of California Davis Ca Usa
Garrard, Mary, RN, MSCN	Oregon State University Or Usa
Garrod, Marjorie G, BS	Medical Pathology Univ Of Ca, Davis Med Center Ca Usa

Personnel Roster

Name, Degree	Department, Non-Host Institution Information
Gee, Shirley, MS	Entomology Uc Davis Ca Usa
Green, Ralph, MD	Medical Pathology Univ Of Ca, Davis Med Center Ca Usa
Haack, Kurt, BS	Center For Ams/Chem, Mat, Life
Haenggi, Daniel, PHD	Department Of Neurosurgery Heinrich-Heine University Germany
Hammock, Bruce, PHD	Entomology Uc Davis Ca Usa
Henderson, Paul T, BS, PHD	Urology Universtity Of California, Davis Ca Usa
Hetts, Steven W., MD	Radiology/Biomedical Imaging Uc San Francisco Ca Usa
Holden, Patricia, PHD	Bren School Of Env Sci/Mgmt Uc Santa Barbara Ca Usa
Hu, Wenwu, PHD	Dept Of Internal Medicine Universtity Of California, Davis Ca Usa
Ilkman, Erhan, BA	Physics Rutgers University Nj Usa
Jan, Kajstura, PHD	Anesthesia And Medicine Brigham And Women'S Hospital Ma Usa
Jiao, Yongqin, PHD	Biology And Biotechnology Division
Kim, Helen, MPH, PHD	Anesthesia/Perioperative Care Uc San Francisco Ca Usa
Kim, Kami, MD	Medicine/Microbio.& Immunology Albert Einstein College Of Medicine Ny Usa
Krueger, Sharon, PHD	The Linus Pauling Institute Oregon State University Or Usa
Kulp, Kristen, PHD	Biosciences And Biotechnology
Lam, Kit, PHD, MD	Universtity Of California, Davis Ca Usa

Personnel Roster

Name, Degree	Department, Non-Host Institution Information
Lango, Jozsef, PHD	Molecular Biosciences Univ Of Ca, Davis Ca Usa
Lao, Victoria	Biosciences & Biotechnology
Lawton, Michael, MD	Neurological Surgery Uc San Francisco Ca Usa
Leri, Annarosa, MD	Anesthesia And Medicine Brigham And Women'S Hospital Ma Usa
Li, Ka Wan, PHD	Mol. & Cell. Neurobiology Vrije Universiteit Netherlands
Li, Tao, PHD	Dept Of Internal Medicine Univ. Of Ca, Davis Ca Usa
Lightsone, Felice C, PHD	Biosciences & Biotechnology Lawrence Livermore National Laboratory Ca Usa
Lin, Cindy, DVM	University Of California Davis Ca Usa
Luo, Juntao, PHD	Universtity Of California, Davis Ca Usa
Macdonald, R. Loch, MD, PHD	Surgery/Neurosurgery University Of Toronto Canada
Mack, Phil, PHD	University Of California Davis Ca Usa
Madrid, Dennis, BA	Microbiology & Immunology Albert Einstein College Of Medicine Ny Usa
Malfatti, Michael A, PHD, MS	Biosciences & Biotechnology Lawrence Livermore National Laboratory Ca Usa
Mccartt, Alan Daniel	Cams/LInI
Mckenna, Michael, MD	Professor Of Otology And Laryn Harvard Medical School, Meei Ma Usa
Mei, Henrik, BS	Drfz Berlin Germany
Mendelson, John, MD	California Pacific Medical Center Research Institute Ca Usa
Miller, Joshua W, PHD	Nutritional Sciences Rutgers University Nj Usa

Personnel Roster

Name, Degree	Department, Non-Host Institution Information
Murnick, Daniel E, PHD	Physics Rutgers University Nj Usa
Nambiar, Krishnan, PHD	Chemistry University Of California, Davis Ca Usa
Navid, Ali, PHD	Biology And Biotechnology Division
Nguyen, Nguyen, BS	Chemistry Uc Davis Ca Usa
Ognibene, Ted, PHD	Center For Ams
Pan, Chong-Xian, MD, PHD	Dept Of Internal Medicine Univ. Of Ca, Davis Ca Usa
Patel, Ketan, PHD	Cancer Studies/Molecular Med. University Of Leicester Uk
Paul, Dipayan, MS	Mathematics University Of Groningen The Netherlands
Petersen, Elijah J.	Biochemical Science Division Nist Md Usa
Pett-Ridge, Jennifer, PHD	Chemical Sciences
Phillips, Don R, PHD	Biochemistry La Trobe University Australia
Piero, Anversa, MD	Anesthesia And Medicine Brigham And Women'S Hospital Ma Usa
Rella, Chris	R&D Picarro, Inc. Ca Usa
Rice, Robert, PHD	Environmental Toxicology University Of California David Ca Usa
Saliz, Mehti, MD	Harvard Medical School Ma Usa
Santos, Felipe, MD	Department Of Otolaryngology Harvard Medical School, Meei Ma Usa
Sarachine Falso , Miranda J., PHD	Div Of Mathematics & Nat Sci Penn State Altoona Pa Usa

Personnel Roster

Name, Degree	Department, Non-Host Institution Information
Schneider, Philip J, MD	Surgical Oncology Univer Of Ca, Davis Ca Usa
Scow, Catherine, PHD	Land Air Water Resources Univ Of Ca, Davis Ca Usa
Semmler-Behnke, Manuela, DVM, PHD	Inst Of Inhalation Biolog Gsf Research Institute Germany
Shabab-Ferdows, Setti	Usda Ca Usa
Spalding, Kirsty L, PHD	Cellular And Molecular Biology Karolinska Institute Sweden
Steiger, Hans-Jakob, PHD	Department Of Neurosurgery Heinrich-Heine University Germany
Stephensen, Charles, PHD	Nutrition Univ. Of California, Davis Ca Usa
Stewart, Benjamin, PHD	Center For Ams
Su, Hua, MD	Anesthesia/Perioperative Care Uc San Francisco Ca Usa
Sudakin, Dan, MD	Oregon State University Or Usa
Thomas, Avi T., BS	
Turteltaub, Kenneth W, PHD	Biology And Biotechnology Division
Van Minnen, Jan, PHD	Mol. & Cell. Neurobiology Vrije Universiteit Netherlands
Vander Griend, Donald, PHD	Dept. Of Surgery University Of Chicago Il Usa
Wang, Yanchun, PHD, MD	University Of California Davis Ca Usa
Williams, David, PHD	Environ & Molecular Toxicology Oregon State University Or Usa
Willis, Dianna E, PHD	Nemours Biomedical Research Alfred I Dupont Hospital For Children De Usa
Wirries, Ina, MS	Dörner Germany

Personnel Roster

Name, Degree	Department, Non-Host Institution Information
Young, William L., MD	Anesthesia/Perioperative Care Uc San Francisco Ca Usa
Zhang, Hongyong, DVM, PHD	University Of California Davis Ca Usa

Collaborative Research Subproject Descriptions

ASSESSMENT OF VITAMIN B12 BIOAVAILABILITY FROM EGG (0054)

BTR Unit:	Collaborative Research	Resource Assigned ID:	RR proj #62
%BTR \$:	0.000%		

Investigator	Department/Non-Host Information
ALLEN, LINDSAY, PHD	
GARROD, MARJORIE G, BS	Medical Pathology UNIV OF CA, DAVIS MED CENTER, CA USA
GREEN, RALPH, MD	Medical Pathology UNIV OF CA, DAVIS MED CENTER, CA USA
MILLER, JOSHUA W, PHD	Nutritional Sciences RUTGERS UNIVERSITY, NJ USA

Subproject Description

Vitamin B12 (B12) is an essential nutrient required for the normal functioning of mammalian cells, particularly in the hematological and neurological systems. All naturally occurring B12 is synthesized exclusively by bacteria since both plants and animals lack the necessary enzymes for the biosynthesis of B12. Intestinal bacteria synthesize B12, which is absorbed and incorporated into animal tissues, as well as milk and eggs, making these products primary dietary sources of B12 for humans. However, there is only limited information available on the bioavailability of B12 from these dietary sources. A major technological advance, accelerator mass spectrometry (AMS), available through collaborative arrangements with the Lawrence Livermore National Laboratories, provides the capacity to detect trace levels of carbon-14 (¹⁴C) in biological samples at attomolar (10⁻¹⁸) concentrations. The instrument is therefore uniquely suited for assessing the biological fates of ¹⁴C-labeled substances after oral ingestion. In ongoing NIH-sponsored human studies, we are assessing the absorption, metabolism, and turnover of a single oral dose of ¹⁴C-labeled B12 (¹⁴C-B12) dissolved in water. In a pilot feasibility study funded by the National Cattlemen's Beef Association (NCBA), we have successfully enriched beef muscle and liver in vivo with ¹⁴C-B12. The goal of this proposal is to enrich chicken eggs in vivo with ¹⁴C-B12 and feed the ¹⁴C-B12-enriched eggs to healthy human subjects to determine B12 bioavailability from eggs. Eggs and dairy products are the only non-meat sources of vitamin B12.

Eggs

Chicken eggs enriched in vivo with ¹⁴C-B12 were fed to human volunteers. Baseline and post-ingestion blood, urine and stool samples were collected over a one-week period and assessed for ¹⁴C-B12 content using accelerator mass spectrometry. Bioavailability is being determined by measurement of the fraction of the total oral dose appearing in the stool, as well as calculation of the area under the curve (AUC) reflecting the appearance and disappearance of ¹⁴C-B12 from plasma. The hypothesis to be tested is that an accurate value for the bioavailability of B12 from chicken eggs can be determined, which in turn can allow us to definitively determine the nutritional value of eggs as a source of B12. Data is currently being analyzed.

Bread

Cobalamin (vitamin B12) deficiency is highly prevalent in the US and worldwide. Deficiency is most common in the elderly, with an average prevalence ≈25% over age 60 y. The situation is similar in California; in a representative sample of 1545 Latinos aged ≥60 y in the Sacramento region, we observed 6.5% deficiency and an additional 16% marginal status. About 40% of older persons with low serum cobalamin have food cobalamin malabsorption (F-CM), in which gastric atrophy and/or dysfunction is implicated. The gastric atrophy is often the result of chronic infection with *Helicobacter pylori*, which over time causes hypochlorhydria and diminished production of gastric pepsin, followed by

loss of mucosal integrity, and subsequent overgrowth of bacteria in the stomach and upper intestine. The hypochlorhydria and failure of pepsin production impairs release of cobalamin from proteins in food, and the bacterial overgrowth may compete for uptake of cobalamin.

Most elderly with F-CM can still absorb synthetic, crystalline forms of the vitamin when added to fortified cereals or used as supplements. It is generally recommended that elderly consume a higher proportion of their cobalamin in fortified foods than younger people. However there is considerable debate about whether crystalline cobalamin is absorbed as well by elderly with F-CM, especially if added as a fortificant to food. Elderly in general seem to need substantially higher intakes than the RDA to restore or maintain their cobalamin status, even when the vitamin is given as a supplement. The question of whether the most at-risk elderly, those with gastric atrophy, can absorb vitamin cobalamin added as a fortificant to cereals is especially timely based on recent reports that those with deficiency but high serum folate have five times the risk of cognitive impairment and anemia compared to those with normal cobalamin and folate status. Another problem is that when low serum cobalamin is diagnosed in older people, it is often ignored by medical practitioners until depletion progresses further, or there are symptoms of deficiency (such as nerve damage). Alternatively high oral doses, or injections, are prescribed for the rest of life. Diagnosis of F-CM is rarely attempted. It has not yet been investigated whether treating the *Helicobacter pylori* and bacterial overgrowth, achievable with the same medications, will improve ability to absorb cobalamin from food or in the crystalline form.

Our hypothesis is: Older adults with markers of gastric atrophy will absorb less ¹⁴C-cobalamin added as a fortificant to bread than will older adults with no sign of gastric atrophy. The research will employ a novel method to assess absorption using ¹⁴C-cobalamin that has very low levels of radioactivity which will be quantified by accelerator mass spectrometry. The ¹⁴C-cobalamin will be added to bread as a fortificant, at a level similar to that which might be added in flour fortification. Persons aged ≥60 y will be screened to identify those with low cobalamin, who will then be assessed for serum markers of gastric atrophy. Ten with evidence of gastric atrophy, and ten without, will consume the labeled bread, and absorption of cobalamin compared by quantifying radioactivity in plasma and urine during the next 24 h.

The main limitation of the study is that assessment of absorption of cobalamin from endogenously labeled food is not possible with the limited resources available, but this will be basis of future research proposals. However, we will be able to determine whether cobalamin added as a fortificant to bread is equally well absorbed by older persons with gastric atrophy and those without, and whether absorption of fortificant cobalamin can be improved by treatment for *Helicobacter pylori* and bacterial overgrowth.

Subproject Progress

Two papers were submitted for the Egg project: one on the ¹⁴C-B12 in vivo labeling of eggs and one on the bioavailability of ¹⁴C-B12 in vivo labeled egg in humans. Based on comments of reviewers the papers are being combined into a single one.

**CARBON-14 BASED AGE ANALYSIS OF METASTATIC PROSTATE
CANCER SAMPLES (0059)**

BTR Unit:	Collaborative Research	Resource Assigned ID:	RR Project #67
%BTR \$:	0.500%		

Investigator

BOVA, G. STEVEN, MD

Department/Non-Host Information**Subproject Description**

We have previously shown that genomic copy number analysis of multiple metastases from men with metastatic prostate cancer can be used to distinguish clonal from nonclonal genomic changes. Recent carbon-14 based studies have proven valuable in establishing the rate of cell turnover in myocardium and other tissues. The primary aim of a carbon-14 based study of metastatic prostate cancer DNA is to establish the relative age of various metastases in a given individual with metastatic prostate cancer, to provide valuable new insight into the natural history of metastatic cancer lesions.

The scientific purpose is to understand the natural history of metastatic cancer, so that diagnostic methods and treatment can be better tailored to each person with metastatic cancer. Do all metastases in an individual have a similar profile, or are they measurably different in age, and do these differences in age correspond to differences in clonal and subclonal copy number changes? Genomic DNA samples for the study are from the Johns Hopkins Integrated Clinical –Genomic Autopsy Study of Lethal Prostate Cancer. DNA samples studied include at least three anatomically separate metastatic sites and at least one noncancerous DNA sample from up to 12 subjects. The AMS technique is the only method with sufficient accuracy and precision to measure bomb-pulse carbon-14 in relatively small DNA samples.

Subproject Progress

Manuscript is in preparation.

STRATEGIES FOR PERSONALIZING LEUKEMIA TREATMENT (0074)

BTR Unit:	Collaborative Research	Resource Assigned ID:	RR Project #81
%BTR \$:	0.500%		

Investigator

BROWN, KAREN, PHD

DYER, MARTIN, BCH, DPHL, MB

PATEL, KETAN, PHD

Department/Non-Host Information

Cancer Biomarkers & Prevention

U OF LEICESTER, UK

Cancer Studies/Molecular Med.

UNIVERSITY OF LEICESTER, UK

Cancer Studies/Molecular Med.

UNIVERSITY OF LEICESTER, UK

Subproject Description

B-Cell chronic lymphocytic leukaemia (CLL) is a heterogeneous disease with variable clinical course. A fundamental drawback of CLL management is the lack of sensitive early indicators of disease progression to guide and monitor intervention strategies in individual patients. Recent studies have identified a proliferative compartment in every patient, indicating that increased cell birth rate, as well as resistance to apoptosis, contributes towards rising lymphocyte count.

This project aims to examine the potential clinical value of a newly-designed technique to measure CLL cell birth and death rates in vivo. In this approach, [¹⁴C]-labelled aspartic acid is incorporated into the DNA of actively proliferating CLL cells. The degree of ¹⁴C incorporation and subsequent loss of labelled cells from the circulation of an individual may identify prospectively patients destined to develop progressive disease. The strategy, which utilizes accelerator mass spectrometry (AMS), offers significant advantages over labelling methods involving deuterated water, due to vastly superior sensitivity, which permits kinetic profiling of smaller numbers of cells. The technique may also help assess sensitivity to different forms of chemotherapy by monitoring induction of apoptosis and/or inhibition of proliferation caused by chemotherapeutic agents, permitting the rational introduction of personalised therapy.

Subproject Progress

New project.

**CELL TURNOVER USING A NOVEL METHOD FOR THE
RETROSPECTIVE BIRTH DATING OF CELLS (0025)**

BTR Unit:	Collaborative Research	Resource Assigned ID:	RR Project #035
%BTR \$:	2.000%		

Investigator

BUCHHOLZ, BRUCE A, PHD

ALKASS, K, BS

BERGMANN, OLAF I, MD

DRUID, HENRIK, MD, PHD

FRISEN, JONAS, MD, PHD

SPALDING, KIRSTY L, PHD

Department/Non-Host Information

Center For Ams

Dept Of Forensic Medicine
KAROLINSKA INSTITUTE, SWEDENCellular & Molecular Biology
KAROLINSKA INSTITUTE, SWEDENDept Of Forensic Medicine
KAROLINSKA INSTITUTE, SWEDENCell And Molecular Biology
KAROLINSKA INSTITUTE, SWEDENCellular And Molecular Biology
KAROLINSKA INSTITUTE, SWEDEN**Subproject Description**

Neurogenesis is known to occur in specific regions of the adult animal brain, but the extent and comparability of neurogenesis in the adult human brain is much harder to determine, and to date largely unknown. Traditional methods used for dating cells are limited in the information they provide, or are not appropriate for human use. Thus, currently there is no method available to study cellular turnover in man. We propose to develop a method for the retrospective birth dating of cells. We are interested in using bomb pulse carbon-14 (C14) dating as a method for measuring the approximate age of specific populations of cells in the adult human brain. This method is based on establishing the proportion of the isotope C14 in genomic DNA. C14 measurements will be made using the highly sensitive accelerator mass spectrometer (AMS).

After a cell has terminally differentiated it does not divide again. Since the last cell division represents the last time point when the cell synthesized DNA, its chromosomal DNA will reflect the age when the cell was born. Traditionally, the slow decay of C14 relative to other carbon species has given it a temporal resolution of many years, however due to nuclear tests in the late 1950s and early 1960s, the level of C14 in the atmosphere has increased dramatically. This level has since dropped off in an exponential fashion, allowing one to resolve C14 differences in the range of years. Because DNA has a C14 content reflective of the time when it was synthesized, establishing the C14 content of chromosomal DNA will enable us to retrospectively birth date cells, and thus establish cellular turnover.

Crucial to the understanding of basic biological processes, is information about cellular turnover. As well as having an interest in normal cellular turnover, many diseases are thought to be affected in their generation of new cells. Information about cellular turnover in disease states may provide novel insights into the pathological processes of the disease, and possibly suggest new therapeutic strategies. Particular populations of cells will then be isolated using FACS analysis (which allows specific cell populations to be isolated e.g. one can sort for neurons using neuronal specific markers such as NeuN). The technique has also been applied to cardiomyocytes and adipocytes. We are investigating the turnover of lipids within adipocytes also. The use of enamel as a forensic tool to establish date of birth has been under investigation the past couple years.

Subproject Progress

Additional areas of the adult human brain have been examined. We expect additional publications soon.

We are examining a wide variety of teeth to establish a forensic dating technique that can be used in law enforcement applications.

**CANCER STEM CELL LONGEVITY AND METASTATIC POTENTIAL
IN BLADDER CANCER (0062)**

BTR Unit:	Collaborative Research	Resource Assigned ID:	RR Project #69
%BTR \$:	3.000%		

Investigator	Department/Non-Host Information
BUCHHOLZ, BRUCE A, PHD	Center For Ams
DEVERE WHITE, RALPH DR, BCH, MB, OTH	Urology Dept; Ucd Cancer Cente UNIV. OF CA, DAVIS, CA USA
SARACHINE FALSO , MIRANDA J., PHD	Div Of Mathematics & Nat Sci PENN STATE ALTOONA, PA USA

Subproject Description

Recent studies on stem-like cancer cells (SLCC) suggest a potential reason for the low efficacy of current cancer therapies and a way to improve them. Current cancer chemo- and radio-therapies target the rapidly-dividing cells in a tumor, however a tumor is a heterogeneous population of cells. Comprising a small amount of the population, SLCC remain relatively static and initiate the proliferative cells that account for the majority of the tumor mass. These SLCC are essentially dormant and resistant to chemo- and radio-therapy. SLCC remain after therapy and retain their ability to initiate proliferative cancer cells; therefore it is believed SLCC are the source of metastasis and relapse. However, there is controversy over the definition of SLCC and their role in cancer development and progression. Using bladder cancer as a model, we aim to show SLCC in bladder cancer tumors are much older than the bulk of the tumor, resistant to conventional chemo- and radio-therapy and responsible for metastasis and relapse.

The first part of the project involves isolating stem cell populations from three bladder cancer cell lines with a range of responses to the chemotherapeutic agent, cisplatin. The cycling of these cells will be examined in addition to their ability to demonstrate properties associated with stem cells as demonstrated through colony-forming assays, long term proliferation, differentiation and response to chemotherapy and radiation. Initially we plan to isolate CD44+, CD66C- as our stem cell population, as this has the strongest support in the literature. A side-scatter population has also been reported to possess stem cell capabilities in several bladder cancer lines and may also be investigated.

The second goal of the project is to investigate SLCC in fresh human bladder tumor samples. The age of SLCC isolated from fresh tumor samples along with non-stem cells will be determined using the ¹⁴C bomb curve with AMS. The elevation of ¹⁴C/¹²C in the DNA of these sorted cells will be used to assign a date of synthesis. This date of synthesis can be compared to the medical history of the patient in order to determine where on the timeline of tumor development SLCC fall.

Finally, we will develop a new tool for use in cancer research by producing SLCC with highly labeled DNA. Labeled cancer stem cells will be produced from one of the lines used in the initial part of the project based upon the percentage of stem cells and cell cycle properties. These labeled SLCC can then be used in research on their metastatic potential and as a tracer in cancer research. AMS will allow us to follow individual cells.

This work has the power to transform the current methods used to treat cancer. Existing therapies are developed primarily against the highly proliferative cells that make up the bulk of the tumor. By definitively measuring the longevity of SLCC, they can be confirmed as the reason for relapse and metastasis. The creation of labeled SLCC will provide a new tool to be used in cancer research and change the direction of the field.

Subproject Progress

A variety of surface markers and the aldefluor assay were used to analyze three bladder cancer cell lines with different sensitivity to cisplatin. Aldefluor seems to be the most promising marker as it isolates a small population from the resistant cell lines that demonstrate properties of stem cells.

Work is being done in order to optimize the number of cancer stem cells that can be isolated from human bladder tumors. The number of SLCC was too small to obtain a DNA age. The separated SLCC are currently frozen and are being saved for dating in the future or for use in a xenograft transplant.

DETERMINATION OF THE AGE OF ARTERIOVENOUS MALFORMATIONS (AVMS) (0075)

BTR Unit:	Collaborative Research	Resource Assigned ID:	RR Project #83
%BTR \$:	1.000%		

Investigator	Department/Non-Host Information
COOKE, DANIEL, MD	Radiology/Biomedical Imaging UC SAN FRANCISCO, CA USA
BUCHHOLZ, BRUCE A, PHD	Center For Ams
ETMINAN, NIMA, PHD	Department Of Neurosurgery UNIVERSITY OF DUESSELDORF, GERMANY
HETTS, STEVEN W., MD	Radiology/Biomedical Imaging UC SAN FRANCISCO, CA USA
KIM, HELEN, MPH, PHD	Anesthesia/Perioperative Care UC SAN FRANCISCO, CA USA
LAWTON, MICHAEL, MD	Neurological Surgery UC SAN FRANCISCO, CA USA
MACDONALD, R. LOCH, MD, PHD	Surgery/Neurosurgery UNIVERSITY OF TORONTO, CANADA
SU, HUA, MD	Anesthesia/Perioperative Care UC SAN FRANCISCO, CA USA
YOUNG, WILLIAM L., MD	Anesthesia/Perioperative Care UC SAN FRANCISCO, CA USA

Subproject Description

We will employ retrospective birth dating of cells using bomb pulse carbon-14 (¹⁴C) dating as a method for measuring the approximate age of arteriovenous malformations (AVMs). Brain AVMs (BAVMs) are complexes of tortuous, tangled vessels with connections between arteries to veins without a true capillary bed. AVMs are much larger than aneurysms so it is possible to date DNA of vascular cells comprising them and the structural collagen. AVMs are retrieved during surgery at UCSF and enter the tissue bank. The tissue would be disposed under usual circumstances. 20 AVM samples will be analyzed initially. Collagen and DNA will be extracted at UCSF and then analyzed for ¹⁴C by AMS to determine the age of collagen and DNA using the bomb pulse. By measuring the average age of DNA and collagen from AVMs we will determine molecular turnover and elucidate the progression and longevity of AVMs.

Subproject Progress

New project.

ROLE OF GENETIC AND NON-GENETIC MECHANISMS IN FURAN RISK (0053)

BTR Unit: Collaborative Research
%BTR \$: 0.000%**Resource Assigned ID:** RR Project #61

Investigator
DEKANT, WOLFGANG, PHD**Department/Non-Host Information**
Dept Of Toxicology
UNIVERSITY OF WURTZBURG, GERMANY

Subproject Description

Furan induces high incidences of cholangiocarcinoma and hepatocellular carcinomas in rats, but the mechanisms of tumor formation by furan in rodents are not well understood. The objective of this study is to assess DNA and protein binding after single oral administration of [3,4-¹⁴C]-furan to rats at doses close to human exposure. Thereby, information will be obtained on dose-response and molecular mechanisms of furan carcinogenicity as a basis for risk assessment of human furan exposures with food. This study WP 1.1 is designed to establish whether furan is genotoxic or if carcinogenicity may be a consequence of toxicity induced by protein-binding. Therefore DNA and proteins isolated from liver will be analysed to determine their ¹⁴C-content. Rats will be used due to their high sensitivity and the specific pathology induced by furan in this species.

Subproject Progress

no progress to report

LIFETIME OF EFFECTOR B CELLS (0057)

BTR Unit: Collaborative Research
%BTR \$: 1.000%

Resource Assigned ID: RR Proj #65

Investigator

DOERNER, THOMAS
 MEI, HENRIK, BS

Department/Non-Host Information**Subproject Description**

Human body integrity is largely based on a healthy immune system fighting pathogens. One key feature of the immune system is expression of memory, i.e. maintaining the ability to fight a pathogen upon its re-encounter effectively, even after years of pathogen absence. Based on that, vaccinations can prevent from severe infections. In pathogen defense, proteins binding specifically to the pathogen (antibodies) play a vital role. Antibodies tag the pathogen thus cause highly efficient degradation of the pathogen by specialized immune cells or neutralize it. The physical half-life of antibodies is less than 4 weeks. By contrast, specific antibodies induced by vaccination or infection can be readily detected in human blood serum after decades of antigen absence (humoral memory). This suggests that serum antibody levels are regulated at the stage of antibody secreting cells, and their specific precursors (memory B cells). For both cell types, longevity vs. continuous renewal is discussed as means to maintain the entire cell population and its composition, but both concepts were neither proven nor disproven physically.

In this project we want to measure ¹⁴C content in the DNA of antibody-secreting cells and memory B cells obtained from donors exposed to elevated levels of atmospheric ¹⁴C in Europe during the 1960s. Elevated levels of the ¹⁴C isotope can still be detected in cells being quiescent since then, revealing their age. ¹⁴C analysis will require accelerator mass spectrometry (AMS) ensuring a precise quantitation of very low levels of ¹⁴C from minimum DNA samples. In analogy to Spalding et al (Cell, Vol. 122, 133–143, July 15, 2005), this analysis will determine the in vivo age of candidate subsets of antibody-secreting cells and memory B cells and prove or disprove longevity of these cells, arguing in favor or against B cell longevity as a key element of humoral memory. The expected results will define new requirements for the successful development of effective vaccines.

AMS will be used in this project to determine the content of ¹⁴C in DNA samples isolated from immune cells, thereby revealing their average age. AMS is indispensable as the cell populations to analyze are very rare, thus yielding very small DNA sample size. According to previous studies, the concentration of the ¹⁴C within the sample is again very low. Comparable analysis has already been performed at the LLNL and thus we believe to conduct a successful project in close cooperation with the resource. Except for the very small sample size, no additional development of methodology appears to be required. The results from ¹⁴C analyses will be combined with different results derived from various immunological methods to provide new insight in functionally different effector B cells and mechanisms of humoral memory. AMS results are a vital part of the whole study.

Human blood and tissue will be obtained and specific cells will be extracted. From these cells, DNA will be isolated using established protocols compatible with latter ¹⁴C quantitation. DNA will be sent to the resource for ¹⁴C analysis.

Subproject Progress

Cell separation and purification techniques have been developed. Anticipate ¹⁴C measurements in the near future.

**DETERMINATION OF AGE OF RUPTURED AND UNRUPTURED
INTRACRANIAL ANEURYSMS (0066)**

BTR Unit:	Collaborative Research	Resource Assigned ID:	RR Proj#73
%BTR \$:	2.000%		

Investigator	Department/Non-Host Information
ETMINAN, NIMA, PHD	Department Of Neurosurgery UNIVERSITY OF DUESSELDORF, GERMANY
BRUCKNER, PETER, PHD	
DREIER, RITA, PHD	
HAENGGI, DANIEL, PHD	Department Of Neurosurgery HEINRICH-HEINE UNIVERSITY, GERMANY
MACDONALD, R. LOCH, MD, PHD	Surgery/Neurosurgery UNIVERSITY OF TORONTO, CANADA
STEIGER, HANS-JAKOB, PHD	Department Of Neurosurgery HEINRICH-HEINE UNIVERSITY, GERMANY

Subproject Description

Traditional methods used for dating cells are limited in the information they provide, or are not appropriate for human use. We developed a method for the retrospective birth dating of cells using bomb pulse carbon-14 (¹⁴C) dating as a method for measuring the approximate age of specific populations of cells in the adult human brain and other tissues. This method is based on establishing the proportion of the isotope ¹⁴C in genomic DNA.

After a cell has terminally differentiated it does not divide again. Since the last cell division represents the last time point when the cell synthesized DNA, its chromosomal DNA will reflect the age when the cell was born. Traditionally, the slow decay of ¹⁴C relative to other carbon isotopes has given it a temporal resolution of many years, however due to nuclear tests in the late 1950s and early 1960s, the level of ¹⁴C in the atmosphere doubled. This level has since dropped off in an exponential fashion, allowing one to resolve ¹⁴C differences in the range of years. Because DNA has a ¹⁴C content reflective of the time when it was synthesized, establishing the ¹⁴C content of chromosomal DNA will enable us to retrospectively birth date cells, and thus establish cellular turnover.

Rather than dating DNA, the objective of this study is to establish a method to date ruptured and unruptured intracranial aneurysms in patients treated with aneurysm clipping and excision. Subarachnoid hemorrhage (SAH) due to ruptured intracranial aneurysm is a major cause of morbidity and mortality in patients with cerebrovascular incidents, especially in younger patients. It accounts for 10% of strokes and affects 10 of every 100,000 inhabitants in North-America and Europe. Despite the efforts to optimize treatment, aneurysmal SAH continues to have a case fatality of 25 to 50%. Due to the poor prognosis of aneurysm rupture, there has been great interest in methods to detect and treat intracranial aneurysms prior to rupture. This study tests the hypothesis that ruptured aneurysms rapidly increase in size shortly before the time of rupture, and that their actual size thus would not correspond to the size of the aneurysm at any time before rupture. We hypothesize that rupture of intracranial aneurysms occurs as a result of instability and growth. Since the aneurysm grew acutely before rupture, the ruptured aneurysm should consist mainly of younger or new aneurysmal tissue. This hypothesis is supported by the observation that there are a large number of small aneurysms found after SAH in epidemiological studies, suggesting that a high proportion of aneurysms arise rapidly and quickly progress to rupture. Contrary to patients with SAH due to small ruptured aneurysms, epidemiological data show there are older patients with larger

incidental aneurysms. These cases might be explained through a selection effect where if aneurysm growth does not result in rupture, it could lead to an enlarged but stable aneurysm. Until further investigation it will remain controversial whether older patients who present with unruptured aneurysms that measure above average in diameter (i.e. more than 7-8 mm in diameter), have older aneurysms or whether these aneurysms are young and have progressed and formed rapidly.

The objective of this study is to establish a method to date ruptured and unruptured intracranial aneurysms in patients treated with aneurysm clipping and excision. However, these data are theoretical or observational and to date no study has analysed aneurysms from patients to determine the age of the aneurysm. We will test the hypothesis that unruptured aneurysms are older than ruptured aneurysms. The answer to our hypothesis would bear on the question of use of radiological screening of the population for incidental aneurysms. If aneurysms form rapidly and rupture or stabilize and never rupture, then screening may not be very useful. The results of the present study seek to determine whether this occurs.

We will determine age of ruptured and unruptured intracranial aneurysms using ¹⁴C dating of aneurysmal collagen, on the condition that the tissue is ultra-pure and not mixed with other tissues or cells. Hence, after isolation and purification of collagen from aneurysm walls excised from patients undergoing surgery, we will determine the age of the collagen using ¹⁴C birth dating in order to understand aneurysm formation in patients with ruptured aneurysms compared to unruptured aneurysms.

Subproject Progress

Experimental progress:

A total of 40 additional intracranial aneurysms were surgically removed. To date, 20 of these have undergone AMS for birth dating and the collagen from the remaining 20 aneurysms has been purified and will be sent for AMS within the next 14 days. A total of 8 mouse tendons served as collagen controls for AMS. The additional ¹⁴C data from the first 20 aneurysms confirms our previous findings that intracranial aneurysms contain mainly contemporary collagen, irrespective of their rupture status. This suggests that cerebral undergo constant collagen turnover or remodeling, which may underline previous mathematical models and assumptions on structural instability due to non-linear development.

Manuscripts

Etminan N, Dreier R, Buchholz B, Brucker P, Steiger HJ, Hänggi D, Macdonald RL, Exploring the age of intracranial aneurysms-Preliminary Results. Stroke. 2013 Jan 17. [Epub ahead of print] PMID: 23329209

**ID AND DEV OF BIOLOGICAL MARKERS OF HUMAN EXPOSURE TO THE
INSECTICIDE PERMETHRI (0027)**

BTR Unit:	Collaborative Research	Resource Assigned ID:	RR Proj #037
%BTR \$:	0.500%		

Investigator	Department/Non-Host Information
HAMMOCK, BRUCE, PHD	Entomology UC DAVIS, CA USA
BUCHHOLZ, BRUCE A, PHD	Center For Ams
GEE, SHIRLEY, MS	Entomology UC DAVIS, CA USA

Subproject Description

Agricultural workers, gardeners and homeowners are routinely exposed to the insecticide permethrin. Also, military personnel are exposed to the permethrin when using the DOD Insect Repellent System and over-the-counter lice soaps use permethrin as the active ingredient. A urinary metabolite of permethrin, that is in high abundance and is relatively stable, may be an ideal biomarker of exposure to this pesticide. In addition, the ratio of one metabolite to another may vary, according to the route of administration. The results of this study would be used to identify candidates for the development of a rapid, sensitive immunochemical based analytical method that can be used to routinely monitor human exposure to permethrin.

Objectives: The purpose of this study is to determine the human metabolite(s) of permethrin in urine following dermal exposure that are in greatest abundance and are the most stable. Accelerator mass spectrometry is a method for measuring levels of ¹⁴C several orders of magnitude more sensitive than liquid scintillation counting. With this high sensitivity we will conduct human metabolism studies at biologically relevant doses.

SPECIFIC AIMS: I. Develop an LC/MS method for separation of permethrin and its putative human metabolites. II. Determine the human metabolite profile of permethrin using accelerator mass spectrometry (AMS). III. Develop an immunoassay to the key metabolite identified in Objective II as a biomarker of human exposure to permethrin.

METHODOLOGY: For specific aim I, synthesize metabolite standards; develop an HPLC method to separate the putative pyrethroid metabolites using ultraviolet detection; determine the feasibility of an HPLC/mass spectrometry method for analysis of pyrethroid metabolites. For specific aim II, clinical exposure of humans to radiolabeled permethrin dermally and collection of urine, blood and saliva; separation of samples by methods developed in specific aim I and analysis of separated samples by accelerator mass spectrometry; identification of most prevalent metabolite from resultant data. For specific aim III, synthesis of haptens; development of antibodies; use of the haptens and antibodies in the development of an immunoassay for the most prevalent metabolite; validation of immunoassay.

EXPECTED PRODUCTS (MILESTONES): Literature review of putative human metabolites of permethrin; small quantities of synthesized metabolites of permethrin; an HPLC method for separating permethrin metabolites in human urine or saliva; identification of the most abundant human metabolite(s) by accelerator mass spectrometry; an immunoassay to detect the targeted human metabolite of permethrin

STATUS/RESULTS TO DATE: Literature review has been completed and putative major metabolites identified. All of the major metabolites have been synthesized or acquired. Using the metabolite standards a high performance liquid chromatography method for

their analysis has been developed. This method will later be used to identify metabolites found in human urine samples and the liquid chromatography-mass spectrophotometric method used for validation. Using the chemical knowledge from the preparation of metabolites, synthesis of haptens for immunoassay detection of these molecules is complete. Immunoassays for 3-phenoxybenzoic acid, the glycine conjugate of 3-phenoxybenzoic acid, the glycine conjugate of dichlorovinylchrysanthemic acid (DCCA) and the glucuronide conjugate of 3-phenoxybenzyl alcohol (see publication below) have been developed. An assay for free DCCA is in progress (manuscript in preparation). The assay for 3-phenoxybenzoic acid has been adapted to a sensitive, high throughput method (see publication below). Clinical exposures have been completed. All samples have been measured by AMS for total carbon-14. An estimate of the total dose absorbed (for 4 subjects) ranged from 0.06 to 0.27%. The permethrin is eliminated from the blood with a half life of about 12-24 hours. The urinary half life averaged 24 hours. Saliva was sampled, but permethrin does not appear to be excreted by that route. Liquid chromatography analysis of the metabolite pattern in urine is underway.

Conclusion: The results of this study will be used to identify candidates for the development of a rapid, sensitive immunochemical based analytical method that can be used to routinely monitor human exposure to permethrin. The ability to carefully monitor the presence of absorbed doses of permethrin will be a useful tool to prevent the possibility of human health effects due to permethrin exposure.

Subproject Progress

Manuscript is in preparation.

METHODS MONITOR TOXIC SUBSTAN AND/OR INDICATORS OF PRESENCE IN HUMANS&OTHER SPE (0038)

BTR Unit:	Collaborative Research	Resource Assigned ID:	RR Project #008
%BTR \$:	1.000%		

Investigator	Department/Non-Host Information
HAMMOCK, BRUCE, PHD	Entomology UC DAVIS, CA USA
AHN, KICHANG	
BUCKPITT, ALAN, PHD	Veterinary Medicine, Molecular UNIV OF CA, DAVIS, CA USA
DENNISON, MICHAEL, PHD	Environmental Toxicology UNIV CALIFORNIA, DAVIS, CA USA
RICE, ROBERT, PHD	Environmental Toxicology UNIVERSITY OF CALIFORNIA DAVID, CA USA

Subproject Description

Anthropogenic environmental toxins, even at low doses, cause some measure of biological change to take place, within plants, animals, microbes, or even humans. The goal of the UC Davis Superfund Program is to discover ways to observe and quantify these biomarkers of environmental impacts, so that the sources and causes of these impacts can be understood, assessed, traced, and remediated. To that end, the Program includes AMS quantitation as one of its competencies in its analytical core. This analytical core serves several of the Program projects, including Soil and Waste Transport, development of Immunochemical Biomarkers, Pulmonary Biomarkers, and Reproductive Biomarkers.

Accelerator mass spectrometry (AMS) plays an important role in the assessment of human exposure to toxic substances and in probing the mechanistic basis of toxicity in humans and in other host species. It is a core technology in our program of using biomarkers of environmental exposures to toxic substances from agricultural and industrial activities. We define urinary, pulmonary, reproductive, and circulating biomarkers of specific toxic exposures that are quantifiable using assays such as immunoassays, protein mass spectrometry, chromatography, and direct quantitation of isotope labeled toxins with AMS. AMS also provides calibration of the other assays through correlation of isotope label incorporation from toxins into a host. Quantitation of a derived biomarker is then calibrated by the uptake of toxin indicated by the AMS measurements.

In the case of Transport, the investigators are assessing the biological activity of the recently used fuel additive, methyl-tert-butyl-ether (MTBE), which leaked into the ground from fuel depots over the past decade. The binding of ¹⁴C-MTBE to mammalian protein is being studied to determine if the compound presents a threat to cellular systems. These laboratory studies are freely done with the levels of ¹⁴C needed to interact with cell cultures, but much of the Program is concerned with quantifying biomarkers in natural settings where radiotracer release is not possible. The preferred technology or quantifying recognizable biomarkers is the immunoassay which can eventually be made into field-usable kits. It is important to choose the right target for immunoassay development, such as the most likely metabolite or hormonal response of a chemical exposure. AMS is a particularly valuable technology for the discovery of optimal immunoassay targets because it reveals all metabolites of an isotope-labeled xenobiotic, even at low dose exposures. We found that the di-dealkyl mercapturate metabolites of atrazine were the most prominent lasting biomarkers of this ubiquitous herbicide in humans. Additionally, although 3-phenoxybenzoic acid is commonly used as a biomarker of exposure to pyrethroid insecticides, a conjugated form predominates. Immunoassays are developed for these

biomarkers.

There are "marker" species in ecosystems which are sensitive to environmental change, much like the canaries of past centuries in coal mines. An increasing number of pollutants are being seen as hormonal mimics that act as "poison" to a species by impairing its reproductive success. We are using small quail as one such example and are finding the metabolites of testosterone or cortisone in their fecal droppings, which are used as sample so as to avoid stress effects in a captured bird. The pattern of metabolites will be quantified to find which might be signs of slowly developing environmental stresses. The birds are small, and cannot be heavily dosed, so the sensitivity of AMS is needed.

Pulmonary responses to environmental chemicals need to be studied from respiration of environmentally relevant doses. The dose deposition in specific proteins of lung tissue of model animals is poorly quantified by present methods that provide a large exposure followed by protein separation on two-dimensional gels followed by long term (1 month) autoradiography. AMS has the sensitivity for appropriate doses and sequential gel separations have been worked out to maximize target protein discovery.

The AMS core serves to identify prominent biomarkers of exposure for fieldable assay development and quantifies exposures to labeled compounds for the Program researchers.

Subproject Progress

Two subprojects have used AMS in the past year to measure adduct formation in target tissues. Prof. Bob Rice used the common antimicrobial found in bar soap, ¹⁴C-TCC (triclocarban), in culture with human keratinocytes to measure protein adduct formation at baseline and with P450 induction. The adduct level was much higher with P450 induction, suggesting possible causes for sensitization and contact dermatitis after skin exposure to TCC. The work is found in a paper published in 2012 and was presented at the 2012 SOT meeting.

Prof. Alan Buckpitt and Dr. Buchholz (LLNL) have conducted initial ex vivo exposures of mouse and rat respiratory tissues to naphthalene (NA) in search of DNA-NA adducts. NA forms protein adducts abundantly. Initial work has measured DNA-NA adducts in tissues that produced tumors after chronic exposures to NA. The work was presented in a poster at the 2012 SOT meeting. Work is being done to ensure DNA separations are free of protein that could give erroneous results.

**DEVELOP ASSAY TO QUANT PLAT-DNA ADDUCTS & PREDICT
RESPONSE TO CHEMOTHERA (0048)**

BTR Unit:	Collaborative Research	Resource Assigned ID:	RR Project #057
%BTR \$:	2.500%		

Investigator

HENDERSON, PAUL T, BS, PHD

GANDARA, DAVID, MD

MACK, PHIL, PHD

PAN, CHONG-XIAN, MD, PHD

WANG, YANCHUN, MD, PHD

ZHANG, HONGYONG, DVM, PHD

Department/Non-Host Information

Urology

UNIVERSITY OF CALIFORNIA, DAVIS, CA USA

Dept Of Internal Medicine

UNIV. OF CA, DAVIS, CA USA

Subproject Description

AMS will be applied to measuring the platinum-DNA adducts for drug pharmacokinetics. In spite of the importance of platinum-based anticancer drugs (cisplatin, carboplatin and oxaliplatin), their mechanisms of action, repair of damaged DNA and pharmacokinetics are unclear because of the detection limit of conventional methods (conventional methods have failed in quantifying Pt-DNA adducts with cells incubated with a pharmacological dose of the anticancer agent, which is the reason we need the sensitivity of AMS). In order to address these important issues, ¹⁴C-labeled carboplatin and oxaliplatin will be administered to E. coli, human cells, and bladder cancer patients, which may overcome the previous detection limits even at sub-pharmacological doses. The goals are to use AMS to elucidate their in vivo mechanism of action, to correlate Pt-DNA adduct level with cell death using a variety of human cancer cells, to determine the pharmacokinetics of the patients dosed with ¹⁴C-labeled carboplatin and oxaliplatin, and to ultimately correlate the pharmacokinetic results to individual outcome (patient survival). Experimentally, after dosing a number of human cancer cells or cancer patients with radioactive platinum-based anticancer drugs, cell lysis and extracted platinumated DNA will be measured by AMS. These 'real-time pharmacokinetics' will allow determination of which cancer patients will benefit from platinum treatment and which will be resistant to the drugs. Because of the high sensitivity, AMS is the very best technology for realizing these challenging goals.

Subproject Progress

A UC Davis clinical study aimed at developing a test to predict response to platinum-based chemotherapy was expanded to include three additional sites: UCSF, UCLA and USC. To date eleven patients have been accrued to the study. All of the patients were given a microdose of carboplatin that was radiocarbon labeled. Blood and tumor biopsy samples were taken within 24h after microdosing. Within four weeks patients began platinum-based chemotherapy and were monitored for objective responses including reduction in tumor volume, progression-free survival and overall survival. Response data are starting to be acquired, and the expected trend that the highest microdose-induced drug-DNA adduct levels correlate to response to therapy was observed. However, the trend is not yet statistically significant and we need to accrue additional patients.

FEASIBILITY OF NANOPARTICLE-MEDIATED PACLITAXEL DELIVERY (0073)

BTR Unit:	Collaborative Research	Resource Assigned ID:	RR Project #66
%BTR \$:	0.500%		

Investigator

HENDERSON, PAUL T, BS, PHD

HU, WENWU, PHD

LAM, KIT, MD, PHD

LUO, JUNTAO, PHD

MALFATTI, MICHAEL A, MS, PHD

PAN, CHONG-XIAN, MD, PHD

Department/Non-Host Information

Urology

UNIVERSITY OF CALIFORNIA, DAVIS, CA USA

Dept Of Internal Medicine

UNIVERSITY OF CALIFORNIA, DAVIS, CA USA

Biosciences & Biotechnology

LAWRENCE LIVERMORE NATIONAL

LABORATORY, CA USA

Dept Of Internal Medicine

UNIV. OF CA, DAVIS, CA USA

Subproject Description

Paclitaxel (Taxol) is a standard and effective chemotherapeutic for many cancer types, including breast cancer, ovarian cancer, small cell lung cancer and non-small cell lung cancer. Since paclitaxel (PTX) has very limited solubility in water, the formulation of this drug requires Cremophor EL which causes significant side effects, such as allergic reactions.

Consequently, patients receiving PTX require premedication with histamine blockers and steroids. We proposed to use a novel water-soluble nanoparticle-based formulation to enable

better drug delivery of PTX. This proprietary formulation has been demonstrated to be safer

and more effective in preclinical studies compared to currently available formulations. For example, some tumor-bearing mice exposed to PTX-nanoparticles were cured of cancer, which was not observed for the Cremophor formulation. The goal of this study is to advance

the use of PTX-nanoparticles into Phase 0 clinical studies by using ¹⁴C-paclitaxel to label the

nanoparticles followed by absorption, distribution, metabolism and excretion (ADME) studies

in nude mice with human tumor xenografts. The particles will be synthesized according to established protocols which includes addition of the ¹⁴C-paclitaxel in the final self-assemble

step. Mice will be dosed with PTX-nanoparticles of sufficient specific activity to allow tracing by liquid scintillation counting (LSC). The LSC experiments will allow calculation of the specific activity needed for the studies to be repeated using AMS-based detection of the ¹⁴C-paclitaxel. AMS is needed as part of the project in order to predict dose formulations and develop methods for use in clinical studies (to be proposed later). It is anticipated that LSC has sufficient sensitivity for human phase 0 studies, but this needs to be established empirically with the proposed animal studies. If successful, the feasibility study data will be submitted to FDA for an exploratory IND application in order to determine the pharmacokinetics of PTX-nanoparticles in humans in order to justify subsequent Phase 1 studies.

Subproject Progress

Background: A multifunctional telodendrimer-based micelle system was characterized for delivery of imaging and chemotherapy agents to mouse tumor xenografts. Previous optical

imaging studies demonstrated qualitatively that these classes of nanoparticles, called nanomicelles, preferentially accumulate at tumor sites in mice. The research reported herein describes the detailed quantitative imaging and biodistribution profiling of nanomicelles loaded with a cargo of paclitaxel.

Methods: The telodendrimer was covalently labeled with ¹²⁵I and the nanomicelles were loaded with ¹⁴C-paclitaxel, which allowed measurement of pharmacokinetics and biodistribution in the mice using microSPECT/CT imaging and liquid scintillation counting, respectively.

Results: The radio imaging data showed preferential accumulation of nanomicelles at the tumor site along with a slower clearance rate than paclitaxel formulated in Cremophor EL (Taxol®). Liquid scintillation counting confirmed that ¹⁴C-labeled paclitaxel sequestered in nanomicelles had increased uptake by tumor tissue and slower pharmacokinetics than Taxol.

Conclusion: Overall, the results indicate that nanomicelle-formulated paclitaxel is a potentially superior formulation compared with Taxol in terms of water solubility, pharmacokinetics, and tumor accumulation, and may be clinically useful for both tumor imaging and improved chemotherapy applications.

This research was extended to AMS by the use of cannulated rats for a microdosing/therapeutic dosing comparison study as a test of the feasibility of a Phase 0 human study. Rats were dosed with 1 percent of the therapeutic dose of paclitaxel and a therapeutically relevant dose. The drug was formulated in Chremophor, nanomicelles and a new crosslinked derivative of nanomicelles. As observed in the previous mouse study, the area under the curve (total in vivo drug exposure) was increased by both types of nanomicelles compared to the same dose of drug in Chremophor. However, there was nonlinearity in the kinetics between therapeutic and microdoses, which is currently under investigation.

TROPHIC TRANSFER OF 14C-LABELED CARBON NANOTUBES (0076)

BTR Unit:	Collaborative Research	Resource Assigned ID:	RR Project #80
%BTR \$:	1.000%		

Investigator	Department/Non-Host Information
HOLDEN, PARTICIA, PHD	Bren School Of Env Sci/Mgmt UC SANTA BARBARA, CA USA
BUCHHOLZ, BRUCE A, PHD	Center For Ams
PETERSEN, ELIJAH J.	Biochemical Science Division NIST, MD USA

Subproject Description

The potential environmental impacts of nanotechnology are mostly unknown, but could include bioaccumulation of nanoparticles in environmental organisms, biotransformation of nanoparticles, trophic transfer via predator-prey interactions, and toxicity. In prior research, bioaccumulation of CdSe quantum dot nanoparticles was quantified in *Pseudomonas aeruginosa* bacteria; trophic transfer and biomagnification of nanoparticles and of Cd was then quantified in the protozoan predator, *Tetrahymena thermophila*. Both the prey and predator were intoxicated, while nanoparticles substantially remained intact, during the bioaccumulation and predation-associated biomagnification processes. Here, we will study multiwalled carbon nanotubes (MWCNTs), which are increasingly incorporated into consumer goods and thus entering the environment where they can impact organisms and water quality, and thus human health. This research regards the use of 14C-labeled MWCNTs that can be sensitively traced through prey, predator, and into transformation products using accelerator mass spectrometry (AMS) at LLNL. The 14C-labeled MWCNTs, synthesized by collaborators at the National Institutes for Standards and Testing (NIST), are provided to UCSB researchers who administer the nanoparticles via aqueous growth media to *Pseudomonas aeruginosa*. AMS is used to sensitively quantify 14C label incorporated into bacterial cells. Bacteria are fed to their protozoan predator, wherein time course samples are acquired during feeding, and for which food vacuoles—the protozoan digestive compartment where nanoparticles may be decomposed or translocated to the cytoplasm and organelles—are separately analyzed for 14C label incorporation. The overarching hypothesis to be tested is that MWCNTs are trophically transferred and biomagnified in this two-step food chain, with little MWCNT decomposition in the process. Further, we hypothesize that protozoan feeding on nanoparticle-contaminated bacteria will result in toxicity, whereas direct indiscriminate uptake of nanoparticles in rich media without bacteria will result only in expulsion of nanoparticle-rich fecal pellets. The results of this research will provide novel insights into the potential ecotoxicology of MWCNTs, and the fates of these materials as they accumulate in the environment.

Subproject Progress

Doctoral student training and generation of bacterial growth curves in growth media.
Literature review on MWCNTs' effects on bacteria and protozoa. Determination of MWCNT exposure concentration to bacteria and protozoa.

**NOVEL AGENTS FOR GRAM-NEGATIVE BIODEFENSE
PATHOGENS (0070)**

BTR Unit:	Collaborative Research	Resource Assigned ID:	RR Project # 77
%BTR \$:	3.000%		

Investigator

LIGHTSONE, FELICE C, PHD

LAO, VICTORIA

MALFATTI, MICHAEL A, MS, PHD

Department/Non-Host Information
Biosciences & Biotechnology
LAWRENCE LIVERMORE NATIONAL
LABORATORY, CA USA

Biosciences & Biotechnology

Biosciences & Biotechnology
LAWRENCE LIVERMORE NATIONAL
LABORATORY, CA USA
Subproject Description

This project is to discover and develop a novel broad-spectrum antimicrobial compound active against the priority bacterial pathogens in the Strategy and Implementation Plan for Chemical, Biological, Radiological and Nuclear Threats. The top priority threats addressed include *F. tularensis* and *Y. pestis*. The aim of the research is to provide a single solution to multiple threats, complementing older antibacterials that are already known to be vulnerable to resistance mechanisms. The overall scope of the work ensures that multiple compounds will advance to optimization, allowing for multiple opportunities for success. The goal in optimization is to advance one to three promising compounds into preclinical evaluation. The net result of this project will be an antimicrobial agent with unprecedented spectrum and safety.

To assess the efficacy of these leads in vivo testing need to be done, including determining the pharmacokinetics (PK) and metabolism of the drug leads. Very low concentrations of drug and metabolites can be readily assessed by the application of Accelerator Mass Spectrometry to metabolite analysis, PK assessment, protein binding and tissue distribution studies. AMS is used to achieve the sensitivity needed for low dose studies of both animal and human samples or tissue distribution studies. Whereas current methods of decay counting usually require high doses with high-specific-activity compounds, AMS is exquisitely sensitive and selective for ¹⁴C analysis. We can quantify with high precision the distribution of lead compounds (and their metabolites) through the body at 100 – 1000-fold lower concentrations than those likely to be required for therapeutic efficacy which is not achievable using decay counting methods.

Subproject Progress

Determining the pharmacokinetics (PK) of a drug is essential when evaluating its efficacy and safety. The ability to obtain PK information early in the drug development process can help decide if future development is warranted before costly clinical trials. Microdosing has been developed to assess PK at ultra low doses with virtually no adverse drug effects, which can then be extrapolated to therapeutic doses. Part 1 of the study assessed the PK of an antimicrobial drug lead at 3 proposed therapeutic doses (3, 10, & 30 mg/kg), to determine dose proportionality. Part 2 compared the PK of a ¹⁴C-microdose (0.01 mg/kg) to a ¹⁴C-

therapeutic dose (3 mg/kg) to determine if a microdose can predict the PK of a therapeutic dose, and to support the effort of predicting human PK. In both studies Sprague Dawley rats were exposed to an IV dose, and blood was collected at set time points. PK parameters were quantified using accelerator mass spectrometry. In part 1 of the study, the plasma PK over the 3 doses was proportional. Over the 10-fold dose range the C_{max} and AUC had a 9.5 and 15.8 fold increase, respectively. In part 2, the PK from rats dosed with a ¹⁴C-microdose vs. a ¹⁴C-therapeutic dose displayed dose linearity. The mean distribution T_{1/2} for both doses was 0.26 h. The volume of distribution was 30.5 L/kg and 30.7 L/kg in the micro and therapeutic dosed animals, respectively. The mean AUC_{0-t} values, when normalized to 1.0 mg/kg, were 244.9 ng•hr/mL for the microdose and 451.7 ng•hr/mL for the therapeutic dose. This 1.8-fold difference in AUC_{0-t} following a 300-fold difference in dose is considered linear across the dose range. Further support of dose linearity was found when dose-normalized values were compared across all doses. Based on the results, the PK from the microdosed animals was considered to be predictive of the PK from the therapeutically dosed animals.

MULTI-SCALE TOXICOLOGY INITIATIVE (0069)

BTR Unit:	Collaborative Research	Resource Assigned ID:	RR Project #76
%BTR \$:	3.000%		

Investigator

MALFATTI, MICHAEL A, MS, PHD

Department/Non-Host InformationBiosciences & Biotechnology
LAWRENCE LIVERMORE NATIONAL
LABORATORY, CA USA

ENRIGHT, HEATHER, PHD

Biosciences And Biotechnology

Subproject Description

The purpose of this project is to develop a suite of new capabilities for assessing the safety of materials designed for use in humans or to which humans will be exposed. Assessing potential safety/toxicity of nanomaterials will be the initial focusing problem for this effort.

The goals are to combine existing measurement capabilities into a unique multiscale toxicology assessment pipeline, to integrate data from different end-point studies into a comprehensive model that links the in vitro cellular effects to the in vivo whole animal response and most importantly to develop a suite of new high throughput technologies that, when combined, will add new insights into the potential toxicities of materials developed for use in humans. Such insights should reduce the time it takes to complete safety assessments and thus reduce the time it takes to make new products available to the public. It should also improve the accuracy of predicting human toxicity. Importantly, the end products of this research will be of similar value for assessing the safety of chemical

agents used in commerce as well as those found in the environment. Nanoparticles are a class of materials that is receiving increasing interest for a variety of applications ranging from electronics, catalysis, photovoltaic materials to drug delivery devices. Research on the

application of nanomaterials is intensifying and over 200 potential product uses are being investigated. One general area of use that has received much attention is in health care. Certain properties of nanomaterials make them ideal candidates for use in drug delivery, medical diagnostics & imaging, implantable devices, biomaterials or as pharmaceuticals themselves. While much emphasis has been invested in exploring applications of nanomaterials, relatively little effort has been directed towards understanding the health risks associated with their use. Use of nanomaterials in pharmaceuticals and medical devices will require safety evaluations as part of the development process prior to FDA approval.

Subproject Progress

Silica dioxide nanoparticles (SiNPs) are used for a wide variety of commercial and biomedical applications, yet little is known about their toxicology and safety. An important aspect in better understanding SiNP safety is characterization of the factors that affect SiNP biodistribution. Currently, comprehensive studies on biodistribution are lacking, most likely due to the lack of suitable analytical methods. To investigate the relationship between administered dose, PK, and long-term biodistribution, the technique of accelerator mass spectrometry (AMS) was used to determine the PK and tissue distribution of ¹⁴C-SiNPs in mice. AMS quantifies extremely low concentrations of radiolabeled chemicals, and new here labeled nanoparticles, enabling long-term kinetic studies. Mice were administered a single intravenous dose of ¹⁴C-SiNPs. Blood and tissue were collected at specific time points following dose administration over an 8-week time period, and SiNP concentrations determined by quantifying the radiocarbon equivalents in each sample by AMS. Plasma PK analysis showed that SiNPs were rapidly cleared from the central compartment with a half-life of 0.38 h. The large apparent volume of distribution of 2058.1 ml/kg indicated extensive distribution to tissues. SiNPs were rapidly distributed to tissues of the reticuloendothelial system including liver, spleen, kidney, lung, and bone marrow, and

persisted in the tissue over the 8-week time course, raising questions about the potential for bioaccumulation and associated long-term effects. SiNP elimination occurred by both renal and biliary routes. These results indicate that AMS is an effective tool to accurately and precisely quantify the long-term kinetics and biodistribution of SiNPs in vivo.

LASER BASED 14C COUNTING FOR BIOMEDICAL STUDIES (0032)

BTR Unit:	Collaborative Research	Resource Assigned ID:	RR Project #042
%BTR \$:	2.000%		

Investigator	Department/Non-Host Information
MURNICK, DANIEL E, PHD	Physics RUTGERS UNIVERSITY, NJ USA
BACHA, TULA, PHD	
OGNIBENE, TED, PHD	Center For Ams
PAUL, DIPAYAN, MS	Mathematics UNIVERSITY OF GRONINGEN , THE NETHERLANDS

Subproject Description

The use of isotopic labeling with carbon 14 is widespread in biological and medical research, medical diagnostics and drug discovery and development. The aim of this project is to complement AMS with a device, suitable for routine laboratory use, for atom counting of the tracer ^{14}C . The $^{14}\text{CO}_2$ instrumentation will have sensitivity orders of magnitude greater than possible with scintillation (decay) detection and will compete with typical tandem accelerator mass spectrometers (AMS) that have demonstrated sensitivity at the picomole to the attomole level. The enhanced sensitivity is important for low dose and small sample tracer studies, long-term metabolic studies, pharmacokinetics studies and is being studied as a possible tool for protein sequencing and micro-imaging studies. The system developed is based on intracavity optogalvanic spectroscopy (ICOGS) with a non-linear electrical response to $^{14}\text{CO}_2$ - laser interactions. Such a system can be used to quantitatively measure small samples of ^{14}C -enriched carbon dioxide. Results of measurements with unknown samples will quantify improvements required to achieve ultimate sensitivity.

Subproject Progress

In the past year two prototype instruments have been used for instrumentation tests and calibration studies. Major advances have been made on theoretical studies to allow better separation of signal and background and to develop robust signal handling algorithms.

We have been testing the new algorithms primarily using standards, with improved calibration results. The figure below, for example is consistent with earlier calibration results, but much more robust. The points include a blank from samples prepared at LLNL as well as a sample

that was expected to be ~ 75 M; but appears on our calibration curve at ~ 125 M.

The figure shows ICOGS extracted signal (arbitrary units) plotted against AMS values in units of Modern. The point at .001 M is "dead" CO_2 indicating a limit of detection near 10-15 $^{14}\text{C}/^{12}\text{C}$. The point at 0.1M is the blank used for isotope dilution in oxidizing samples at the LLNL Bio-AMS lab. The highest point is an "unknown" estimated by preparation to be ~ 75 M, but appearing on the calibration curve closer to ~ 125 M.

Other "unknown" samples have been split for analysis at The BioAMS facility and with the ICOGS instrument. Agreement has been reasonable, $\pm \sim 20\%$, due to instabilities in the ICOGS instrument as well as due to losses in CO_2 in transferring oxidized material from Livermore to Newark.

QUANTIFICATION OF 14C BY OPTICAL SPECTROMETRY (0077)

BTR Unit:	Collaborative Research	Resource Assigned ID:	RR Project #82
%BTR \$:	3.000%		

Investigator	Department/Non-Host Information
OGNIBENE, TED, PHD	Center For Ams
MCCARTT, ALAN DANIEL	Cams/LInI
RELLA, CHRIS	R&D PICARRO, INC., CA USA

Subproject Description

Accelerator Mass Spectrometry (AMS) is currently the most sensitive method for quantitation of radiocarbon in biological samples and can routinely quantify an attomole of ¹⁴C-labeled agent with a precision of around 1% in real samples. This technology has been used in a variety of studies over the last 20 years where very high sensitivity is needed. However, AMS instrument costs are on the order of several millions of dollars and they require support from a highly trained technical staff and location in specialized facilities. Additionally, traditional AMS ion sources require that the sample be presented as a solid graphitic target. This necessitates the time-consuming conversion of carbonaceous material to graphite in a technically complex procedure that limits overall measurement throughput and can keep per sample charges unreasonably high. AMS instrumentation requires expert knowledge and specialized facilities which are only available at a very few places in the world. Development of a method that has a footprint of a few square feet and that can be operated without the need for a staff of trained operators and physicists and without the need for expensive and large instrumentation is a gap that, if filled, would open new avenues of research and clinical studies by its low cost, ease of use and broad availability.

Quantification of ¹⁴C via optical spectroscopy offers an approach that eliminates many of the shortcomings of an accelerator-based system. This technique can exploit the selectivity of the ro-vibrational transitions in the infrared spectral region and their isotopic shifts. The sensitive infrared-based approach utilizes an intra-cavity laser technique to increase the optical path-length called Cavity Ring Down Spectroscopy (CDRS).

We will develop a table-top sized spectroscopic method to quantify ¹⁴C in milligram sized biological samples at levels down to the isotopic natural abundance (¹⁴C:C ratio of 1:1012) using a technique called Cavity Ring Down Spectroscopy with excitation lines in the mid-infrared.

Subproject Progress

New project.

**METABOLITE PROFILING OF CELLULOLYTIC MICROORGANISMS
FOR BIOFUEL PRODUCTION. (0065)**

BTR Unit:	Collaborative Research	Resource Assigned ID:	RR Proj #70
%BTR \$:	2.500%		

Investigator	Department/Non-Host Information
PETT-RIDGE, JENNIFER, PHD	Chemical Sciences
JIAO, YONGQIN, PHD	Biology And Biotechnology Division
NAVID, ALI, PHD	Biology And Biotechnology Division
STEWART, BENJAMIN, PHD	Center For Ams

Subproject Description

For established cellulose-based H₂-producing co-culture systems, modest H₂ production has been achieved in bioreactors with syntrophic microorganisms. However, the metabolic interactions between the organisms are poorly understood. The goal of this project is to characterize metabolic interactions between H₂-producing organisms in order to enable system optimization for improving H₂ production efficiency.

Subproject Progress

A manuscript is currently in preparation demonstrating increased H₂ production in a co-culture system compared to pure culture on cellulose.

TURNOVER OF CELLS IN THE HUMAN MYOCARDIUM (0064)

BTR Unit:	Collaborative Research	Resource Assigned ID:	RR Proj #71
%BTR \$:	0.500%		

Investigator	Department/Non-Host Information
PIERO, ANVERSA, MD	Anesthesia And Medicine BRIGHAM AND WOMEN'S HOSPITAL, MA USA
BUCHHOLZ, BRUCE A, PHD	Center For Ams
JAN, KAJSTURA, PHD	Anesthesia And Medicine BRIGHAM AND WOMEN'S HOSPITAL, MA USA
LERI, ANNAROSA, MD	Anesthesia And Medicine BRIGHAM AND WOMEN'S HOSPITAL, MA USA

Subproject Description

For nearly 4 decades, the human heart has been considered a post-mitotic organ composed of a predetermined number of myocytes, which is established at the end of gestation. According to this Old Paradigm, the generation of myocytes ceases at birth and their number is preserved throughout life until death of the organ and organism. Cardiac growth postnatally and organ hypertrophy in the adult occur only by myocyte enlargement. On this premise, the age of myocytes corresponds to the age of the organ and organism, i.e., cellular, organ and organism age coincide. Recent results from our laboratory and others have documented that tissue specific stem cells reside in the human heart. Human cardiac stem cells (hCSCs) are self-renewing and multipotent in vitro and in vivo; hCSCs differentiate in myocytes, and vascular smooth muscle cells (SMCs) and endothelial cells (ECs) organized in coronary vessels. The recognition that the human heart possesses a stem cell compartment has imposed a reevaluation of cardiac homeostasis, aging and pathology. The New Paradigm refutes the conviction that myocytes are formed only during embryonic development and suggests that the replacement of coronary vascular SMCs and ECs is regulated by differentiation of hCSCs rather than by the ability of these mature cells to divide. A novel conceptual framework of the heart has emerged; the Heart Is a Self-renewing Organ characterized by a compartment of resident stem cells. This discovery has laid the ground work for the use of hCSCs in the treatment of the failing heart. Currently, two phase I clinical trials are in progress (ClinicalTrials.gov Identifier: NCT00474461; Identifier: NCT00893360).

Our understanding of the cellular processes implicated in the maturation, homeostasis and repair of the human heart is extremely deficient and the need for basic information is striking. Findings in nematodes, fruit flies, zebra fish and rodents have often been translated to human beings with little caution, emphasizing the necessity to study the fundamental principles that regulate the plasticity of the myocardium during the lifespan of women and men. Moreover, the mechanisms modulating the response of the female and male heart to ischemic and non-ischemic myocardial injury and the principal factors conditioning end-stage heart failure and death in humans are at present unknown. Thus, the major objective of this application is to establish the rate of myocyte and non-myocyte turnover mediated by hCSC activation and differentiation in the developing, adult, aging and failing heart. To achieve this goal, we will employ retrospective ¹⁴C birth dating of cardiac cells to establish the average age of myocytes and non-myocytes. This information will be complemented by defining the age distribution of myocytes and non-myocytes utilizing a mathematical model of age-structured cell populations. These data will offer a novel comprehensive perspective of the cellular processes which govern the lifespan of the human heart. This information is critical for the recognition of the mechanisms that control the dynamics of the human heart, its growth reserve, adaptation to stress and failure.

Subproject Progress

New program project grant starting winter 2013 investigating aging of heart.

**OTOPATHOLOGY BY LIGHT MICROSCOPY AND MOLECULAR
TECHNIQUES (0072)**

BTR Unit:	Collaborative Research	Resource Assigned ID:	RR Project # 79
%BTR \$:	0.500%		

Investigator

SANTOS, FELIPE, MD

EDGE, ALBERT, PHD

MCKENNA, MICHAEL, MD

SALIZ, MEHTI, MD

Department/Non-Host InformationDepartment Of Otolaryngology
HARVARD MEDICAL SCHOOL, MEEI, MA USAProfessor Of Otology And Laryn
HARVARD MEDICAL SCHOOL, MEEI, MA USA**Subproject Description**

Retrospective ¹⁴C birth dating of cells is based on the increase in ¹⁴C in the atmosphere during aboveground nuclear testing in the 1950s and early 1960s and the dramatic drop in ¹⁴C after the agreement on nuclear testing was signed in 1963. Atmospheric ¹⁴C levels have decreased exponentially at a rate of ~50% per 11 years. ¹⁴C is not directly assimilated by humans but it is absorbed by plants and through daily ingestion is integrated in replicating cells in S phase. The decrease in ¹⁴C is constantly monitored and the average birth date of cells can be defined by tracing back the year in which an equivalent atmospheric ¹⁴C was detected. ¹⁴C birth dating has been applied to teeth, brain, fat, pancreas, and heart. ¹⁴C birth dating provides information similar to that generated by the incorporation of thymidine analogs in animal models, an analysis that has only been rarely possible in human beings.

The turnover of cells within the inner ear is difficult to study in humans. Techniques used in animals involving incorporation of radiolabeled nucleotides cannot be used in humans because of potential toxicity. Absence of mitotic figures on histology cannot be used to exclude the possibility of cell turnover because mitosis constitutes a small fraction of time within the cell cycle.

It has been traditionally assumed that the sensory and non-sensory cells of the inner ear are post-mitotic, but this dogma has never been adequately tested in the human. Recent work has suggested that there may be low-grade cellular turnover occurring in non-sensory supporting cells of the organ of Corti in the human, based on detection of cells expressing immunomarkers of proliferation. There is also evidence that stem cells may persist in the cochlea and vestibular end organs in the adult human. Knowledge about the fundamental question of cell turnover has far reaching implications for the emerging field of regenerative medicine and cell replacement therapies as they pertain to hearing loss and vestibular problems.

Recently, a method has been developed and validated that allows the analysis of cell turnover in humans based on incorporation of radioactive ¹⁴C from nuclear bomb tests into genomic DNA. Levels of ¹⁴C in the atmosphere were relatively stable until the Cold War, when above ground nuclear bomb tests in the 1950s and 1960s caused a notable increase. Since the nuclear test ban treaty of 1963, these levels have dropped exponentially by diffusion from the atmosphere (not because of radioactive decay). The ¹⁴C in the atmosphere reacts with oxygen to form carbon dioxide, which is then incorporated into organic molecules by plant photosynthesis, and ultimately consumed by animals and humans. It has been shown that the ¹⁴C concentration in the human body closely parallels that in the atmosphere at any given point in time. Because DNA is relatively stable after a cell has gone through its last cell division, the ¹⁴C level in DNA

serves as a date mark for when a cell was born, and this can be used to retrospectively derive the birth date of cells and deduce the percentage turnover in particular tissues. The goal is to study the dynamics of sensory and non-sensory cell turnover in the human cochlea and vestibular organs. AMS is the only technique that can measure the small DNA samples expected with the precision required to determine cell turnover.

Subproject Progress

A set of samples was analyzed in the summer of 2012. Yield was low and additional samples are being stored at -80C while DNA yield improvement is being evaluated.

TRANSPORT OF 14C-TCC ACROSS THE PLACENTAL BARRIER (0068)

BTR Unit:	Collaborative Research	Resource Assigned ID:	RR Project #75
%BTR \$:	3.000%		

Investigator	Department/Non-Host Information
SARACHINE FALSO , MIRANDA J., PHD	Div Of Mathematics & Nat Sci
BUCHHOLZ, BRUCE A, PHD	PENN STATE ALTOONA, PA USA
	Center For Ams

Subproject Description

The objective of this project is to determine the extent and effects of prenatal exposure to an endocrine-disrupting compound (EDC) at the concentrations demonstrated in our water supply. The goal is to develop the methodology to screen EDC at the low levels present in the water supply to determine if they pose a threat to the developing fetus. Initial studies will be done using a human cell culture model. For assay development, triclocarban (TCC; 3,4,4'-trichlorocarbanilide), a compound that has been found in the water supply at very low concentrations and has demonstrated potential to act as an EDC, will be used. TCC is an antimicrobial agent widely used in personal care products such as antibacterial soaps and deodorants. The concentrations of TCC and other EDCs released back into the environment are so low that an analytical method with extremely high sensitivity is needed to accurately assess them. Wastewater treatment plant (WWTP) effluent contains a concentration ranging from 300 pM to 20 nM of TCC which is then diluted into the environment. Accelerator mass spectrometry (AMS) is a unique capability available at LLNL that allows for the administration and accurate measurement of compounds at these extremely low levels. This is done by tracing a 14C-labeled compound.

The BeWo cell line is an immortalized human trophoblastic cell line that forms polarized, confluent monolayers when in culture. This cell line has been used as an in vitro model of the rate-limiting barrier to maternal-fetal exchange. Results have been obtained with the cell line that agree with ex vivo results obtained using a placental perfusion assay validating this cell culture method as a strong model for placental transport. This line is commercially available from ATCC. The BeWo cells will be cultured on polyester transwell inserts with pores coated with human placental collagen. Once the cells form monolayers on the transwell filters as confirmed by measuring the transepithelial electrical resistance (TEER), 14C-TCC will be added to the apical side of the filter in the cell culture media at environmentally-relevant concentrations within the 300 pM to 20 nM range detected in WWTP effluent. The cells will be incubated for various time periods, and then the apical and basolateral media will be obtained along with lysates of the cells.

We will also test placental transfer of TCC in a CD-1 mouse model. Pregnant females will be exposed to 100 nM – 1 pM TCC in their drinking water throughout gestation. Some mice will be sacrificed just before birth and tissues will be harvested from both the mother and offspring. These tissues will be measured by AMS to determine the distribution and transfer of TCC. Other mice will be carried to birth and raised in order to determine TCC's effects on

development and later reproduction.

Subproject Progress

To date, we have successfully developed a cell culture model of the human placental barrier and determined the transfer of low environmentally relevant concentrations of TCC. We observed that <10% of the doses tested (10, 1 and 0.1nM) were capable of transferring into the fetal compartment over an 8 hour time course. In addition to the in vitro studies, we have developed and validated drinking bottles for the in vivo TCC exposure studies. We have also completed and analyzed the in utero transfer of TCC from mother to offspring. For this study, TCC was detected in fetal tissue (~1pmol TCC/g), and placental tissue (<2pmol TCC/g) in utero (day 18). The studies entailing exposure through lactation are currently underway.

**ANALYSIS OF COLD WAR C14 LEVELS IN DNA FROM HUMAN
PROSTATE TISSUES (0061)**

BTR Unit:	Collaborative Research	Resource Assigned ID:	RR Project #68
%BTR \$:	1.000%		

Investigator

VANDER GRIEND, DONALD, PHD

Department/Non-Host Information

Dept. Of Surgery
UNIVERSITY OF CHICAGO, IL USA

Subproject Description

Introduction: Prostate diseases such as prostate cancer and prostatic enlargement (i.e. benign prostatic hyperplasia, or BPH) are major health problems among American men. The growth of the prostate gland occurs in various stages throughout the lifespan of male individuals, and both prostate cancer and BPH are the result of aberrant cellular turnover and growth. However, this process of cellular turnover is very poorly understood, particularly in humans. The goal of this project is to use AMS to measure Carbon-14 levels in the DNA of prostate cells as a result of Cold War atomic bomb testing. Measurement of Carbon-14 in the post-bomb testing era allows an accurate assessment of tissue turnover and cellular birth-dates; such an approach allows important questions in prostate biology research to be answered regarding the etiology of prostate disease.

Methods: DNA will be extracted from tissue specimens, quality controlled, and submitted for Carbon-14 analyses using AMS. Tissue specimens will include archived tissue specimens from our institution (1955 – present), as well as fresh prostate tissue specimens from patients currently at our institution. Carbon-14/Carbon-12 ratios will be compared to the known atmospheric Carbon-14 levels, as previously reported, and tissue and cellular “dates of birth” will be determined.

Anticipated Results: We anticipate that AMS will accurately determine the rate of turnover within the prostate cellular compartments, and that such Carbon-14 levels will indicate the time of disease development when compared between normal prostate, prostate cancers, and BPH. Furthermore, such an approach will document the average lifespan of the various cell types within the prostate gland, such as the long-lived prostate stem cell. Finally, data generated from these studies will greatly facilitate the generation of new hypotheses regarding prostate function and disease etiology.

Impact: Data generated from these studies will have an immediate impact on the field of prostate cancer research and will facilitate answers to essential - and as yet unanswered - questions pertaining to prostate growth, maintenance, and the etiology of prostate disease.

Subproject Progress

We have prepared a manuscript and are discussing additional experimental approaches to elucidate the role of formalin fixation and paraffin embedding on contaminating carbon within human prostate tissues.

Services Subproject Descriptions

**MICRONUTRIENT KINETICS IN HUMANS AT PHYSIOLOGIC
DOSES (0019)**

BTR Unit: Services	Resource Assigned ID: RR Project #017
%BTR \$: 0.000%	

Investigator	Department/Non-Host Information
CLIFFORD, ANDREW J, PHD	Nutrition UC DAVIS, CA USA
OGNIBENE, TED, PHD	Center For Ams

Subproject Description

A steady supply of dietary micronutrients (vitamins) is needed to maintain normal biochemical reactions within humans. Folate is needed to maintain purine and pyrimidine synthesis, and metabolism of homocysteine to methionine. Carotene is a commonly consumed plant pigment (polyene) that is a biological antioxidant and a nutritional precursor of vitamin A. These and other micronutrients have generally been studied within humans at high, non-physiologic, doses in the past using stable or radioactive isotopes. While safe for use in humans, stable isotopes produce kinetic signals that quickly approach natural isotopic levels after a single dose. Radioisotopic labels are sufficiently distinct to provide traceable levels for longer periods, but the risks of radiation exposure limit such uses to populations that are less concerned with possible damage, such as elderly or infirm men. Accelerator mass spectrometry has such high sensitivity for quantifying long-lived isotopes that ¹⁴C-labeled micronutrients can now be traced in healthy young people, including particularly women of child-bearing age.

Volunteers are given microgram doses of ¹⁴C-labeled compounds at radiative doses of 100-200 nCi (6-12 kBq). Blood is frequently collected from an indwelling catheter in the arm during the first day or two to provide high density data reflecting the absorption and initial metabolism phases of the pharmacokinetics. Urinary and fecal collections are made daily during the first two weeks past exposure to provide the quantitative bioavailability of the compound. Blood is then collected occasionally by venipuncture over the next 4-7 months post exposure. Circulating metabolites of the labeled nutrient are separated by HPLC. All samples are quantified for ¹⁴C concentration by AMS measurement of at least 0.5 mg carbon from the sample.

Studies have been performed to examine the metabolism of various nutrients including: beta-carotene, folate and vitamin E. Compartmental models are devised for the nutrient based on present best understanding of the absorption, metabolism, and elimination processes. The models are then modified to fit the dense data set of AMS measurements. Such models provide insight into the slowest turning (and thus the most sustained) pools of nutrients in the volunteers.

Subproject Progress

Has been no progress.

**B12 ABSORPTION, KINETICS AND TRANSCOBALAMIN
GENOTYPE (0043)**

BTR Unit: Services	Resource Assigned ID: RR Proj #051
%BTR \$: 0.000%	

Investigator

MILLER, JOSHUA W, PHD

GREEN, RALPH, MD

Department/Non-Host Information

Nutritional Sciences

RUTGERS UNIVERSITY, NJ USA

Medical Pathology

UNIV OF CA, DAVIS MED CENTER, CA USA

Subproject Description

The overall goal of our research is to utilize carbon-14 labeled vitamin B12 (14C-B12) and accelerator mass spectrometry (AMS) to assess the absorption and turnover of vitamin B12

in humans, as well as the bioavailability of vitamin B12 from foods. With respect to absorption and turnover, we have dosed 6 healthy control subjects with 14C-B12 (50 nCi, 1.3 µg) and collected baseline and post-dose blood, urine, and stool samples for assessment

of 14C-B12 by AMS. The data show relatively consistent patterns of absorption and turnover among the subjects, including first appearance of 14C-B12 in the plasma at about

3 hours, peak plasma levels between 6 and 8 hours, followed by similar plasma elimination

curves. A surprising finding has been our observation of high levels of 14C in the urine (30-

40% of the administered dose), which is in direct contrast to previous literature reports of 0.1-0.5% of the administered dose in studies using 57Co-labeled vitamin B12. We have hypothesized that we are observing breakdown of the vitamin B12 in the gastrointestinal track that was not possible to observe when using 57Co-B12. This finding has potential implications for the bioavailability of B12 from pills and from foods to which vitamin B12 has

been added as a fortificant. Future studies will focus on determining if 14C-B12 can be used to diagnose vitamin B12 malabsorption syndromes (e.g. pernicious anemia) and developing a model for vitamin B12 absorption and turnover in humans. In addition, we will attempt to determine how vitamin B12 is broken down in the gastrointestinal tract and

what are the products of this breakdown that seem to be appearing in the urine.

For assessment of vitamin B12 bioavailability from foods, we are enriching chicken eggs in vivo with radioactively labeled vitamin B12 to a level that allows us to feed the enriched eggs to humans and determine how much of the vitamin B12 is digested and absorbed into

the body. The results of our first experiment indicate that we can inject radioactively labeled vitamin B12 into a laying hen and detect the radioactive vitamin B12 in the eggs at

a level sufficient for feeding to humans in a bioavailability study. The in vivo labeled eggs have been prepared for human consumption and a single dose of ~20 nCi in labeled scrambled egg was fed to a human subject. We were able to track the appearance of 14C-

B12 in plasma, urine and feces at this level of enrichment. We are currently recruiting additional subjects for further study.

Subproject Progress

no progress to report

MICRO-DOSING TO DETERMINE PHARMACOKINETICS OF PAH'S (0067)

BTR Unit:	Services	Resource Assigned ID:	RR Proj #74
%BTR \$:	2.500%		

Investigator

WILLIAMS, DAVID, PHD

CORLEY, RICHARD, PHD

Department/Non-Host Information

Environ & Molecular Toxicology
OREGON STATE UNIVERSITY, OR USA

Biological Sciences
PACIFIC NORTHWEST NATIONAL LABORATORY,
WA USA

Subproject Description

PAHs (polycyclic aromatic hydrocarbons) are widespread pollutants that result from the burning of material such as cigarettes, diesel, coal, or gasoline. They are also found in foods such as smoked meat and cheese, or liquor aged in charred wood barrels. In animals, high levels of PAHs can cause cancer of the skin, breast and lung. In humans, PAHs are linked to cancer and other health problems such as asthma. It is unknown how fast PAHs are absorbed into the body when eaten, or how long they stay in the body. Even though PAHs are ubiquitous environmental pollutants of potential concern to human health, there is little or no information on the pharmacokinetics of PAHs in humans. With the utilization of Accelerator Mass Spectrometry (AMS), it is possible to measure ¹⁴C-isotopically-labeled PAHs in human tissues following administration of micro-doses. The results from this study will be used to model the pharmacokinetics of polycyclic aromatic hydrocarbons (PAHs) in humans. This work has never been done before and the potential benefit for regulatory agencies is very significant. As of now, all they can rely on is animal data. As PAHs are increasing in the environment, it is crucial that we have the best information possible to make decisions concerning safe exposure by inhalation or by diet.

Subproject Progress

¹⁴C- Dibenzo[def,p]chrysene (DBC) from the National Cancer Institute was utilized in a pharmacokinetic study employing micro-dosing with analysis by Bio-AMS Research Resource at Lawrence Livermore National Laboratory. With full IRB and radiation safety approval 9 individuals were enrolled in the study. These individuals were dosed with 5 nCi [¹⁴C]-DBC (29 ng) utilizing the Integrative Health Sciences Facility Core and blood and urine collected over 72 hours. The volunteers enrolled in this study were males and females between 20 to 65 years old with BMIs of 23.5 - 34.8. Despite the variability expected in a human study, including any unidentified polymorphisms, a variety of ages, BMI, and sexes, the pharmacokinetic results were remarkably consistent. This represents the first dataset of a carcinogenic PAH in humans at an environmentally relevant level of exposure.

Technological Research & Development Subproject Descriptions

PROJECT 2 QUANTITATIVE CELL BIOLOGY (0002)

BTR Unit:	Technological Research and Developn	Resource Assigned ID:	RR Project #002
%BTR \$:	15.000%		

Investigator	Department/Non-Host Information
BENCH, GRAHAM, PHD	Center For Ams
BUCHHOLZ, BRUCE A, PHD	Center For Ams
NAVID, ALI, PHD	Biology And Biotechnology Division
STEWART, BENJAMIN, PHD	Center For Ams
TURTELTAUB, KENNETH W, PHD	Biology And Biotechnology Division

Subproject Description

The research area of Cell Biology focuses on gaining an understanding of both the composition and function of individual cells, how cells change over their lifetime and in response to signals from their environment as well as determining how cells form a whole organism. Biochemical studies of cells and their contents depend on collecting, defining, identifying, and quantifying the biomolecules responsible for a certain function, structure, or biochemical signal. Such well-defined biomolecular samples often contain little material. To address this paucity of material, a variety of sensitive instrumentation, including flow cytometers and capillary electrophoresis (CE) systems, are available to provide sensitivity for studying these materials. Such techniques typically employ chemical reactions to enable fluorescent labeling and conventional or laser-induced fluorescence for detection. While such methods have detection sensitivities as low as several zeptomole for proteins, not all moieties within a cell can be fluorescently labeled and quantitation often relies on molecular properties of the analyte.

Isotope labeling strategies provide an alternative approach to protocols that employ fluorescence detection. The isotope label can be analytically measured independent of molecular structure to quantitate the amount of a substance. ¹³C and ¹⁴C are frequently used in isotope labeling experiments primarily because they are isotopes of an element naturally present in almost all molecules of biological interest. Cells can be cultured in enriched ¹³C or ¹⁴C media so that, in principle, all cellular constituents are uniformly labeled. This means that all compounds within the cell can be efficiently labeled. AMS, owing to its ability to measure small quantities of material at high sensitivity and with accurate analytical quantitation, is well suited to make valuable contributions to cell biology.

Successful use of AMS for cell biology will not only require adequate sample preparation methods, but also will require that the biological aspects of the experiments be designed and conducted for compatibility with AMS analysis. This project will develop methods: 1) to routinely couple cell biology experiments to AMS and; 2) to quantitate components within cells that have been labeled with ¹⁴C. Emphasis will be placed on the development of separatory methods to speciate metabolites and molecules from pools of cells for accurate quantification by AMS. Specifically, we will develop robust methods to:

- 1) Isolate sub-cellular fractions and speciate individual metabolites from pools of cells.
- 2) Couple enzyme kinetics and metabolic flux measurements to AMS.
- 3) Couple AMS to quantitative measurement of post translational modifications and adducts

formed by the interaction of reactive metabolites with cellular macromolecules.

4) Couple AMS to cell turnover in human tissues using the 14C bomb curve.

Subproject Progress

Aims 1-3: 1). Isolate sub-cellular fractions and speciate individual metabolites from pools of cells; 2). Couple enzyme kinetics and metabolic flux measurements to AMS; and 3). Couple AMS to quantitative measurement of post translational modifications and adducts formed by the interaction of reactive metabolites with cellular macromolecules.

We used 14C-labeled precursors to characterize metabolic fate and cellular effects of metabolites in model organisms. In the yeast *Saccharomyces cerevisiae*, we traced the fate of the reactive dicarbonyl methylglyoxal under low-glucose, normal glucose, and high glucose growth conditions. We observed that the glyoxalase pathway was primarily responsible for detoxification of methylglyoxal under these growth conditions. We measured formation of chemical adducts produced by the interaction of 14C-methylglyoxal with yeast macromolecules. These results have been accepted for publication in the journal *Yeast*. In addition, a collaborative project was completed in which metabolites were measured in a microbial co-culture system for hydrogen production. The results of this project were published in the *International Journal of Hydrogen Energy*.

Quantitation of low-abundance protein modifications involves significant analytical challenges, especially in biologically-important applications such as studying the role of post-translational modification in biology and measurement of the effects of reactive drug metabolites. Research performed under Specific Aim 3 involved measurement of proteins and peptides alkylated with 14C-iodoacetamide and measurement by liquid chromatography-tandem mass spectrometry and liquid sample AMS. Labeling techniques and separation methods were developed to allow measurement of proteins and peptides using the liquid sample interface. We performed real-time 14C quantitation of HPLC separations by liquid sample accelerator mass spectrometry (LS-AMS). We demonstrated that direct HPLC-AMS coupling overcomes several major limitations of conventional HPLC-AMS where individual HPLC fractions must be collected and converted to graphite before measurement. These results are currently under review for publication in the journal *Analytical Chemistry* (Thomas, A. T.; Stewart, B. J.; Ognibene, T. J.; Turteltaub, K. W. and Bench, G. Directly coupled HPLC-AMS measurement of chemically-modified protein and peptides.

Aim 4: Couple AMS to cell turnover in human tissues using the 14C bomb curve.

Projects coupling AMS to cell and protein turnover in human tissues using the 14C bomb curve were advanced in Specific Aim 4. Specific sample definition is the biggest challenge in these dating and turnover projects. Four general classes of samples were studied: DNA, proteins, lipids, and dental enamel. We developed a method for the retrospective birth dating of cells using bomb pulse 14C dating as a method for measuring the approximate age of specific populations of cells in the adult human brain and other tissues (Spalding, et al., 2005). This method is based on establishing the proportion of the isotope 14C in genomic DNA. After a cell has terminally differentiated it does not divide again. Since the last cell

division represents the last time point when the cell synthesized DNA, its chromosomal DNA reflects the age when the cell was born. Atmospheric nuclear tests in the late 1950s and early 1960s doubled the level of ^{14}C in the atmosphere followed by an exponential decline since 1963, allowing one to resolve ^{14}C differences in the range of years. Because DNA has a ^{14}C content reflective of the time when it was synthesized, establishing the ^{14}C content of chromosomal DNA enables retrospectively birth dating of cells, and thus establishes cellular turnover. Establishing a marker to specifically identify and separate specific cell types has been the biggest challenge in these studies. Pancreatic beta cells were separated using pro-insulin as a marker to establish turnover ceases after age 30 (Perl, et al., 2010). Long time collaborator at the Karolinska Institute, Kirsty Spalding, continues to probe specific brain regions for neurogenesis using NeuN+ as a positive nuclear surface marker for neurons. Spalding has also advanced investigations of lipid turnover in health and disease to determine that lipid turns over every 1.5 years (Arner, et al., 2011) while fat cells live about 10 years (Spalding, et al., 2008) in healthy individuals. The carbon in most proteins is replaced relatively rapidly. The lens of the eye is an exception, with cells and structural proteins formed in utero lasting a lifetime. The lens grows throughout life adding cells to the outer layers. Using relatively simple separation of water-soluble (crystalline) proteins from water-insoluble (mostly membrane) and molecular weight spin filters to remove small molecules, Stewart, et al. (2013) quantified turnover of water-soluble crystalline proteins to be ~0.5-1% annually while membrane proteins were not renewed. The finding is significant because it contradicts dogma and demonstrates a natural process of renewing carbon in elderly crystalline proteins. We also recently developed a technique to extract collagen from blood vessels. Collagen is typically extracted from bone by dissolving the mineral phase in acid and used to date archeological remains. Here collagen was extracted from blood vessels to date ruptured and unruptured intercranial aneurysms (Etrminan, et al., 2013). The collagen was less than 5 years old in all cases with larger aneurysms being slightly older. Finally, bomb pulse dental enamel to determine date of birth in forensic cases has been systematically extended by combining enamel dating with other analyses. Aspartic acid racemization of teeth can provide an estimate of date of death when combined with enamel dating (Alkass, et al., 2010). Stable isotope can provide geographical information of residence during tooth formation (Alkass, et al., 2011). As the forensic community becomes aware of enamel dating, it is being applied to cold cases with other modern analytical techniques to solve cases (Speller, et al., 2012).

PROJECT 3: QUANTITATIVE ENDPOINTS IN HUMANS (0003)

BTR Unit:	Technological Research and Developn	Resource Assigned ID:	RR Project #003
%BTR \$:	15.000%		

Investigator

MALFATTI, MICHAEL A, MS, PHD

GANDARA, DAVID, MD

HENDERSON, PAUL T, BS, PHD

MACK, PHIL, PHD

PAN, CHONG-XIAN, MD, PHD

TURTELTAUB, KENNETH W, PHD

WANG, YANCHUN, MD, PHD

ZHANG, HONGYONG, DVM, PHD

Department/Non-Host Information

Biosciences & Biotechnology
LAWRENCE LIVERMORE NATIONAL
LABORATORY, CA USA

Urology
UNIVERSITY OF CALIFORNIA, DAVIS, CA USA

Dept Of Internal Medicine
UNIV. OF CA, DAVIS, CA USA

Biology And Biotechnology Division

Subproject Description

This project is focusing on developing and validating methods and techniques to demonstrate the applicability of AMS to assess human responses to drugs, xenobiotics and endogenous compounds. We are conducting studies using AMS to develop and validate methods for use in absorption, distribution, metabolism and elimination studies (ADME) using drugs alone and in combination. We will investigate how best to conduct basic metabolism studies as well as to work with isolated macromolecular fractions such as DNA modified by chemotherapy drugs. We will work in cell culture, animal models, and humans to assess what types of samples can be utilized and how best to process them for AMS analysis.

Subproject Progress

Pharmacokinetics of Nanoparticles: The PK properties and the quantitative long-term tissue

distribution of amorphous spherical ¹⁴C-SiNPs, was investigated using the (AMS) for quantification.

Silica dioxide nanoparticles (SiNPs) are used for a wide variety of commercial and biomedical

applications, yet little is known about their toxicology and safety. An important aspect in better understanding SiNP safety is characterization of the factors that affect SiNP biodistribution. Currently, comprehensive studies on biodistribution are lacking, most likely

due to the lack of suitable analytical methods. To investigate the relationship between administered dose, PK, and long-term biodistribution, the technique of accelerator mass spectrometry (AMS) was used to determine the PK and tissue distribution of ¹⁴C-SiNPs in mice. AMS quantifies extremely low concentrations of radiolabeled chemicals, and new here

labeled nanoparticles, enabling long-term kinetic studies. Mice were administered a single intravenous dose of ¹⁴C-SiNPs. Blood and tissue were collected at specific time points following dose administration over an 8-week time period, and SiNP concentrations determined by quantifying the radiocarbon equivalents in each sample by AMS. Plasma PK analysis showed that SiNPs were rapidly cleared from the central compartment with a half-

life of 0.38 h. The large apparent volume of distribution of 2058.1 ml/kg indicated extensive distribution to tissues. SiNPs were rapidly distributed to tissues of the

reticuloendothelial system including liver, spleen, kidney, lung, and bone marrow, and persisted in the tissue over the 8-week time course, raising questions about the potential for bioaccumulation and associated long-term effects. SiNP elimination occurred by both renal and biliary routes. These results indicate that AMS is an effective tool to accurately and precisely quantify the long-term kinetics and biodistribution of SiNPs in vivo.

Accelerating Drug Development: To understand the in vivo pharmacokinetics (PK) of a drug

lead three studies were conducted at LLNL. The objective of the first study was to determine the dose proportionality of the drug in male Sprague Dawley rats. The second study was done to determine the PK of the drug following micro or therapeutic dose administrations in the male Sprague-Dawley rat. Finally, experiments were done to determine the mass balance and tissue distribution of the drug in the male Sprague-Dawley rat.

Dose proportionality: The pharmacokinetics following a single intravenous administration of

3, 10, or 30 mg/kg drug in rat exhibits a multi-compartment profile, which initially shows a rapid decline in plasma concentrations post-dose followed by a slower terminal phase with a

mean apparent terminal half-life of 4.40 to 7.90 hrs. Across the 3 doses studied, total clearance of drug from plasma (CL) was 42.1 to 68.0 mL/min/kg and the apparent volume

of distribution at steady state (V_{ss}) was 10400 to 17700 mL/kg suggesting rapid and extensive distribution beyond the plasma compartment. Overall, for an increase in dose from 3 to 30 mg/kg, the increases in mean C_{max} (9.51-fold) and AUC_{0-t} (15.8-fold) values were within a factor of two and considered dose proportional.

Micro vs therapeutic dose PK: The pharmacokinetic profiles following a micro (0.01 mg/kg, 7.7 nCi/kg radiolabeled compound) or a therapeutic (3 mg/kg, 7.7 nCi/kg radiolabeled compound) intravenous (IV) dose in rats are similar. Both dose groups exhibit a multi-compartment profile, which initially shows a rapid decline in plasma concentrations post-dose followed by a slower terminal phase with a mean apparent terminal half-life of 5.4 to 13.2 hrs. The apparent volume of distribution (V_d) was 30510 and 30747 mL/kg for the 0.01 mg/kg and 3.0 mg/kg dose groups, respectively, suggesting rapid and extensive distribution beyond the plasma compartment. When dose normalized, there was a 1.8 fold difference in AUC_{0-t} and a 2.9-fold difference in C_{max} between dose groups. Overall, for an increase in dose from 0.01 to 3.0 mg/kg (300-fold), the increases in mean AUC_{0-t} (1.8-fold) and C_{max} (2.9-fold) values were within a factor of two and three, respectively, and considered dose linear.

Tissue distribution and excretion: Analysis of blood determined that the majority of the dose

is found in the plasma compartment with very little in the red blood cells. The cumulative plasma concentration of drug over the 96 hr exposure time was 8.2 $\mu\text{g/ml}$. Comparison of mean plasma AUC_{0-t} and $AUC_{0-\infty}$ values for the drug indicates that approximately 72%

of the compound was eliminated from the plasma within 96 hr post dose. The mean (\pm SD)

total percentage of dose excreted within 96 hrs was 33.4 % \pm 11.6%, of which 30.66% \pm

11.4% was eliminated in the feces and 2.81% \pm 1.64% was excreted in the urine. The average drug feces/urine ratio was calculated as 10.9:1. Most of the urinary and fecally

excreted drug was eliminated within 4 and 24 hours postdose, respectively.

In the tissue the lung the highest mean concentration of drug at $38.2 \text{ ng/mg} \pm 2.8$ at 1 hr

post dose followed by the kidney, spleen and liver suggesting rapid distribution to the tissues. At 96 hr post dose the liver had the highest mean concentration at $2.5 \text{ ng/mg} \pm 0.1$ followed by the spleen and kidney

Overall the drug is rapidly distributed from the plasma compartment to tissues with the lung having the greatest concentration. Excretion is rapid, with 24 hr post dose. The pharmacokinetics show a multi-compartment profile which is consistent with the tissue distribution data.

PROJECT 1 SAMPLE-SPECTROMETER INTERFACE DEVELOPMENT (0001)

BTR Unit:	Technological Research and Developn	Resource Assigned ID:	RR Project #001
%BTR \$:	28.000%		

Investigator	Department/Non-Host Information
OGNIBENE, TED, PHD	Center For Ams
BENCH, GRAHAM, PHD	Center For Ams
DALEY, PAUL, PHD	Environmental Restoration
THOMAS, AVI T., BS	
TURTELTAUB, KENNETH W, PHD	Biology And Biotechnology Division

Subproject Description

Project 1 seeks to improve our capability to quantitate very small samples. These capabilities will use interfaces developed for our new gas-accepting ion source, which, while recently installed onto our existing 1-MV AMS system, has not yet been fully characterized. This source makes more efficient use of the sample and requires less sample handling. It reduces the minimum sample size required for analysis while improving analysis throughput using appropriate interfaces to directly couple standard bioanalytical techniques to AMS. One goal of Project 1 is to develop an on-line combustion interface to our gas-accepting ion source to provide a direct analysis of small, well-defined biochemical samples.

While the use of gaseous samples will prove beneficial for many small radiolabeled samples, it may not fulfill all of our requirements for the analysis of DNA and proteins from specific cell types. The relative small size (<100 µg C) and low ¹⁴C/¹²C enrichment (1-2 Modern) of DNA or protein samples analyzed in bomb-pulse dating requires the use of solid graphite targets. The LLNL designed high-output solid graphite Cs-sputter source and HVEE FN-class tandem electrostatic AMS system at LLNL routinely achieves 15% total system efficiency, so 1% measurement precision of recent biological material is possible with samples containing as little as 10 µg C, if these small samples can be reduced to graphite and effectively consumed in the ion source.

Subproject Progress

We demonstrated the real-time ¹⁴C quantitation of HPLC separations by liquid sample accelerator mass spectrometry (LS-AMS) by quantifying low-abundance protein modifications. To demonstrate LS-AMS and compare the new technology to traditional solid sample AMS (SS-AMS), reduced and native bovine serum albumin (BSA) was modified by ¹⁴C-iodoacetamide with and without glutathione present, producing adducts on the order of 1 modification in every 106 to 108 proteins. ¹⁴C incorporated into modified BSA was measured by solid carbon AMS and LS-AMS. Reduced and native BSA peptides were generated by tryptic digestion with analysis of HPLC-separated peptides performed in parallel by LS-AMS, fraction collection combined with SS-AMS, and (for peptide identification) electrospray ionization and tandem mass spectrometry (ESI-MS/MS). LS-AMS enabled ¹⁴C quantitation from ng sample sizes and was 100 times more sensitive to ¹⁴C incorporated in HPLC-separated peptides than SS-AMS, resulting in a lower limit of quantitation of 50 zmol ¹⁴C/peak. Additionally LS-AMS turnaround times were minutes instead of days, and HPLC trace analyses required 1/6th the AMS instrument time required for analysis of graphite fractions by SS-AMS.

Publications Supported

*Center Cited

Abstracts**SPIDs**

1. Ricciardi M Pesapane A Polverino Arranto C Arranto Palano G Lam H Castano A Matsuda A Rota M Leri A Rosas I Perrella M Buchholz BA Kajstura J Loscalzo J Anversa P. Birth Dating of Human Lung Cells by Accelerator Mass Spectrometry CIRCULATION RESEARCH 2012; 111:E382.

0064

2. OgOrek B Hosoda T Rondon C Gurusamy N Gatti A Bardelli S Quaini F Bussani R Silvestri F Daniela C Beltrami AP del Monte F Rota M Urbanek K Buchholz BA Leri A Beltrami CA Anversa P Kajstura J. Myocyte Turnover in the Aging Human Heart. CIRCULATION RESEARCH 2012; 111(4) Supplement: S Meeting Abstract: 19

0064

R. Green & J. Miller. New insights into cobalamin absorption and metabolism using accelerator mass spectrometry A12 Clin Chem Lab Med 2012;50(2):A1 A69.

0043

Books**SPIDs**

1. Buchholz B. A. Sarachine Falso M. J. Stewart B. J. Haack K. W. Ognibene T. J. Bench G. Salazar Quintero G. A. Malfatti M. A. Kulp K. S. Turteltaub K. W. and Lyubimov A. V. (2012). Bioanalytics for Human Microdosing Encyclopedia of Drug Metabolism and Interactions John Wiley & Sons Inc.

2. Stewart B. J. Bench G. Buchholz B. A. Haack K. W. Malfatti M. A. Ognibene T. J. and Turteltaub K. W. (2012). Accelerator Mass Spectrometry in Pharmaceutical Development Mass Spectrometry Handbook John Wiley & Sons Inc. pp. 259-269.

Journals**SPIDs**

PMID22400017 Chang JS, Lara PN Jr, Pan CX. Progress in personalizing chemotherapy for bladder cancer. Adv Urol. 2012;2012:364919. doi: 10.1155/2012/364919. Epub 2012 Feb 13. PubMed

0003, 0048

PMID22845409 Dall'Era MA, Cheng L, Pan CX. Contemporary management of muscle-invasive bladder cancer. Expert Rev Anticancer Ther. 2012 Jul;12(7):941-50. doi:10.1586/era.12.60. Review. PubMed

PMID23329209 Etminan N, Dreier R, Buchholz BA, Bruckner P, Steiger HJ, Hagi D, Macdonald RL. Exploring the age of intracranial aneurysms using carbon birth dating: preliminary results. Stroke. 2013 Mar;44(3):799-802. doi:10.1161/STROKEAHA.112.673806. Epub 2013 Jan 17. PubMed

0066

PMID22955965 Kajstura J, Rota M, Cappetta D, Og B, Arranto C, Bai Y, Ferreira-Martins J, Signore S, Sanada F, Matsuda A, Kostyla J, Caballero MV, Fiorini C, D'Alessandro DA, Michler RE, del Monte F, Hosoda T, Perrella MA, Leri A, Buchholz BA, Loscalzo J, Anversa P. Cardiomyogenesis in the aging and failing human heart. Circulation. 2012 Oct 9;126(15):1869-81. doi: 10.1161/CIRCULATIONAHA.112.118380. Epub 2012 Sep 6. PubMed

0064

PMID23075393 Malfatti MA, Palko HA, Kuhn EA, Turteltaub KW. Determining the pharmacokinetics and long-term biodistribution of SiO₂ nanoparticles in vivo using accelerator mass spectrometry. Nano Lett. 2012 Nov 14;12(11):5532-8. doi:10.1021/nl302412f. Epub 2012 Oct 17. PubMed

0003, 0069

Journals		SPIDs
PMID23077194 PMC3497961	*Novotny JA, Fadel JG, Holstege DM, Furr HC, Clifford AJ. This kinetic,bioavailability, and metabolism study of RRR-a-tocopherol in healthy adultssuggests lower intake requirements than previous estimates. J Nutr. 2012Dec;142(12):2105-11. doi: 10.3945/jn.112.166462. Epub 2012 Oct 17. PubMed	
PMID23359559 PMC3555406	Sarachine Falso MJ, Buchholz BA. Bomb Pulse Biology. Nucl Instrum Methods PhysRes B. 2013 Jan 1;294:666-670. Epub 2012 Sep 15. PubMed	0055
PMID22711420 PMC3522462	Schebb NH, Buchholz BA, Hammock BD, Rice RH. Metabolism of the antibacterialtriclocarban by human epidermal keratinocytes to yield protein adducts. J BiochemMol Toxicol. 2012 Jun;26(6):230-4. doi: 10.1002/jbt.21411. PubMed	0038
PMID23361949 PMC3569098	*Stewart BJ, Navid A, Kulp KS, Knaack JL, Bench G. D-Lactate production as afunction of glucose metabolism in Saccharomyces cerevisiae. Yeast. 2013Feb;30(2):81-91. doi: 10.1002/yea.2942. Epub 2013 Jan 30. PubMed	0002
PMID23441119 PMC3580966	Stewart DN, Lango J, Nambiar KP, Falso MJ, Fitzgerald PG, Rocke DM, HammockBD, Buchholz BA. Carbon turnover in the water-soluble protein of the adult human lens. Mol Vis. 2013;19:463-75. Epub 2013 Feb 25. PubMed	0038, 0055
PMID22605931 PMC3352867	Xiao W, Luo J, Jain T, Riggs JW, Tseng HP, Henderson PT, Cherry SR, Rowland D,Lam KS. Biodistribution and pharmacokinetics of a telodendrimer micellarpaclitaxel nanoformulation in a mouse xenograft model of ovarian cancer. Int JNanomedicine. 2012;7:1587-97. doi: 10.2147/IJN.S29306. Epub 2012 Mar 27. PubMed	0073

Published This Year	Citation			Public Access			
	Total	Cited	Not Cited	PMCID	MSID	PMC Journal	Policy Not Applicable
Abstracts	3	0	3				
Books	2	0	2				
Journals	11	2	9	11	0	0	0
Total	16	2	14				

Source of Investigators' Support**Non-Federal****Foundation**

Investigator Organization	Grant/Contract	Total Funding SPIDs
Doerner, Thomas GERMAN RESEARCH COUNCIL	SFB650, SFB421, DFG GRANT	\$250,000 0057
Etminan, Nima PHYSICIANS' SERVICES, INC. FOUNDATION	N/A	\$0 0066
Frisen, Jonas LINNE CENTER OF EXCELLENCE		\$0 0025
Henderson, Paul T SUSAN AND GERRY KNAPP FOUNDATION		\$25,000 0003
UC DAVIS	201121855	\$79,122 0003
Malfatti, Michael A NIH/NCRR	NCRR 5 P41 RR013461	\$0 0003
Spalding, Kirsty L VINNOVA VINNMER MARIE CURIE OUTGOING		\$0 0025
SVEN AND EBBA-CHRISTINA HAGBERG STIFETELSEN STRATEGISK FORSKNING FRAMTIDENS FORSKNINGSLEDARE AWARD		\$0 0025
VETENSKAPSRADET		\$0 0025
NARSAD: THE MENTAL HEALTH RESEARCH ORGANIZATION	200702008	\$0 0025
Vander Griend, Donald NORTHWESTERN/UNIVERSITY OF CHICAGO	SPORE PROGRAM	\$50,000 0061
AMERICAN CANCER SOCIETY UNIVERSITY OF CHICAGO	GRANT EARLY CAREER FUNDS	\$100,000 0061 \$100,000 0061

Non-Federal**Other Non-Federal**

Investigator Organization	Grant/Contract	Total Funding SPIDs
Malfatti, Michael A LLNL LDRD	PLS-11-ERD-012	\$400,131 0003
BATTELLE MEMORIAL INSTITUTE	PNNL/284	\$357,805 0003, 0069
LLNL-LDRD	13ER-1100	\$550,000 0003
Miller, Joshua W AMERICAN EGG BOARD		\$0 0043

Non-Federal

Other Non-Federal

Investigator	Organization	Grant/Contract	Total Funding SPIDs
Sarachine Falso , Miranda J.	LLNL LDRD	11-LW-018	\$150,000 0068
Stewart, Benjamin	LLNL LDRD	08-ERI-02	\$0 0002

Federal

Investigator Organization	Grant/Contract	Total Funding SPIDs
Federal - Non-PHS		
Hammock, Bruce DOD	DAMD17-01 1 0769	\$250,000 0027
Henderson, Paul T DHHS	HHSN261201200048C	\$750,000 0003, 0048
DHHS	SBIR CONTRACT HHSN261201200084 C	\$200,000 0003, 0048
Malfatti, Michael A DOD	HHSN272200800042C	\$1,200,000 0070, 0071
DOD	DTRA	\$2,000,000 0070, 0071
Murnick, Daniel E NSF	0922872	\$200,000 0032
Pett-Ridge, Jennifer DOE	KP160103	\$750,000 0065

Federal

Investigator Organization	Grant/Contract	Total Funding SPIDs
Federal - PHS		
Corley, Richard NIH	5R01HL073598-08	\$1,624,470
Edge, Albert NIH	5R01DC007174-08	\$369,581
Fitzgerald, Paul NIH	5R01EY015560-07	\$346,500
Gandara, David NIH	5U10CA046441-26	\$229,013
Hammock, Bruce NIH	5P42ES004699-26	\$2,587,449
	5R01ES002710-32	\$348,494
	2T32GM008799-11	\$179,393
Kim, Helen NIH	5K23NS058357-05	\$187,380
Kim, Kami NIH	5R01AI087625-04	\$650,114
Lam, Kit NIH	5R01EB012569-03	\$478,449 0073
	5R21NS072084-02	\$367,407 0073
	3R01CA115483-08S1	\$38,683
	3R01CA115483-08S2	\$77,226
	1R33CA160132-01A1	\$353,500
	5R01CA115483-08	\$310,460
	5R21HL108300-02	\$207,900
	3R01CA115483-07S1	\$58,992
Leri, Annarosa NIH	1R01HL114346-01	\$422,700
	5R01HL065577-09	\$371,250
	5R01AG037490-03	\$337,806
Mckenna, Michael NIH	5R01DC009837-04	\$311,237 0072
	5U24DC011943-02	\$546,756 0072
Piero, Anversa NIH	1R01HL105532	\$300,000 0064
Stephensen, Charles NIH	5R21AT006082-02	\$74,250
Su, Hua NIH	5R21NS070153-02	\$189,263
Turteltaub, Kenneth W NIH	8P41GM103483-14	\$1,709,225

Federal

Investigator Organization	Grant/Contract	Total Funding SPIDs
Federal - PHS		
Williams, David		
NIH	5P42ES016465-04	\$2,888,367 0067
NIH	3P42ES016465-04S1	\$10,800 0067
NIH	5R01HL038650-24	\$313,023 0067

Resource Summary: Subprojects

The following only includes information associated with subprojects.

	Collab	Dissem & Training	Services	Tech R & D	Total (Excludes duplicates)
Number of Subprojects	25	0	3	3	31
Number of Investigators	73	0	6	15	80
Number of Published	10	0	1	3	12
%Non-AIDS Dollars	39.500	0.000	2.500	58.000	100.000
% Total Dollars	39.500	0.000	2.500	58.000	100.000

Resource Summary: Administrative

	On Subprojects	Not On Subprojects
Host Personnel	80	27

GEOGRAPHICAL USAGE BY INVESTIGATORS AT NON-HOST INSTITUTIONS

Foreign Investigators by Country

AUSTRALIA	1
CANADA	1
GERMANY	10
NETHERLANDS	2
SWEDEN	5
THE NETHERLANDS	1
UK	4

USA Investigators by State

CA	42
DE	1
IL	1
MA	7
MD	2
NJ	5
NY	3
OR	5
PA	1
WA	1

GEOGRAPHICAL USAGE BY INVESTIGATORS AT NON-HOST INSTITUTIONS

Foreign Investigators by Country

AUSTRALIA	1
CANADA	1
GERMANY	10
NETHERLANDS	2
SWEDEN	5
THE NETHERLANDS	1
UK	4

USA Investigators by State

CA	42
DE	1
IL	1
MA	7
MD	2
NJ	5
NY	3
OR	5
PA	1
WA	1

92

Resource Summary: Publication/Support

Publications

	Citation			Public Access			
	Total	Cited	Not Cited	PMCID	MSID	PMC Journal	Policy Not Applicable
Abstracts	3	0	3				
Books	2	0	2				
Journals	11	2	9	11	0	0	0
Total	16	2	14				

Investigator Support

NON-FEDERAL

	\$1,457,936
FOUNDATION	\$604,122
NON-FEDERAL	2,062,058

FEDERAL

NON-PHS

DHHS	\$950,000
DOD	\$3,450,000
DOE	\$750,000
NSF	\$200,000
NON-PHS	5,350,000

PHS

AG	\$337,806
AI	\$650,114
AT	\$74,250
CA	\$1,067,874
DC	\$1,227,574
EB	\$478,449
ES	\$5,835,110
EY	\$346,500
GM	\$1,888,618
HL	\$3,239,343
NS	\$744,050
PHS	15,889,688

TOTAL SUPPORT **\$23,301,746**

TABLE OF CONTENTS

PROTECTION AGAINST RESEARCH RISKS	2
PERSONNEL ROSTER	3
SUBPROJECT DESCRIPTIONS	10
<i>COLLABORATIVE RESEARCH</i>	
ALLEN, LINDSAY	
- ASSESSMENT OF VITAMIN B12 BIOAVAILABILITY FROM EGG (0054)	2
BOVA, G. STEVEN	
- CARBON-14 BASED AGE ANALYSIS OF METASTATIC PROSTATE CANCER SAMPLES (0059)	4
BROWN, KAREN	
- STRATEGIES FOR PERSONALIZING LEUKEMIA TREATMENT (0074)	5
BUCHHOLZ, BRUCE A	
- CELL TURNOVER USING A NOVEL METHOD FOR THE RETROSPECTIVE BIRTH DATING OF CELLS (0025)	6
- QUANTITATION OF PROTEIN TURNOVER IN THE HUMAN LENS USING THE 14C BOMB-PULSE (0055)	8
- CANCER STEM CELL LONGEVITY AND METASTATIC POTENTIAL IN BLADDER CANCER (0062)	9
COOKE, DANIEL	
- DETERMINATION OF THE AGE OF ARTERIOVENOUS MALFORMATIONS (AVMS) (0075)	11
DEKANT, WOLFGANG	
- ROLE OF GENETIC AND NON-GENETIC MECHANISMS IN FURAN RISK (0053)	12
DOERNER, THOMAS	
- LIFETIME OF EFFECTOR B CELLS (0057)	13
ETMINAN, NIMA	
- DETERMINATION OF AGE OF RUPTURED AND UNRUPTURED INTRACRANIAL ANEURYSMS (0066)	14
HAMMOCK, BRUCE	
- ID AND DEV OF BIOLOGICAL MARKERS OF HUMAN EXPOSURE TO THE INSECTICIDE PERMETHRI (0027)	16
- METHODS MONITOR TOXIC SUBSTAN AND/OR INDICATORS OF PRESENCE IN HUMANS&OTHER SPE (0038)	18
HENDERSON, PAUL T	
- DEVELOP ASSAY TO QUANT PLAT-DNA ADDUCTS & PREDICT RESPONSE TO CHEMOTHERA (0048)	20
- FEASIBILITY OF NANOPARTICLE-MEDIATED PACLITAXEL DELIVERY (0073)	21
HOLDEN, PARTICIA	
- TROPHIC TRANSFER OF 14C-LABELED CARBON NANOTUBES (0076)	23
LIGHTSONE, FELICE C	
- NOVEL AGENTS FOR GRAM-NEGATIVE BIODEFENSE PATHOGENS (0070)	24
- NEW BROAD SPECTRUM ANTIMICROBIAL FOR MULTI-DRUG RESISTANT BIOWARFARE PATHOGENS (0071)	26

COLLABORATIVE RESEARCH

MALFATTI, MICHAEL A - MULTI-SCALE TOXICOLOGY INITIATIVE (0069)	27
MURNICK, DANIEL E - LASER BASED 14C COUNTING FOR BIOMEDICAL STUDIES (0032)	29
OGNIBENE, TED - QUANTIFICATION OF 14C BY OPTICAL SPECTROMETRY (0077)	30
PETT-RIDGE, JENNIFER - METABOLITE PROFILING OF CELLULOLYTIC MICROORGANISMS FOR BIOFUEL PRODUCTION. (0065)	31
PIERO, ANVERSA - TURNOVER OF CELLS IN THE HUMAN MYOCARDIUM (0064)	32
SANTOS, FELIPE - OTOPATHOLOGY BY LIGHT MICROSCOPY AND MOLECULAR TECHNIQUES (0072)	34
SARACHINE FALSO, MIRANDA J. - TRANSPORT OF 14C-TCC ACROSS THE PLACENTAL BARRIER (0068)	36
VANDER GRIEND, DONALD - ANALYSIS OF COLD WAR C14 LEVELS IN DNA FROM HUMAN PROSTATE TISSUES (0061)	38
<i>SERVICES</i>	
CLIFFORD, ANDREW J - MICRONUTRIENT KINETICS IN HUMANS AT PHYSIOLOGIC DOSES (0019)	40
MILLER, JOSHUA W - B12 ABSORPTION, KINETICS AND TRANSCOBALAMIN GENOTYPE (0043)	41
WILLIAMS, DAVID - MICRO-DOSING TO DETERMINE PHARMACOKINETICS OF PAH'S (0067)	43
<i>TECHNOLOGICAL RESEARCH & DEVELOPMENT</i>	
BENCH, GRAHAM - PROJECT 2 QUANTITATIVE CELL BIOLOGY (0002)	45
MALFATTI, MICHAEL A - PROJECT 3: QUANTITATIVE ENDPOINTS IN HUMANS (0003)	48
OGNIBENE, TED - PROJECT 1 SAMPLE-SPECTROMETER INTERFACE DEVELOPMENT (0001)	51
PUBLISHED: ABSTRACTS, BOOKS, JOURNALS	61
SOURCE OF INVESTIGATORS' SUPPORT	63
RESOURCE SUMMARY: SUBPROJECTS	68
RESOURCE SUMMARY: ADMINISTRATIVE	69
RESOURCE SUMMARY: PUBLICATION/SUPPORT	70

4/01/2012-3/31/2013 8 P41 GM103483-14 RESOURCE FOR THE DEVELOPMENT OF BIOMEDICAL MASS SPECTROMETRY

ADMINISTRATIVE INFORMATION

ADMINISTRATION & ALLOCATION OF RESOURCE ACCESS

The Principal Investigator, Kenneth W. Turteltaub, makes Resource allocation with advice from the Internal Executive Committee and the External National Advisory Committee (refer to Advisory Committee pages for list of members). The Internal Executive Committee members are taken from the Resource's key personnel list. The Internal Executive Committee, along with Resource staff and associated members of the Lawrence Livermore National Laboratory (LLNL) Center for Accelerator Mass Spectrometry, meet weekly to discuss project effort and prioritization, review collaborative/service requests, and deal with other issues related to Biomedical AMS. The External National Advisory Committee strives to meet once a year. The Annual Advisory Committee Meeting is focused on the progress being made on the Technology Development projects, the allocation of resources, and the Resource's future. Ad Hoc discussions with individual members of the External National Advisory Committee take place when needed by phone, email or fax.

The Resource makes Biomedical AMS available to the research community through Collaborative and Service subprojects. The operational procedures for access to the Resource are available to the public on the Resource website at <http://bioams.llnl.gov/>. Prior to formally applying for access to the Resource, an investigator must discuss a proposal concept with an appropriate Resource staff member for evaluation of project compatibility with AMS. Only after pre-screening is an application for access to the Resource submitted to the Resource Administrator. This process has served to eliminate the need to reject applications. New subprojects, which have been accepted into the Resource, are categorized as Collaborative or Service. Collaborative subprojects align with the scientific focus of the Technology Development projects (subprojects 0001, 0002, and 0003) and Resource members play a key role in the project. All other projects are classified as Service. Service subprojects are supported by the Resource for initial feasibility and assisted as needed to ensure successful utilization of AMS in furthering the investigator's studies. Individual Resource staff members provide oversight to all Collaborative and Service subprojects, as they relate to their areas of research interest and expertise.

As a condition for use of the Biomedical AMS Resource, it is expected that investigators will pursue publication of their project's results within one year of successfully completing the studies. When using data in publications and/or presentations, they must acknowledge NIH Resource for Biomedical Accelerator Mass Spectrometry and the supporting NIH/NIGMS grant funding. In compliance with NIH policy, all publications are made publically available. Additionally, copies of preprints for review and release by LLNL and reprints are to be submitted to the Resource Administrator. This past year,

work from 25 active subprojects has resulted in 25 publications, of which 3 abstracts, 20 journal articles and 2 book chapters have cited the Resource. (Nine of the publications are in the process of being deposited in PubMed Central).

IACUC AND IRB COMPLIANCE

The Research Resource and its subprojects are in compliance with the Lawrence Livermore National Laboratory's Institutional Animal Care and Use Committee (IACUC) guidelines as well as the Institutional Review Board (IRB) requirements. The requisite approvals are on file and copies of the approval letters are available upon request.

WEBSITE

The Biomedical AMS Resource maintains a website at <http://bioams.llnl.gov/>. The website was refreshed to reflect latest updates to the Resource forms, equipment, images and figures, publications, as well as personnel. In addition, a new section titled "In the News" was added to feature highlights of our recent work. The website will be updated again this spring to reflect relevant changes at NIH and LLNL.

The Resource is also featured on the Physical and Life Sciences Directorate website at https://www-pls.llnl.gov/?url=about_pls-centers_and_institutes-bioams.

AWARDS, HONORS, SPECIAL RECOGNITIONS

None

COMMITTEE REPORTS

The External National Advisory Committee met on March 9, 2012. Six of the committee members were in attendance: Tom Baillie, University of Washington; Tom Brenna, Cornell University; Al Burlingame, UCSF; Jim Felton, LLNL retiree; John Hayes; Woods Hole; Matt Jacobsen, UCSF. A full day of presentations detailing scientific progress of the project and future directions were given by the RR staff, postdocs and students. This meeting has resulted in a input to the resource staff regarding new directions and upcoming grant submissions.

DISSEMINATION

PRESENTATIONS GIVEN BY RESOURCE STAFF

Members of the Resource have contributed to the dissemination of the technological developments of the Resource through presentations of their work. Presentations have been given at professional society meetings and to various research groups in the U.S. and abroad. In some cases the abstracts have been published in the proceedings of the meetings. The presentations are listed below by presenter.

Ken Turteltaub and Graham Bench presented:

- “Accelerator Mass Spectrometry: Peeking at Particles for Human Health”. Presented to ~150 community college students at Los Positas Community College, Livermore CA 3/2012

Ken Turteltaub presented:

- “An Overview of Biosciences at LLNL” at the NIH Campus, Bethesda MD, 11/2012

Buchholz, B.A.:

- B.A. Buchholz. Exploiting the ^{14}C Bomb Pulse with Accelerator Mass Spectrometry. American Nuclear Society NORCAL Section Meeting. December 6, 2012.

Malfatti, M.:

- LLNL, BBTD Division seminar. Title: Multi-Scale Toxicology Initiative: An integrated approach for assessing the toxicity of nanoparticles 5/31/12
- The Use of Cytochrome P450-embedded Nanodisks to Enhance Metabolism and Elimination of Chemical Toxicants, Society of Toxicology meeting, March 2013

Stewart, B.:

- LLNL, 8/11/2011. Title: Calculating Life: In silico Systems Biology strives to make sense of the deluge of biological data
- Methylglyoxal Production and Metabolism in *Saccharomyces cerevisiae* as a Function of Glycolytic Flux, Society of Toxicology meeting, 14 March 2012
- Absolute Quantitation of Low-Abundance Protein Adducts using a Novel Accelerator Mass Spectrometry Liquid Sample Interface, Society of Toxicology meeting, March 2013

Henderson, P.T.:

- UC Davis Molecular Toxicology Lecture. Title: Overcoming Cancer Drug Resistance through Nanotechnology and Accelerator Mass Spectrometry. Title: Overcoming Cancer Drug Resistance through Nanotechnology and Accelerator Mass Spectrometry. Date: September 15, 2012
- LLNL Departmental Seminar. Title: Application of Advanced Mass Spectrometry and Nanoparticle Technologies to Overcoming Cancer Drug Resistance.” Date: November 15, 2012

Presentations, posters and seminars:

The technology of the AMS Resource was disseminated through the presentations of Resource staff and collaborators. Their presentations are listed below.

SPID0003

- Society of Toxicology Annual meeting. Date: March 11-15, 2012
Title: Long-term pharmacokinetics and biodistribution of silica nanoparticles using Accelerator Mass Spectrometry in vivo
Authors: Heather Palko, Ed Kuhn, Ken Turteltaub, Mike Malfatti
- ICAAC annual meeting. Date: Sept. 9-12, 2012
Title: Use of Microdosing and Accelerator Mass Spectrometry to Evaluate the Pharmacokinetics of a Novel GyrB/ParE Inhibitor
Authors: M. A. Malfatti, V. Lao, K.W. Turteltaub, C.L. Hall, V.S. Ong

SPID0032

- International Isotope Society. Date: 9 to 13 Sept 2012. Title: Laser Based Radiocarbon Analysis in Drug Discovery- Status and Outlook
Authors: Daniel Murnick
- 15th Annual Symposium, Clinical & Pharmaceutical Solutions through Analysis. Date: 1 to 4 Oct 2012. Title: Laser Based Radiocarbon Analysis in Drug Discovery- Status and Outlook
Authors: Daniel Murnick
- Radiocarbon 2012. Date: 9 to 12 July 2012
Title: Intra Cavity Opto Galvanic Spectroscopy: a new way of ¹⁴C measurement
Authors: MURNICK D.E, BACHA T, ILKMEN E, DEGUZMAN M

SPID0043

- Advances and Controversies in B-Vitamins and Choline. Liepzig, Germany. Date: March 5-8, 2012. Title: New insights into cobalamin absorption and metabolism using accelerator mass spectrometry
Authors: R. Green & J. Miller

SPID0048

- Eastern Analytical Symposium and Exposition 2012, November 12-15, 2012.
Title: Carboplatin Microdosing for Mechanistic and Diagnostic Studies in Cancer
Authors: Paul Henderson, George Cimino, Sisi Wang, Tao Li, Ralph de Vere White, Cindy Lin, Mike Malfatti, Kenneth Turteltaub, Chong-xian Pan
- Fall 2012 Philadelphia ACS National Meeting (August 19-23, 2012) Abstract 12984. Title: Challenges and triumphs of starting and building a cancer diagnostics company (oral)
Authors: Paul T. Henderson, George Cimino, Chong-xian Pan

SPID0054

- Neurogenesis, Keystone Symposia Conference, Santa Fe, NM. Date: February 3-6, 2013. Title: Neurogenesis and Gliogenesis in the Adult CNS
- Jonas Frisé. Neural stem cells and neurogenesis in the adult, Stem Cell Diabetes Research Workshop, Korean Academy of Science and Technology, Seoul, South Korea, February 27, 2013.
- Jonas Frisé. Neurogenesis and Gliogenesis in the Adult CNS. Neurogenesis, Keystone Symposia Conference, Santa Fe, NM. February 3-6, 2013.
- Jonas Frisé. Neural stem cells and neurogenesis, International Congress on Stem Cells and Tissue Formation, Dresden, Germany, July 18-20, 2012.
- Jonas Frisé. Cardiac Regeneration. Wenner-Gren Symposia, Stockholm, Sweden, May 9-12, 2012.
- Alkass K, Buchholz BA, Druid H. How teeth can be used to estimate date of birth and geographical origin of unknown dead bodies. Annual meeting of Nordic Forensic Odontologists, Stockholm, Sweden, May 30, 2012.
- Alkass K, Buchholz BA, Spalding KL, Druid H. Chemical and isotopic analysis of teeth to assist in identification work. Oral presentation at the triannual Nordic Conference on Forensic Medicine, Aarhus, Denmark, June 14, 2012.

- Kirsty Spalding, Radiocarbon analysis of tooth enamel as a forensic tool to facilitate identification of unknown decedents. Women in Police forensics meeting, Newfoundland, Canada, September 9-14, 2012.
- Kirsty Spalding, Adipose tissue turnover in man. Adiposcience meeting, Osaka, Japan, August 23-25, 2012.
- Kirsty Spalding, Radiocarbon analysis of cell and tissue regeneration in humans. 21st International Radiocarbon Conference, Paris, France, July 8-14, 2012.
- Alkass K, Saitoh H, Buchholz BA, Holmlund G, Senn DR Spalding K, Druid Henrik. Date of Birth Estimation of Dead Bodies - A Compilation of C14 Reference Levels in Enamel to Assist in Identification work. American Academy of Forensic Sciences 65th Annual Meeting, Washington DC, February 21, 2013.

SPID0064

- Scientific Sessions of the American-Heart-Association, Los Angeles, CA. Date: November 3-7, 2012. Title: Birth Dating of Human Lung Cells by Accelerator Mass Spectrometry
- Basic Cardiovascular Sciences Scientific Session, American Heart Association. Date: July 23-26, 2012. Title: Myocyte Turnover in the Aging Human Heart Authors: OgOrek, B, Hosoda, T, Rondon, C, Gurusamy, N, Gatti, A, Bardelli, S, Quaini, F, Bussani, R, Silvestri, F, Daniela, C, Beltrami, AP, del Monte, F, Rota, M, Urbanek, K, Buchholz, BA, Leri, A, Beltrami, CA, Anversa, P, Kajstura, J. Myocyte

SPID0066

- Etminan N, Dreier R, Bruckner P, Steiger HJ, Hänggi D, Macdonald RL: Determining the age of aneurysms using radiocarbon birth-dating. Cerebrovascular Section meeting of the German Society of Neurosurgery, Zürich, Switzerland. April 2012
- Etminan N, Dreier R, Bruckner P, Steiger HJ, Hänggi D, Macdonald RL: Determining the age of aneurysms using radiocarbon birth-dating. 6th European-Japanese-Joint Conference on Stroke Surgery, Utrecht, The Netherlands. June 2012
- Etminan N, Dreier R, Bruckner P, Steiger HJ, Hänggi D, Macdonald RL: The age of aneurysms. Annual meeting of German Society of Neurosurgery, Leipzig, Germany. Juli 2012

SPID0067

- Society of Toxicology 52nd Annual Meeting. Date: March 10-14, 2013. Title: In Vivo human pharmacokinetics of dibenzo[def,p]chrysene (DBC) following microdosing. Bridging the gap between high dose animal data and environmentally relevant human exposures. Authors: Madeen, E. P.; Corley, R.; Turteltaub, Kenneth W.; Ognibene, T.; Malfatti, M.; Garrard, M.; Sudakin, K.; Mcquistan, T.; Williams, D. E.
- Environmental and Molecular Toxicology Research Day. OSU. Date: January 11, 2013. Title: Human Pharmacokinetics of the High Molecular Weight Polycyclic Aromatic Hydrocarbon Dibenzo(def,p)chrysene: Utilizing Sensitive Accelerator Mass Spectrometry to Verify Rodent Model Based Modeling Authors: E.P. Madeen, R. Corley, K. Turteltaub, T. Ognibene, M. Malfatti, M. Garrard, D. Sudakin, T. McQuistan, D.E. Williams
- Superfund Research Program Annual Meeting. Date: October 21-24 2012. Title: In Vivo human pharmacokinetics of dibenzo (def,p) chrysene following microdosing: Bridging the gap between high dose animal data and environmentally relevant human exposures Authors: E.P. Madeen, R. Corley, K. Turteltaub, T. Ognibene, M. Malfatti, M. Garrard, D. Sudakin, T. McQuistan, D.E. Williams

SPID0068

- Society of Toxicology. Date: March 2012 Title: Transfer of Triclocarban across the Placental Barrier Authors: Falso MJS, Walsworth V, Buchholz BA

SPID0069

- Society of Toxicology Annual meeting. Date: March 11-15, 2012 Title: Long-term pharmacokinetics and biodistribution of silica nanoparticles using Accelerator Mass Spectrometry in vivo Authors: Heather Palko, Ed Kuhn, Ken Turteltaub, Mike Malfatti
- Society of Toxicology Annual meeting. Date: March 2013 Title: Pharmacokinetics and biodistribution of Iron Oxide nanoparticles using Accelerator Mass Spectrometry in vivo Authors: Heather Enright, P. Nallathamby, V. Mikheev, B. Forsythe, W. Wang, Ed Kuhn, S. Retterer, Ken Turteltaub, Mike Malfatti
- Society of Toxicology Annual meeting. Date: March 2013 Title: Radiolabeled, Superparamagnetic, Nanoparticles for Bio-distribution Studies in Life Sciences. Authors: P. Nallathamby, H. Enright, Mike Malfatti, S. Retterer, W. Wang

SPID0070

- ICAAC annual meeting. Date: Sept. 9-12, 2012 Title: Use of Microdosing and Accelerator Mass Spectrometry to Evaluate the Pharmacokinetics of a Novel GyrB/ParE Inhibitor Authors: M. A. Malfatti, V. Lao,, K.W. Turteltaub, C.L. Hall, V.S. Ong

Resource Bibliography

Publications (in Pubmed central):

1. J. S. Chang, P. N. Lara Jr., and C.-X. Pan, "Progress in Personalizing Chemotherapy for Bladder Cancer," *Advances in Urology*, vol. 2012, Article ID 364919, 10 pages, 2012. doi:10.1155/2012/364919 **(SPID 0003;SPID 0048)**
2. M. A. Dall'Era, L. Cheng and C.-X. Pan, Contemporary management of muscle-invasive bladder cancer, *Expert Review of Anticancer Therapy* July 2012, Vol. 12, No. 7, Pages 941-950 , DOI 10.1586/era.12.60 (doi:10.1586/era.12.60) **(SPID 0003)**
3. N. Etmnan, R. Dreier, B.A. Buchholz, P. Bruckner, H.-J. Steiger, D. Hänggi, R.L. MacDonald. (2013) Exploring the age of intercranial aneurysms using carbon birth dating: preliminary results. *Stroke* in press. Online January 18, 2013, DOI: 10.1161/STROKEAHA.112.673806. **(SPID 0066)**
4. M.J.S. Falso, B.A. Buchholz. (2013) Bomb pulse biology. *Nucl. Instr. and Meth. B* **294**, 666-670. **(SPID 0055)**
5. Kajstura, J, Rota, M, Cappetta, D, Ogorek, B, Arranto, C, Bai, YN, Ferreira-Martins, J, Signore, S, Sanada, F, Matsuda, A, Kostyla, J, Caballero, MV, Fiorini, C, D'Alessandro, DA, Michler, RE, del Monte, F, Hosoda, T, Perrella, MA, Leri, A, Buchholz, BA, Loscalzo, J, Anversa, P. Cardiomyogenesis in the Aging and Failing Human Heart. *CIRCULATION* 2012; 126:1869-U238 **(SPID 0064)**
6. Malfatti, M. A., Palko, H. A., Kuhn, E. A., Turteltaub, K. W. (2012) Determining the pharmacokinetics and long-term biodistribution of SiO₂ nanoparticles *in vivo* using accelerator mass spectrometry. *Nano Letters*, 12, 5532-5538 **(SPID 0003)**
7. Novotny JA, Fadel JG, Holstege DM, Furr HC, Clifford AJ. This kinetic, bioavailability, and metabolism study of RRR- α -tocopherol in healthy adults suggests lower intake requirements than previous estimates. *J Nutr*. 2012Dec;142(12):2105-11. doi: 10.3945/jn.112.166462. Epub 2012 Oct 17. PubMed
8. N.H. Schebb, B.A. Buchholz, B.D. Hammock, R.H. Rice. Metabolism of the antibacterial triclocarban by human epidermal keratinocytes to yield protein

- adducts. *J. Biochem. Mol. Tox.* **26** (2012) 230-234. DOI: 10.1002/jbt.21411
(**SPID 0038**)
9. Stewart BJ, Navid A, Kulp KS, Knaack JL, Bench G. D-Lactate production as a function of glucose metabolism in *Saccharomyces cerevisiae*. *Yeast*. 2013Feb;30(2):81-91. doi: 10.1002/yea.2942. Epub 2013 Jan 30. PubMed (**SPID 0002**)
 10. D.N. Stewart, J. Lango, K.P. Nambiar, M.J.S. Falso, P.G. FitzGerald, B.D. Hammock, B.A. Buchholz. (2013) Carbon turnover in water-soluble protein of the adult human lens. *Molecular Vision* 2013; 19:463-475
<http://www.molvis.org/molvis/v19/463> (**SPID 0055;SPID 0038**)
 11. W. Xiao, J.Luo, T. Jain, J. W. Riggs, H. P Tseng, P. T Henderson, S. R. Cherry, D. Rowland, K. S. Lam Biodistribution and pharmacokinetics of a telodendrimer micellar paclitaxel nanoformulation in a mouse xenograft model of ovarian cancer *Int J Nanomedicine*. 2012; 7: 1587–1597. Published online 2012 March 27. doi: 10.2147/IJN.S29306 (**SPID 0073**)

Publications (not yet deposited in Pubmed central):

12. Sarachine Falso MJ, Buchholz BA, deVere White R. Characterization of the Populations Isolated Using the Aldefluor Assay in Three Bladder Cancer Cell Lines with Differential Sensitivity to Cisplatin. *Anticancer Research* 32 (3): March 2012. (**SPID 0062**)
13. Zhang H, Aina OH, Lam KS, de Vere White R, Evans C, Henderson P, Lara PN, Wang X, Bassuk JA, Pan CX. Identification of a bladder cancer-specific ligand using a combinatorial chemistry approach. *Urol Oncol*. 2012 Sep;30(5):635-45. doi:10.1016/j.urolonc.2010.06.011. Epub 2010 Oct 2. PubMed
14. Cimino GD, Pan CX, Henderson PT. Personalized medicine for targeted and platinum-based chemotherapy of lung and bladder cancer. *Bioanalysis*. 2013Feb;5(3):369-91. doi: 10.4155/bio.12.325. PubMed (**SPID 0048**)
15. Jiao, Y., Navid, A., Stewart, B. J., McKinlay, J. B., Thelen, M. P. and Pett-Ridge, J. (2012). Syntrophic metabolism of a co-culture containing *Clostridium cellulolyticum* and *Rhodospseudomonas palustris* for hydrogen production. *International Journal of Hydrogen Energy* **37**, 11719-11726. (**SPID 0065**)
16. Neuwirth, C., Mosesso, P., Pepe, G., Fiore, M., Malfatti, M., Turteltaub, K., Dekant, W., Mally, A, Furan carcinogenicity: DNA binding and genotoxicity of furan in rats in vivo, *MOLECULAR NUTRITION & FOOD RESEARCH* 2012,56 (9)1363-1374

17. T. J. Ognibene and G. A. Salazar (2013). "Installation of hybrid ion source on the 1-MV LLNL BioAMS spectrometer." *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* 294(0): 311-314. **(SPID 0001)**
18. G. Salazar and T. Ognibene (2013). "Design of a secondary ionization target for direct production of a C⁻ beam from CO₂ pulses for online AMS." *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* 294(0): 300-306. **(SPID 0001)**
19. C.F. Speller, K.L. Spalding, B.A. Buchholz, D. Hildebrand, J. Moore, R. Mathewes, M.F. Skinner, D.Y. Yang. (2012) Personal identification of cold case remains through combined contribution from anthropological, mtDNA and bomb-pulse dating analyses. *J. Forensic Sci.* **57**, 1354-1360. **(SPID 0054)**
20. A.T. Thomas, B. J. Stewart, T. J. Ognibene, K. W. Turteltaub and G. Bench (2012). " Directly coupled HPLC-AMS measurement of chemically-modified protein and peptides" *Analytical Chemistry*, in press. **(SPID 0001)**

Book Chapter Published:

1. Buchholz, B. A., Sarachine Falso, M. J., Stewart, B. J., Haack, K. W., Ognibene, T. J., Bench, G., Salazar Quintero, G. A., Malfatti, M. A., Kulp, K. S., Turteltaub, K. W. and Lyubimov, A. V. (2012). Bioanalytics for Human Microdosing, *Encyclopedia of Drug Metabolism and Interactions*, John Wiley & Sons, Inc.
2. Stewart, B. J., Bench, G., Buchholz, B. A., Haack, K. W., Malfatti, M. A., Ognibene, T. J. and Turteltaub, K. W. (2012). Accelerator Mass Spectrometry in Pharmaceutical Development, *Mass Spectrometry Handbook*, John Wiley & Sons, Inc., pp. 259-269.

Abstracts Published:

1. Ricciardi, M, Pesapane, A, Polverino, Arranto, C, Arranto, Palano, G, Lam, H, Castano, A, Matsuda, A, Rota, M, Leri, A, Rosas, I, Perrella, M, Buchholz, BA, Kajstura, J, Loscalzo, J, Anversa, P. Birth Dating of Human Lung Cells by Accelerator Mass Spectrometry, *CIRCULATION RESEARCH* 2012; 111:E382.
2. OgOrek, B, Hosoda, T, Rondon, C, Gurusamy, N, Gatti, A, Bardelli, S, Quaini, F, Bussani, R, Silvestri, F, Daniela, C, Beltrami, AP, del Monte, F, Rota, M, Urbanek, K, Buchholz, BA, Leri, A, Beltrami, CA, Anversa, P, Kajstura, J. Myocyte Turnover in the Aging Human Heart. *CIRCULATION RESEARCH* 2012; 111(4) Supplement: S Meeting Abstract: 19
3. R. Green & J. Miller. New insights into cobalamin absorption and metabolism using accelerator mass spectrometry, *A12, Clin Chem Lab Med* 2012;50(2):A1-A69.

Resource Visitors

The Resource welcomes visitors and has had several during this past year. Visitors have included investigators from the Resource's Collaborative subprojects, potential future collaborators, and groups interested in learning more about Biomedical AMS capabilities. Visitors often gave presentations to the Resource staff on their research, followed by discussions, lab tours and any necessary methodology training.

Visitors to the Center for Accelerator Mass Spectrometry by Month:

April 11: Parney Albright, LLNL Director.

April 12: Matt Bruhn, DOE Future Leader individual with the Nuclear Assessment Operations group, currently working in the Forestall Building in DC in emergency response.

April 18: Barbara H. Penland, Deputy Director, Communications and External Relations, ORNL.

May 10: Ward Dougherty and Albert Minnick of the Air Force Technical Applications Center.

May 22: Dick Farnsworth and 13 high school students.

May 30: Kirsti Westphalen, Consul General, Office of the Consulate General of Finland in Los Angeles, and Abdellatif Moufakkir, a journalist with RTM/Moroccan TV

June 7: Doug Wall and Staci Dorsey. Sandia national laboratory, Nuclear Weapons Operations, Assurance and International Programs with SNL/CA academy participants: Midshipmen Zachary Dahlke and Paul Westland.

June 15: Phillip Rankin, Valerie McCarty, Michael Weibel, Terri Kocher, Roderick Fry, Adam Rogers, Joint Program Executive Office for Chemical and Biological Defense

June 20: California Teacher Research Academy with Carey Kopay and Dean Reese

June 26: Fusion/Astrophysics Teacher Research Academy Level 2 program teachers with Dan Burns

June 29: Paul Dodd and Martha Krebs, Office of Research UC Davis

July 10: Dean Reese and summer teacher tour

July 12: Chancellor Linda P.B. Katehi and Vice Chancellor Harris Lewin, UC Davis

July 16: Jim Prosser, Institute of Biological and Environmental Sciences, University of Aberdeen

July 23: Sergey Zykov, Division Head, Technical and Scientific Services, Department of Safeguards, IAEA, Vienna, Austria. Anthony Lavietes, Member of Project Engineering Team, IAEA Department of Safeguards, TSI.

July 30: BBTD Lawrence scholar and summer students

July 31: Kirk Haselton, Ipal, Technical University of Berlin, Germany

August 8: Dr. Beyong-Hwan Ryu works for Device Materials Research Center, Korea Research Institute of Chemical Technology (KRICT)

August 13: Stephen Richardson, Chief Operating Officer, Regional Market Director and Amanda Cashin, VP Life Sciences, San Francisco, Alexandria Real Estates Equities, Inc. (ARE)

September 18: Henry VanBrocklin, UCSF
September 27: Balazs (AKA Ernie) Imre Bodai, M.D., F.A.C.S. Clinical Professor of Surgery, UC Davis
September 27: Major Robert Dunlap: Chemical and Biological National Security Activities Planner, NNSA
October 9: Jason Paragas, Program manager -Medical Countermeasures, DTRA.
October 10: Head of LDRD at NNSA HQ, Bob Meisner.
October 11: Dr. Leor Weinberger, Assistant Professor of Chemistry and Biochemistry, University of California, San Diego.
October 18: J. Manuel Perlado, Full Professor / Chair of Nuclear Physics, Head, Instituto Fusión Nuclear (DENIM)/ Universidad Politécnica de Madrid and Octavio González del Moral, Instituto Fusión Nuclear (DENIM)/ Universidad Politécnica de Madrid.
October 24: Thomas M. Countryman, Assistant Secretary, Bureau of International Security and Nonproliferation, US Department of State
October 26: G8 Global partnership meeting tours for the department of State hosted by CGSR.
October 26: UC Davis Department of Environmental Toxicology graduate student tour.
November 8: Joint Standing Committee on Civil Nuclear Cooperation (JSCCNC) Lawrence Livermore National Laboratory (LLNL) TAIWAN ATOMIC ENERGY COUNCIL (TECRO) AMERICAN INSTITUTE IN TAIWAN (AIT).
November 14: Stanford University, Center for International Security and Cooperation (CISAC) fellows.
November 14: Mr. Stuart Osborne OPM, Deputy Assistant Commissioner and Mr. James Constable, Detective Chief Inspector, Metropolitan Police Scotland Yard, London, UK
November 19: Paul Blustein, reporter for Business Week and The Washington Post.
November 28: Lt. Col Won Kim, Program Manager, Weaponization and Testing Detection, NA22 Office of Nonproliferation and Verification R&D accompanied by NA22 Fellow Sonal Joshi
December 3: Jason Paragas, Senior Advisor for Science, Chemical & Biological Technologies Directorate, DTRA and Giora Fuerstein, Chief Medical and Technical officer USG DTRA
January 7: Clean Energy Research Center on Advanced Coal Technology Consortium (CERC-ACTC)
January 25: Mary Shelton, Superintendent - San Ramon Valley School District
February 7: Joselito S. Ignacio, Captain, U.S. Public Health Service Acting Program Director, Chemical Defense, Health Threats Resilience Division, Office of Health Affairs, Department of Homeland Security
February: 11: Dr. Elizabeth Blackburn (2009 Nobel Prize in Physiology or Medicine), Dr. Brian Mayall, Dr. John Sedat, University of California, San Francisco
February: 11: CDR James Lawler, MD and Chief Scientist at the Naval Medical Research Center, U.S. Navy
February 13: Contra Costa Times reporter Jeremy Thomas
March 21: San Ramon Education Foundation
March 27: David Kramer, Physics Today journalist.

Community/High School/Special Tours by Month:

April 3: George Beck & Nick Williams, Standard Community Tour
April 5: George Beck & Nick Williams, American River College Engineering Club
April 10: Nick Williams & David Meeker, Standard Community Tour
April 12: George Beck & Nick Williams, SIRS, La Morinda
April 17: Don Bartel, George Beck & Nick Williams, Merced Physics Students
May 15: George Beck & Nick Williams, Don Bartel & Steve Fread (UC Merced
Physic Group)
May 17: George Beck & Nick Williams (Autodesk)
May 22: George Beck & Nick Williams (Cosmos Club)
May 23: Don Bartel & Nick Williams (Navy Nuclear Recruits)
May 24: George Beck & Nick Williams (CalTech Physics Research Group)
May 29: Don Bartel, George Beck & Nick Williams (Granada Highschool)
May 31: George Beck & Nick Williams (SJ Medical Alliance)
June 5: Don Bartel & George Beck (El Cerrito Seniors Group)
June 8: Nick Williams & Don Bartel (Air Force Troops)
June 12: Nick Williams & George Beck, Standard Community Tour
June 12: Nick Williams & Steve Fread, Standard Community Tour
June 14: Nick Williams & George Beck (El Cerrito Seniors Group)
June 19: Don Bartel & David Meeker, Standard Community Tour
June 21: Nick Williams & Steve Fread, Standard Community Tour
June 22: Nick Williams, Standard Community Tour
June 26: George Beck & David Meeker, Standard Community Tour
June 27: Nick Williams & David Meeker, (Post-Grad UOP Teachers)
June 28: Nick Williams, George Beck & David Meeker (Fresno State HS Engineering
Camp)
July 10: Nick Williams & George Beck (Misc. Group-13)
July 12: Nick Williams & George Beck (Misc.Group-8)
July 12: Nick Williams & George Beck (McNair Scholars Program)
July 17: Nick Williams & George Beck (Misc.Group-16)
July 19: Nick Williams & David Meeker (SIRS)
July 24: George Beck & Steve Fread (Misc.Group-14)
July 24: Nick Williams & David Meeker (“Bookworms” Librarian Group)
July 26: George Beck & Steve Fread (SIRS)
July 31: Nick Williams & George Beck (Kyungnam Providence Dept of Education)
August 1: Nick Williams (“The Quantum Brain Explorers”-3) Special Community Tour
August 7: George Beck and Nick Williams (Misc. Group-17) Standard Community Tour
August 7: Nick Williams (Misc. Group-3) Standard Community Tour
August 9: George Beck and Nick Williams (Partnership for Environmental Technology
Education) Standard Community Tour
August 14: Don Bartel and George Beck (Misc. Group-17) Standard Community Tour
August 15: George Beck and Nick Williams (ABC Electrical Apprentice Group)
Standard Community Tour
August 21: Don Bartel and Nick Williams (Misc. Group-19) Standard Community Tour

August 23: George Beck and Nick Williams (Walnut Creek SIRS #8-14) Standard Community Tour

August 23: Don Bartel and Nick Williams (Walnut Creek SIRS #8-14) Standard Community Tour

August 28: George Beck and Nick Williams (Genentech-19) Standard Community Tour

August 28: Don Bartel and George Beck (Merced MAPS Scholars) Standard Community Tour

August 30: Nick Williams and Steve Fread (Rossmoor SIRS #81-14) Standard Community Tour

September 11: Don Bartel & George Beck, Standard Community Tour

September 13: George Beck & Nick Williams, SIR Rossmoor Branch #81 Group 1

September 18: Nick Williams & Steve Fread, Standard Community Tour

September 20: Don Bartel & Nick Williams, SIR Rossmoor Branch #81 Group 2

September 25: Don Bartel & George Beck, Standard Community Tour

September 27: George Beck & Nick Williams, ITT Technical Institute Group

October 2: Nick Williams & George Beck (Misc. Group)

October 16: Nick Williams, Don Bartel & Steve Fread (Santa Rosa Day Trippers)

October 18: Nick Williams & David Meeker (UOP Engineering Students)

October 23: Don Bartel & George Beck (Peninsula Vet Center)

October 25: Nick Williams & George Beck (Stanford Energy Resources)

October 30: Nick Williams & Don Bartel (Moraga Parks and Recreation)

November 1: Nick Williams & George Beck (AAUW-14) Standard Community Tour

November 6: Don Bartel & George Beck (Misc. Group-10-14) Standard Community Tour

November 8: Nick Williams & George Beck (UOP Engineering Students-14) Standard Community Tour

November 13: George Beck & Carrie Martin (City of Livermore Safety Coordinators-14) Standard Community Tour

December 11: Don Bartel & George Beck - Standard Community Tour

December 18: Nick Williams & George Beck - Pleasanton Church of Latter-Day Saints

January 15: Nick William, George Beck - Leadership Livermore, Standard Community Tour

January 22: Don Bartel, George Beck - Alamo/Danville Newcomers, Standard Community

January 22: Nick Williams, George Beck - KP IT Dept., Standard Community Tour

January 24: Nick Williams, George Beck- St. Mary's Energy Class, Standard Community Tour

January 29: Don Bartel, George Beck - S. Korean Students, Standard Community Tour

February 7: Nick Williams & Tom Crabtree / Don Bartel & Steve Fread, Ceres High School, Standard Community Tour

February 12: Don Bartel & Tom Crabtree, Misc. Group, Standard Community Tour

February 21: Nick Williams & Steve Fread, DeVry University, Standard Community Tour

February 26: Don Bartel & Nick Williams, American Association of University Women (AAUW), Standard Community tour

March 5: Nick Williams & Tom Crabtree, Standard Community Tour, Misc.
Group

March 12: Nick Williams & Tom Crabtree, Standard Community Tour, Society of
Physics Students at UOP

March 19: Don Bartel & Steve Fread, Standard Community Tour, Woodland Community
College

March 26: Don Bartel & Steve Fread, Standard Community Tour, Alamo/Danville
Newcomers Group

PATENTS, LICENSES, INVENTIONS AND COPYRIGHTS:

none

TRAINING:

The Resource's training manual and associated test continue to be a key tool, used to train investigators working in the Resource's laboratories and to verify their knowledge of the policies, practice and procedures.

Four Ph.D. Students are associated with Resource Projects:

- Avi Thomas, LLNL/UC Davis, SPID001
- Daniel McCartt, LLNL/Stanford University
- Nguyen Nguyen, UC Davis, SPID0055
- Ina Wirries, Charite Berlin, Germany, SPID0057

RESEARCH HIGHLIGHTS

The Resource has chosen to highlight progress and publications from two core projects and one collaborative project for this year's progress report.

Highlight #1:

SPID: 0002

Subproject Title: Quantitative Cell Biology

Investigators: Benjamin Stewart, Ali Navid, Graham Bench

Quantifying and simulating complex biological processes has been a goal in the biomedical sciences for over a decade and is essential for the developing directions in systems biology. Currently, accurate and biologically-relevant simulation is limited by the lack of robust quantitative data to bound the underlying mathematical solutions that form the basis of a simulated outcome. For this year's research highlight, we describe how we used AMS to quantify the metabolism of glucose, a simple sugar, which occurs through biochemical pathways that are highly similar in organisms ranging from yeast to humans. While glucose is essential as a building block and as an energy source for most organisms, excess glucose consumption leads to harmful effects. Adverse effects resulting from excess sugar metabolism are important in medicine and in biotechnology processes. In yeast, high glucose intake results in dramatic metabolic alterations and lifespan reduction. Adverse effects of excessive sugar metabolism may be due in part to the generation of toxic metabolites such as methylglyoxal, a dicarbonyl produced by the spontaneous degradation of sugar metabolites known as triose phosphates. Methylglyoxal can chemically modify cellular macromolecules, potentially disrupting cellular function. Experiments with the yeast *Saccharomyces cerevisiae* showed that healthy cells exposed to physiological levels of methylglyoxal rapidly detoxified this compound, with only 0.04% of administered methylglyoxal binding to cellular macromolecules as measured by ¹⁴C-AMS. Yeast was grown in media containing low, moderate, and high glucose concentrations to determine the relationship between glucose consumption and methylglyoxal metabolism. Cells grown in high-glucose media maintained higher glucose consumption rates than cells grown in moderate-glucose or low-glucose media. Growth in high-glucose media resulted in increased generation of the methylglyoxal metabolite D-lactate and overall lower efficiency of glucose utilization. Computational modeling showed that increased glucose consumption may cause a significant increase in methylglyoxal formation by depletion of the oxidized cofactor NAD⁺, which is essential for the normal metabolism of triose phosphates. These findings suggest biochemical mechanisms responsible for tissue damage resulting from excess sugar intake, and may be applicable to human diseases including type 2 diabetes and cancer.

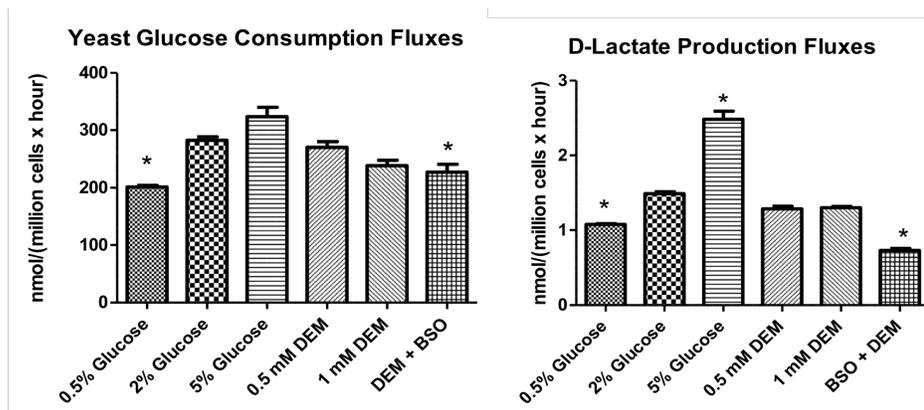


Figure 1: Glucose and D-lactate (a methyl glyoxal metabolite) production fluxes. Increased glucose causes increased formation of the toxic metabolic product of methyl glyoxal.) Mean values for three independent experiments are plotted for each time point with error bars showing standard error of the mean. *, $P < 0.05$. For evaluation of statistical significance, growth conditions are compared to 2% glucose.

Benjamin J. Stewart, Ali Navid, Kristen S. Kulp, Jennifer L. S. Knaack, and Graham Bench, D-Lactate production as a function of glucose metabolism in Saccharomyces cerevisiae. Yeast. 2013 Feb;30 (2):81-91

Highlight #2:

SPID: 0003

Subproject Title: Quantitative Endpoints in Humans

Investigators: Paul Henderson (UCD), Michael Malfatti

Microdose-based predictive diagnostics for chemotherapy

Platinum-based chemotherapy is used to treat over 300,000 patients per year in the US. There are currently no tests available for predicting efficacy of this widely-used class of chemotherapy. **We are developing the *PlatinDx* test, which provides direct measurement of *in vivo* cellular response to a microdose (~1/100th the therapeutic dose) of carboplatin to enable prediction of efficacy.** The microdose safely allows measurement of drug uptake and the formation of drug induced DNA damage, called adducts, before initiation of full-dose chemotherapy. *We hypothesize that the concentration of microdose-induced carboplatin-DNA adducts above a certain threshold is predictive of chemotherapy response.* The test is based on accelerator mass spectrometry (AMS), which is uniquely able to quantify microdoses in clinical samples. Described below are three major accomplishments of subproject 3.

Accomplishment 1: Optimized the clinical protocol for [¹⁴C]carboplatin microdosing.

The goal was to determine the optimal microdose formulation and demonstrate dosing and sample collection in lung and bladder cancer patients. Five patients with lung cancer and four patients with bladder cancer were given 1/100th the therapeutic dose of ¹⁴C-labeled carboplatin. This dose contained 10⁷ dpm of radiocarbon per kg of body mass—resulting in a radiation

exposure equivalent to approximately half of a chest X-ray. This microdose formulation provided drug-DNA damage measurements with sufficient signal-to-noise.

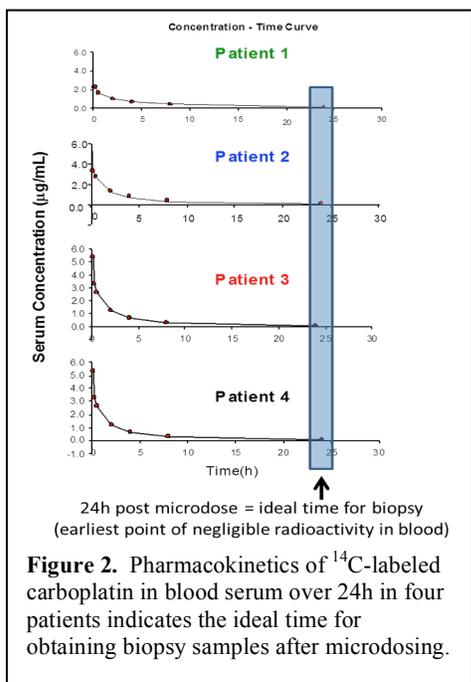
TABLE 1: Carboplatin-DNA monoadducts (per 10^8 nucleotide). PBMC- peripheral blood mononuclear cells. N.M.- not measured due to IRB restrictions. No chemo- chemotherapy not prescribed due to very early or very late stage disease. ¹suspected contaminated sample- protocol revised.

Patient # (cancer type)	PBMC- 8 hrs	PBMC-24 hrs	Tumor- 24 hrs	Responder?
1 (bladder)	0.36	0.33	N.M.	no
2 (bladder)	0.59	0.98	N.M.	yes
3 (lung)	0.29	0.84	N.M.	no
4 (lung)	1.24	1.3	N.M.	yes
5 (bladder)	1.1	0.44	20.7 ¹	No chemo
6 (lung)	0.38	0.46	0.87	No chemo
7 (lung)	0.59	0.94	0.66	Data not avail.
8 (bladder)	0.27	0.08	2.68	Data not avail.
9 (lung)	1.1	0.12	0.05	Data not avail.

Carboplatin-DNA monoadducts were observed in the range of 0.08 to 1.3 adducts per 10^8 nucleotides (5 to 83 adducts per human genome) in PBMC's 2 to 24 hr after dosing. Similar adduct frequencies (0.05 and 2.68 adducts per 10^8 nucleotides) were observed in lung tumor tissue at 24 hours post microdosing (**Table 1**). The first bladder tumor sample for which carboplatin-DNA adduct frequency was determined (Patient 5) had a considerably higher drug-DNA adduct frequency than the PBMC sample from this same individual (20.7 adducts per 10^8 nucleotides in tumor tissue vs. 0.44 adducts per 10^8 nucleotides in PBMC at 24 hrs). This sample may have been contaminated with residual carboplatin that was found in the urine of this patient at 24 hrs post microdosing. This prompted us to update the protocol to include an urine void and washing

of the bladder with PBS prior to biopsy for the second bladder tumor sample (Patient 8).

These findings are significant from two perspectives. First, these AMS measurements represent the first quantitative data assessing carboplatin-DNA adducts in humans given microdoses of a chemotherapeutic agent. It validated the notion that AMS is sensitive enough to measure the very low level of adducts (a few adducts per cell) expected in human tissue after microdosing with a radiolabeled DNA chemotherapeutic agent. Second, it defined the adduct frequency range that occurs in human tissue after carboplatin microdosing.



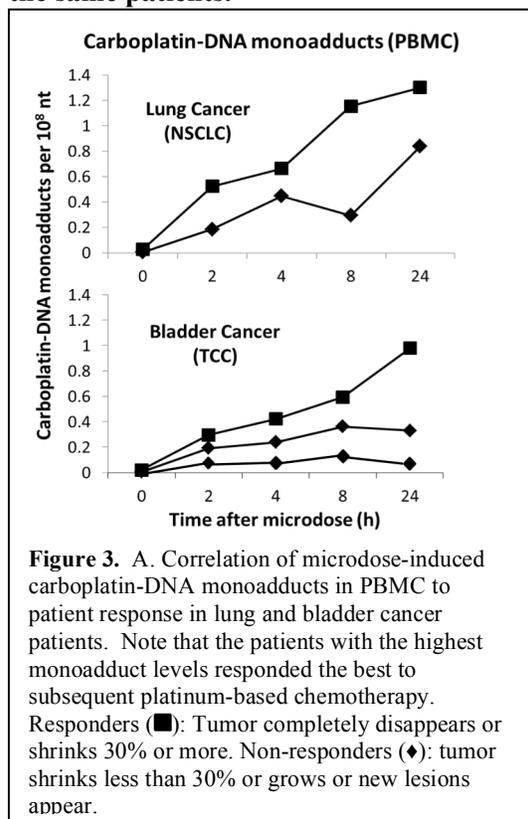
Accomplishment 2: Identified the optimal time post microdosing for tumor biopsy.

The optimal tumor biopsy scheduling was determined by examining serum carboplatin pharmacokinetics (PK) as well as the kinetics of DNA adduct formation in PBMCs from the first four patients. PK in serum was assessed using liquid scintillation counting at time 0, 5 min, 15 min, 30 min, 2h, 4h, 8h and 24h post injection

(**Figure 2**) for the purpose of comparing inter-patient variability and establishing the optimum time point for tumor biopsy sample acquisition. The disintegrations per minute in the sample and specific activity of the microdose formulation were used to calculate the concentration of drug in

each serum sample in mg carboplatin (equivalent) per mL of serum. As expected from literature reports for therapeutic doses of carboplatin, the serum concentration of drug decreased with a half-life of a few hours, and was essentially cleared from the blood within 24h, supporting 24h as the feasible time point for tumor analysis in order to maximize the carboplatin-DNA monoadduct concentration in tumor and minimize the risk of radioactive contamination of the operating room by blood-borne radiocarbon. In contrast, DNA damage in PBMCs continued to accumulate to at least 24 hr even though the serum was cleared of carboplatin, suggesting that intracellular carboplatin was still present and active. Despite the inter-patient differences in PBMC adduct formation (described below), we found that the pharmacokinetics (PK) did not differ across the four patient samples (variation of less than 2-fold [$T_{1/2}$ and AUC]) or between responders and non-responders). Overall, the serum PK and time course PBMC data allowed determination of the best time to dose patients prior to biopsy in order to maximize the signal-to-noise, which was 24h after injection of the microdose. The optimized microdose formulation and dosing schedule was used below in Accomplishment 3 for the preliminary tumor biopsy assessment.

Accomplishment 3. Collect PBMC DNA adduct data and therapeutic outcome data from the same patients.



Of the 9 patients for which the kinetics of carboplatin-DNA adducts were measured, 5 of these patients were followed long enough post carboplatin treatment to determine therapeutic outcome (**Figure 2**). These data are derived from two lung and three bladder cancer patients that were given a microdose of [^{14}C]carboplatin by IV injection. At time points of 2, 4, 8 and 24h blood samples were taken (4 mL) from which PBMC were isolated using commercially available centrifugal sedimentation tubes. After centrifugation, a white band containing PBMC was pipetted out, lysed, treated with RNAase and alcohol precipitated. The resulting DNA pellet was washed repeatedly with ethanol/water to remove unbound drug. The DNA was then quantified by UV-Vis spectrophotometry and the radiocarbon content was measured by AMS. The resulting data allowed calculation of the concentration of carboplatin-modified nucleotides, expressed as carboplatin-DNA monoadducts per 10^8 nucleotides (**Figure 3**). Within four weeks after the microdosing procedure, the patients began platinum-based chemotherapy and were followed for response over approximately two

months. Two patients responded to therapy, while three others were resistant (one lung and one bladder cancer patient in each response groups). As shown in **Figure 3**, there was an overall trend for an increase in microdose-induced monoadduct formation in PBMC for responders over 24h, which supports our hypothesis that there exists a threshold drug-DNA damage level for triggering response to treatment. The response to therapy varied from complete response to disease progression amongst these five patients. The range of drug-DNA adduct values across all patients was greatest at the 24h time point, which supports the conclusion from Accomplishment 2 that this time point is optimal for biopsy collection. There were no adverse effects from the microdose procedure. Although the data display an encouraging trend, many more patients need

to be accrued in order to have statistically significant results from measurements in PBMC and tumor biopsies.

Novelty and significance

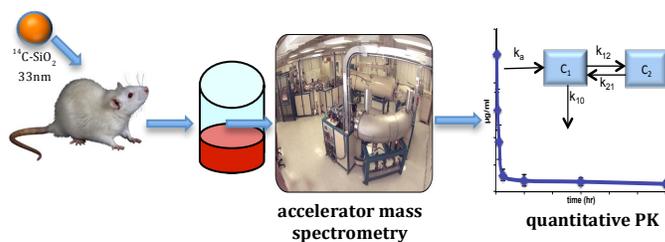
Previous studies to define a predictive platinum chemotherapy biomarker have focused on genetic mutations and gene expression, and have not been very successful owing to the highly complex multifactorial nature of drug resistance to cytotoxic agents. This study focuses on platinum-induced DNA damage, the *in vivo* cumulative pharmacodynamic effect of the action of hundreds of gene products and overall patient health. Several studies using full-dose chemotherapy have shown that the levels of Pt-induced DNA damage correlate to patient response. However, the analytical techniques quantifying Pt-DNA adducts in these previous studies lack the sensitivity to detect adducts using sub-therapeutic doses of platinum agents prior to full dose chemotherapy. These traditional approaches are not useful for treatment planning. In this study, we are using a novel microdosing approach enabled through technical advances in the application of AMS to biological systems. Based on the results of this clinical trial and future studies, it should be possible to classify malignancies into different subgroups according to mechanism of drug resistance and likelihood of response to specific therapy regimens.

SPID: 00069

Subproject Title: Multi-Scale Toxicology Initiative

Investigators: Mike Malfatti, Ken Turteltaub, Heather Enright

The increasing use of nanoparticles (NPs) for a wide variety of commercial, industrial, and biomedical application has led to concerns about their safety. Because of their unique properties such as monodispersity, large surface area, and high drug loading efficiency, silica nanoparticles (SiNPs) have been developed for a vast array of biomedical uses such as optical imaging, cancer therapy, targeted drug delivery, and controlled drug release for genes and proteins. Silica nanoparticles are also found in many personal care products and in certain foods. However, as the potential uses of SiNPs have increased, the evaluation of their biological fate and toxicity has not kept pace. Studies have shown that inhalation of microcrystalline silica may be linked with the pulmonary disease silicosis in humans. Chronic inhalation studies in rats have been associated with pulmonary fibrosis and cancer, and exposure to microscale amorphous silica has been



linked to inflammation, granuloma formation, and emphysema. Overall, however, a complete understanding of the size, shape, and composition-dependent interactions of SiNPs with biological systems is lacking, largely due to suitable analytical methods. In a study published in the October 17

edition of *Nanoletters*, Mike Malfatti, Heather Palko Enright, Ed Kuhn, and Ken Turteltaub reported on accelerator mass spectrometry measurements used to investigate the relationship between administered dose, pharmacokinetics (PK), and long-term biodistribution of ^{14}C -labeled silica nanoparticles (SiNPs) *in vivo*. PK analysis showed that SiNPs were rapidly cleared from the circulatory system (the “central compartment” in PK models) and were distributed to various body tissues, where they persisted over the 8 week time course of the study, raising questions about the potential for bioaccumulation and associated long-term effects.

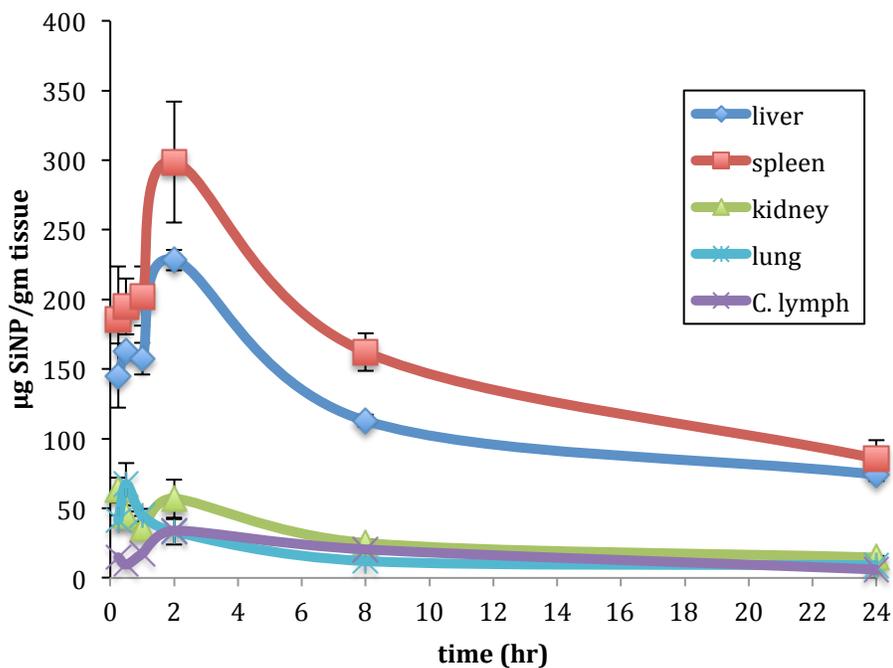


Figure 4. Mean tissue concentration of ¹⁴C-SiNP (33 nm), over a 24 h sampling time, following single 0.5 mg intravenous administration to BALB/c male mice. Data is expressed as the mean of 5 animals ± the standard error of the mean.

Malfatti, M. A., Palko, H. A., Kuhn, E. A., Turteltaub, K. W. (2012) Determining the pharmacokinetics and long-term biodistribution of SiO₂ nanoparticles in vivo using accelerator mass spectrometry. Nano Letters, 12, 5532-5538.

06/01/2012 – 05/31/2013* 8 P41 GM103483-14

RESOURCE FOR THE DEVELOPMENT OF BIOMEDICAL MASS SPECTROMETRY

***Progress as of 3/31/13**

RESEARCH PROGRESS

The goal of the Resource for this funding cycle continues to be to address three major gaps in the continued growth and application of AMS in biomedical research. The first identified gap is the lack of high throughput analysis coupled to molecular speciation, which can be solved by the development of an on-line fully integrated AMS-HPLC system. A second gap is the lack of robust methods for quantifying cellular metabolic endpoints for use in systems and cell biology applications. Understanding how to measure post-translational modifications, metabolic flux and metabolic rate with acceptable sensitivity, precision and reproducibility using substrate concentrations that are within the natural physiological range so that the normal metabolism is not perturbed is likewise important. This should become routine when the coupled AMS-HPLC system is available. Third, the ability to measure biological endpoints in humans is important and AMS offers the ability to do this safely. Our overall goal is to develop AMS and apply its quantitative capabilities to enable effective research in fundamental biology and human health. To that end, this cycle of Resource funding is devoted to pursuing the following specific aims:

- 1.) Increased throughput of AMS through direct coupling to separatory instruments.
 - a.) Conversion of liquid HPLC eluents to gaseous CO₂, directly linking complex chemical analysis to the ion source.
 - b.) Improve the capability to measure very small samples with high precision.

- 2.) Increase the value and information content of AMS measurements by combining molecular speciation with quantitation of defined isolates for pathway analysis from very small cellular, animal, and human samples.
 - a.) Quantitate isotope content and flux in physical and chemical isolates of cells at the individual metabolite and at the pathway level.
 - b.) Quantitate modified or derivatized macromolecules important in cellular metabolism, signaling and control.
 - c.) Provide quantitation of biological systems using multiple isotopic tracers such as ³H, ¹⁴C and other isotopes measured at LLNL.

- 3.) Develop and validate methods for the use of AMS in translational human studies.
 - a.) Demonstrate robust methods for conducting pharmacokinetic/pharmacodynamics in humans and model systems and coupling this information to computational modeling.

- 4.) Provide high throughput precision quantitation for collaborative and service clients.

To accomplish these aims we have organized this center into 3 Core Research and

Development Subprojects and a Collaborative and Service Center. Core Project 1 focuses on our aim to increase AMS throughput via development of a coupled AMS-HPLC system. Our hypothesis is that such a coupled system will significantly increase throughput and enable routine quantification of individual components of complex mixtures thus broadening AMS's utility for the biomedical sciences. Core Project 2 focuses on developing tools to quantitate important metabolic steps and subsystems in cells and to test the hypothesis that quantitative metabolic flux data will improve computational models of metabolic systems and their interactions. It will also work to quantify rates of protein metabolism and post-translational modifications of proteins in cells. Core Project 3 focuses on developing and validating methods to conduct studies in humans, mostly for use in drug development, nutrition and toxicology. This project is working to test the hypothesis that AMS data can improve the accuracy of physiologically-based pharmacokinetic models for predicting human effects of drugs/toxicants or nutrients and can enable earlier human testing of such compounds than is now possible. These core projects are linked to developing the technology to allow more rapid and cost effective analysis of metabolism in cells and higher organisms for use in understanding the dynamic processes that occur and how individual variation, disease, toxicants and drugs affect them. The collaborative and service center oversees the operation of our sample preparation process and accelerator mass spectrometry measurements for our collaborative and service users and assists with study design and data interpretation. These collaborative projects are coupled to the core research and development the center undertakes.

Resource Progress

This past year the three Technology Development projects of the Resource have continued to make good progress in pursuit of the Resource's specific aims. In addition, we have added new collaborative subprojects and they, along with our existing collaborators, are continuing to provide the scientific drivers for the Technology Development projects.

Updates of the Resource Technology Development projects are given below. For updates on Resource Collaborative and Service Subprojects, please see the Subproject section of the 2012 Annual Progress Report (APR).

Project 1 (SPID 0001)

Project 1: Sample-Spectrometer Interface

PI: Ted Ognibene, Co-PIs: Bruce Buchholz, Ken Turteltaub, Kurt Haack

Project 1 seeks to improve our capability to quantitate very small samples. These capabilities will use interfaces developed for our new gas-accepting ion source, which was installed onto our existing 1-MV AMS system. This source makes more efficient use of the sample and requires less sample handling. It reduces the minimum sample size required for analysis while improving analysis throughput while lowering costs.

During the past year, we demonstrated the real-time ^{14}C quantitation of HPLC separations by liquid sample accelerator mass spectrometry (LS-AMS) by quantifying low-abundance protein modifications. To demonstrate LS-AMS and compare the new technology to traditional solid sample AMS (SS-AMS), native and reduced bovine serum albumin (BSA) was modified by ^{14}C -iodoacetamide with and without glutathione present, producing adducts on the order of 1 modification in in every 10^6 to 10^8 proteins. ^{14}C incorporated into modified BSA was measured by solid carbon AMS and LS-AMS. Reduced and native BSA peptides were generated by tryptic digestion with analysis of HPLC-separated peptides performed in parallel by LS-AMS, fraction collection combined with SS-AMS, and (for peptide identification) electrospray ionization and tandem mass spectrometry (ESI-MS/MS). LS-AMS enabled ^{14}C quantitation from ng sample sizes and was 100 times more sensitive to ^{14}C incorporated in HPLC-separated peptides than SS-AMS, resulting in a lower limit of quantitation of 50 zmol ^{14}C /peak. Additionally LS-AMS turnaround times were minutes instead of days, and HPLC trace analyses required 1/6th the AMS instrument time required for analysis of graphite fractions by SS-AMS.

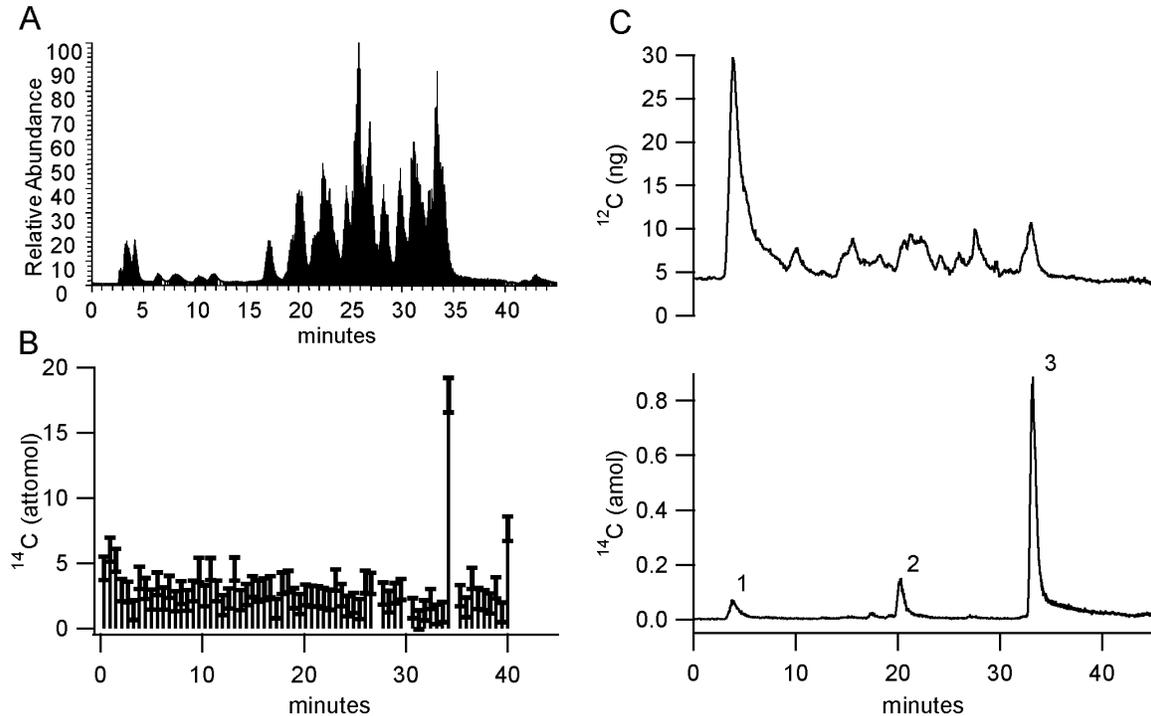


Figure 1. Modification of native bovine serum albumin by ^{14}C -iodoacetamide. **A**, MS/MS relative peptide abundance total ion chromatogram. **B**, LS-AMS ^{12}C trace in units of ng ^{12}C /measurement interval (top) LS-AMS ^{14}C trace in units of amol ^{14}C /measurement interval (bottom). ^{14}C peak 3 is due to the peptide containing Cys 58, GLVLIAFSQYLQQCPFDEHVK. Minor ^{14}C peaks 1 and 2 contain multiple Cys-containing peptides. **C**, Peptide fraction collection and SS-AMS analysis.

Publications:

1. A. T. Thomas, B. J. Stewart, T. J. Ognibene, K. W. Turteltaub and G. Bench (2012). "Directly coupled HPLC-AMS measurement of chemically-modified protein and peptides", *Analytical Chemistry*, accepted for publication.
2. T. J. Ognibene and G. A. Salazar (2013). "Installation of hybrid ion source on the 1-MV LLNL BioAMS spectrometer." *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* 294(0): 311-314.
3. G. Salazar and T. Ognibene (2013). "Design of a secondary ionization target for direct production of a C^- beam from CO_2 pulses for online AMS." *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* 294(0): 300-306.

Project 2 (SPID 0002)

Project 2: Quantitative Cell Biology

PI: Graham Bench, Co-PIs: Ben Stewart, Ali Navid, Bruce Buchholz

The research area of Cell Biology focuses on gaining an understanding of both the composition and function of individual cells, how cells change over their lifetime and in response to signals from their environment as well as determining how cells form a whole organism. Biochemical studies of cells and their contents depend on collecting, defining, identifying, and quantifying the biomolecules responsible for a certain function, structure, or biochemical signal. Such well-defined biomolecular samples often contain little material. To address this paucity of material, a variety of sensitive instrumentation, including flow cytometers and capillary electrophoresis (CE) systems, are available to provide sensitivity for studying these materials. Such techniques typically employ chemical reactions to enable fluorescent labeling and conventional or laser-induced fluorescence for detection. While such methods have detection sensitivities as low as several zeptomole for proteins, not all moieties within a cell can be fluorescently labeled and quantitation often relies on molecular properties of the analyte.

Isotope labeling strategies provide an alternative approach to protocols that employ fluorescence detection. The isotope label can be analytically measured independent of molecular structure to quantitate the amount of a substance. ^{13}C and ^{14}C are frequently used in isotope labeling experiments primarily because they are isotopes of an element naturally present in almost all molecules of biological interest. Cells can be cultured in enriched ^{13}C or ^{14}C media so that, in principle, all cellular constituents are uniformly labeled. This means that all compounds within the cell can be efficiently

labeled. AMS, owing to its ability to measure small quantities of material at high sensitivity and with accurate analytical quantitation, is well suited to make valuable contributions to cell biology.

Successful use of AMS for cell biology will not only require adequate sample preparation methods, but also will require that the biological aspects of the experiments be designed and conducted for compatibility with AMS analysis. This project will develop methods: 1) to routinely couple cell biology experiments to AMS and; 2) to quantitate components within cells that have been labeled with ^{14}C . Emphasis will be placed on the development of separatory methods to speciate metabolites and molecules from pools of cells for accurate quantification by AMS. Specifically, we will develop robust methods to:

- 1) Isolate sub-cellular fractions and speciate individual metabolites from pools of cells.
- 2) Couple enzyme kinetics and metabolic flux measurements to AMS.
- 3) Couple AMS to quantitative measurement of post translational modifications and adducts formed by the interaction of reactive metabolites with cellular macromolecules.
- 4) Couple AMS to cell turnover in human tissues using the ^{14}C bomb curve.

Aims 1-3: 1). Isolate sub-cellular fractions and speciate individual metabolites from pools of cells; 2). Couple enzyme kinetics and metabolic flux measurements to AMS; and 3). Couple AMS to quantitative measurement of post translational modifications and adducts formed by the interaction of reactive metabolites with cellular macromolecules.

We used ^{14}C -labeled precursors to characterize metabolic fate and cellular effects of metabolites in model organisms. In the yeast *Saccharomyces cerevisiae*, we traced the fate of the reactive dicarbonyl methylglyoxal under low-glucose, normal glucose, and high glucose growth conditions. We observed that the glyoxalase pathway was primarily responsible for detoxification of methylglyoxal under these growth conditions. We measured formation of chemical adducts produced by the interaction of ^{14}C -methylglyoxal with yeast macromolecules. These results have been accepted for publication in the journal *Yeast*. In addition, a collaborative project was completed in which metabolites were measured in a microbial co-culture system for hydrogen production. The results of this project were published in the *International Journal of Hydrogen Energy*.

Quantitation of low-abundance protein modifications involves significant analytical challenges, especially in biologically-important applications such as studying the role of post-translational modification in biology and measurement of the effects of reactive drug metabolites. Research performed under Specific Aim 3 involved measurement of proteins and peptides alkylated with ^{14}C -iodoacetamide and measurement by liquid chromatography-tandem mass spectrometry and liquid sample AMS. Labeling techniques and separation methods were developed to allow measurement of proteins and peptides using the liquid sample interface. We performed real-time ^{14}C quantitation of HPLC separations by liquid sample accelerator mass spectrometry (LS-AMS). We demonstrated that direct HPLC-AMS coupling overcomes several major limitations of conventional HPLC-AMS where individual HPLC fractions must be collected and converted to graphite before measurement. These results are currently under review for publication in the journal *Analytical Chemistry* (Thomas, A. T.; Stewart, B. J.; Ognibene, T. J.; Turteltaub, K. W. and Bench, G. Directly coupled HPLC-AMS measurement of chemically-modified protein and peptides.

Aim 4: Couple AMS to cell turnover in human tissues using the ^{14}C bomb curve.

Projects coupling AMS to cell and protein turnover in human tissues using the ^{14}C bomb curve were advanced in Specific Aim 4. Specific sample definition is the biggest challenge in these dating and turnover projects. Four general classes of samples were studied: DNA, proteins, lipids, and dental enamel. We developed a method for the retrospective birth dating of cells using bomb pulse ^{14}C dating as a method for measuring the approximate age of specific populations of cells in the adult human brain and other tissues (Spalding, et al., 2005). This method is based on establishing the proportion of the isotope ^{14}C in genomic DNA. After a cell has terminally differentiated it does not divide again. Since the last cell division represents the last time point when the cell synthesized DNA, its chromosomal DNA reflects the age when the cell was born. Atmospheric nuclear tests in the late 1950s and early 1960s doubled the level of ^{14}C in the atmosphere followed by an exponential decline since 1963, allowing one to resolve ^{14}C differences in the range of years. Because DNA has a ^{14}C content reflective of the time when it was synthesized, establishing the ^{14}C content of chromosomal DNA enables retrospectively birth dating of cells, and thus establishes cellular turnover. Establishing a marker to specifically identify and separate specific cell types has been the biggest challenge in these studies. Pancreatic beta cells were separated using pro-insulin as a marker to establish turnover ceases after age 30 (Perl, et al., 2010). Long time collaborator at the Karolinska Institute, Kirsty Spalding, continues to probe specific brain regions for neurogenesis using NeuN+ as a positive nuclear surface marker for neurons. Spalding has also advanced investigations of lipid turnover in health and disease to determine that lipid turns over every 1.5 years (Arner, et al., 2011) while fat cells live about 10 years (Spalding, et al., 2008) in healthy individuals. The carbon in most proteins is replaced relatively rapidly. The lens of the eye is an exception, with cells and structural proteins formed *in utero* lasting a lifetime. The lens grows throughout life adding cells to the outer layers. Using relatively simple separation of water-soluble (crystalline) proteins from water-insoluble (mostly membrane) and molecular weight spin filters to remove small molecules, Stewart, et al. (2013) quantified turnover of water-soluble crystalline proteins to be ~0.5-1% annually while membrane proteins were not renewed. The finding is significant because it contradicts dogma and demonstrates a natural process of renewing carbon in elderly crystalline proteins. We also recently developed a technique to extract collagen from blood vessels. Collagen is typically extracted from bone by dissolving the mineral phase in acid and used to date archeological remains. Here collagen was extracted from blood vessels to date ruptured and unruptured intercranial aneurysms (Etminan, et al., 2013). The collagen was less than 5 years old in all cases with larger aneurysms being slightly older. Finally, bomb pulse dental enamel to determine date of birth in forensic cases has been systematically extended by combining enamel dating with other analyses. Aspartic acid racemization of teeth can provide an estimate of date of death when combined with enamel dating (Alkass, et al., 2010). Stable isotope can provide geographical information of residence during tooth formation (Alkass, et al., 2011). As the forensic community becomes aware of enamel dating, it is being applied to cold cases with other modern analytical techniques to solve cases (Speller, et al., 2012).

Meetings Attended:

Poster Presentation: Methylglyoxal Production and Metabolism in *Saccharomyces cerevisiae* as a Function of Glycolytic Flux, Society of Toxicology meeting, 14 March 2012

Publications:

- Buchholz, B. A., Sarachine Falso, M. J., Stewart, B. J., Haack, K. W., Ognibene, T. J., Bench, G., Salazar Quintero, G. A., Malfatti, M. A., Kulp, K. S., Turteltaub, K. W. and Lyubimov, A. V. (2012). Bioanalytics for Human Microdosing, *Encyclopedia of Drug Metabolism and Interactions*, John Wiley & Sons, Inc.
- Stewart, B. J., Bench, G., Buchholz, B. A., Haack, K. W., Malfatti, M. A., Ognibene, T. J. and Turteltaub, K. W. (2012). Accelerator Mass Spectrometry in Pharmaceutical Development, *Mass Spectrometry Handbook*, John Wiley & Sons, Inc., pp. 259-269.
- M.J.S. Falso, B.A. Buchholz. (2013) Bomb pulse biology. *Nucl. Instr. and Meth. B* **294**, 666-670.
- N. Etminan, R. Dreier, B.A. Buchholz, P. Bruckner, H.-J. Steiger, D. Hänggi, R.L. MacDonald. (2013) Exploring the age of intercranial aneurysms using carbon birth dating: preliminary results. *Stroke* in press. Online January 18, 2013, DOI: 10.1161/STROKEAHA.112.673806.
- Jiao, Y., Navid, A., Stewart, B. J., McKinlay, J. B., Thelen, M. P. and Pett-Ridge, J. (2012). Syntrophic metabolism of a co-culture containing *Clostridium cellulolyticum* and *Rhodopseudomonas palustris* for hydrogen production. *International Journal of Hydrogen Energy* **37**, 11719-11726.
- C.F. Speller, K.L. Spalding, B.A. Buchholz, D. Hildebrand, J. Moore, R. Mathewes, M.F. Skinner, D.Y. Yang. (2012) Personal identification of cold case remains through combined contribution from anthropological, mtDNA and bomb-pulse dating analyses. *J. Forensic Sci.* **57**, 1354-1360.
- Stewart, B. J., Navid, A., Kulp, K. S., Knaack, J. L. S., Bench, G. D-Lactate Production as a Function of Glucose Metabolism in *Saccharomyces cerevisiae* *Yeast* 2013, In Press.
- D.N. Stewart, J. Lango, K.P. Nambiar, M.J.S. Falso, P.G. FitzGerald, B.D. Hammock, B.A. Buchholz. (2013) Carbon turnover in water-soluble protein of the adult human lens. *Mol Vis* in press.

Project 3 (SPID 0003)

Project 3: Quantitative Endpoints in Humans

PI: Mike Malfatti (LLNL), Co-PIs: Paul Henderson (UC Davis), Ken Turteltaub (LLNL)

This project focuses on developing and validating methods and techniques to demonstrate the applicability of AMS to assess human responses to drugs, xenobiotics and endogenous compounds. We are conducting studies using AMS to develop and validate methods for use in absorption, distribution, metabolism and elimination studies (ADME) using drugs alone and in combination. We will investigate how best to conduct basic metabolism studies as well as to work with isolated macromolecular fractions such as DNA modified by chemotherapy drugs. We will work in cell culture, animal models, and humans to assess what types of samples can be utilized and how best to process them for AMS analysis.

Research Progress (through 3/31/2013):

Pharmacokinetics of Nanoparticles: The PK properties and the quantitative long-term tissue distribution of amorphous spherical ^{14}C -SiNPs, was investigated using the (AMS) for quantification.

Silica dioxide nanoparticles (SiNPs) are used for a wide variety of commercial and biomedical applications, yet little is known about their toxicology and safety. An important aspect in better understanding SiNP safety is characterization of the factors that affect SiNP biodistribution. Currently, comprehensive studies on biodistribution are lacking, most likely due to the lack of suitable analytical methods. To investigate the relationship between administered dose, PK, and long-term biodistribution, the technique of accelerator mass spectrometry (AMS) was used to determine the PK and tissue distribution of ^{14}C -SiNPs in mice. AMS quantifies extremely low concentrations of radiolabeled chemicals, and new here labeled nanoparticles, enabling long-term kinetic studies. Mice were administered a single intravenous dose of ^{14}C -SiNPs. Blood and tissue were collected at specific time points following dose administration over an 8-week time period, and SiNP concentrations determined by quantifying the radiocarbon equivalents in each sample by AMS. Plasma PK analysis showed that SiNPs were rapidly cleared from the central compartment with a half-life of 0.38 h. The large apparent volume of distribution of 2058.1 ml/kg indicated extensive distribution to tissues. SiNPs were rapidly distributed to tissues of the reticuloendothelial system including liver, spleen, kidney, lung, and bone marrow, and persisted in the tissue over the 8-week time course, raising questions about the potential for bioaccumulation and associated long-term effects. SiNP elimination occurred by both renal and biliary routes. These results indicate that AMS is an effective tool to accurately and precisely quantify the long-term kinetics and biodistribution of SiNPs *in vivo*.

Accelerating Drug Development: To understand the *in vivo* pharmacokinetics (PK) of a drug lead three studies were conducted at LLNL. The objective of the first study was to determine the dose proportionality of the drug in male Sprague Dawley rats. The second study was done to determine the PK of the drug following micro or therapeutic dose administrations in the male Sprague-Dawley rat. Finally, experiments were done to determine the mass balance and tissue distribution of the drug in the male Sprague-Dawley rat.

Dose proportionality: The pharmacokinetics following a single intravenous administration of 3, 10, or 30 mg/kg drug in rat exhibits a multi-compartment profile, which initially shows a rapid decline in plasma concentrations post-dose followed by a slower terminal phase with a mean apparent terminal half-life of 4.40 to 7.90 hrs. Across the 3 doses studied, total clearance of drug from plasma (CL) was 42.1 to 68.0 mL/min/kg and the apparent volume of distribution at steady state (V_{ss}) was 10400 to 17700 mL/kg suggesting rapid and extensive distribution beyond the plasma compartment. Overall, the for an increase in dose from 3 to 30 mg/kg, the increases in mean C_{max} (9.51-fold) and AUC_{0-t} (15.8-fold) values were within a factor of two and considered dose proportional.

Micro vs therapeutic dose PK: The pharmacokinetic profiles following a micro (0.01 mg/kg, 7.7 nCi/kg radiolabeled compound) or a therapeutic (3 mg/kg, 7.7 nCi/kg radiolabeled compound) intravenous (IV) dose in rats are similar. Both dose groups exhibit a multi-compartment profile, which initially shows a rapid decline in plasma concentrations post-dose followed by a slower terminal phase with a mean apparent terminal half-life of 5.4 to 13.2 hrs. The apparent volume of distribution (V_d) was 30510 and 30747 mL/kg for the 0.01 mg/kg and 3.0 mg/kg dose groups, respectively, suggesting rapid and extensive distribution beyond the plasma compartment. When dose normalized, there was a 1.8 fold difference in AUC_{0-t} and a 2.9-fold difference in C_{max} between dose groups. Overall, the for an increase in dose from 0.01 to 3.0 mg/kg (300-fold), the increases in mean AUC_{0-t} (1.8-fold) and C_{max} (2.9-fold) values were within a factor of two and three, respectively, and considered dose linear.

Tissue distribution and excretion: Analysis of blood determined that the majority of the dose is found in the plasma compartment with very little in the red blood cells. The cumulative plasma concentration of drug over the 96 hr exposure time was 8.2 μ g/ml. Comparison of mean plasma $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ values for the drug indicates that approximately 72% of the compound was eliminated from the plasma within 96 hr post dose. The mean (\pm SD) total percentage of dose excreted within 96 hrs was 33.4 % \pm 11.6%, of which 30.66% \pm 11.4% was eliminated in the feces and 2.81% \pm 1.64% was excreted in the urine. The average drug feces/urine ratio was calculated as 10.9:1. Most of the urinary and fecally excreted drug was eliminated within 4 and 24 hours postdose, respectively.

04/01/2012 – 03/31/2013 8 P41 GM103483-14 RESOURCE FOR THE DEVELOPMENT OF BIOMEDICAL MASS SPECTROMETRY

ADVISORY COMMITTEE

External National Advisory Committee

The External National Advisory Committee is in place to oversee the Resource's scientific direction and operating policies. In addition, it comments on the needs and plans for adequate instrumentation, technology development, and future scientific direction. It helps recruit investigators for collaboration in new areas of research. The makeup of the committee was weighted by expertise in mass spectrometry to help guide the needs in technology development. It was also weighted by expertise in carcinogenesis and pharmacology/toxicology since these had been the theme areas for the Resource during its initial funding period. The current membership of the External National Advisory Committee is being reviewed to further extend the external expertise into the current scientific focus areas. A.L Burlingame, Ph.D., a senior investigator at University of California at San Francisco and expert in biomedical applications of mass spectrometry, has continued to serve as Chairman of the Committee. The members of the committee are listed below.

<u>Member's Name</u>	<u>Institution</u>	<u>Areas of Expertise</u>
Alma L. Burlingame, Ph.D. Chairman	University of California at San Francisco	Biological Mass Spectrometry
Richard H. Adamson, Ph.D.	American Beverage Association	Cancer Etiology / Consultant Pharmacology / Toxicology
Thomas A. Baillie, Ph.D., D.Sc. Dean, Pharmacy	Washington Unive Drug Metabolism	Mass Spectrometry /
J. Thomas Brenna, Ph.D. Professor	Cornell University Division of Nutritional Sciences	Nutrition / Mass Spectrometry Interfaces
Michael F. Rexach, Ph.D. Associate Professor	University of California at Santa Cruz Dept. Molecular Cell & Developmental Biology	Cell Biology / Yeast genetics Molecular Biology
Vern L. Schramm. Ph.D. Professor	Albert Einstein College of Medicine	Enzymology / Biochemistry

	Dept. of Biochemistry	
Jay Davis	Retired, LLNL Spectrometry	Accelerator Mass
John Hayes	Retird, Scripts	Mass Spec, AMS
Jim Felton	UC Davis	Toxicology

Internal Executive Committee

The Internal Executive Committee members are from the Resource's key personnel list. The Internal Executive Committee, along with Resource staff and associated members of the Lawrence Livermore National Laboratory (LLNL) Center for Accelerator Mass Spectrometry, meet weekly to discuss project effort and prioritization, review collaborative/service requests, and deal with other issues relating to Biomedical AMS. The members of the committee are listed below.

<u>Member's Name</u>	<u>Department</u>
Kenneth W. Turteltaub, Ph.D. Principal Investigator	Biosciences & Biotechnology Division
Graham Bench, Ph.D.	Center for Accelerator Mass Spectrometry
Bruce A. Buchholz, Ph.D.	Center for Accelerator Mass Spectrometry
Mike Malfatti, Ph.D.	Biosciences & Biotechnology Division
Ted J. Ognibene, Ph.D.	Center for Accelerator Mass Spectrometry
Kristen S. Kulp, Ph.D.	Biosciences & Biotechnology Division

The External Advisory Committee, in conjunction with the Internal Executive Committee, strives to meet once a year. Additionally, Partial Committee meetings can occur on *ad hoc* occasions, such as workshops. Discussions also occur as needed by phone, email, or fax. The Annual Advisory Committee Meeting focuses on the progress being made on the Technology Development projects, the allocation of resources, and the Resource's future.