Modification Form for Permit BIO-UWO-0122

Permit Holder: Moshmi Bhattacharya

Approved Personnel
(Please stroke out any personnel to be removed)

Mistre Alemayehu

~~Adel-Aziziyeh~~

~~Timothy Li~~

Cynthia Pape

Additional Personnel
(Please list additional personnel here)

Matt Zajac

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	E. coli DH5 alpha	
Approved Cells	human (established), non-human primate (established), HEK 293, MDA-MB-231, MDA-MB-435S, MDA-MB-468, MCF-7, MCF-10A, MCF-12A, SK-BR-3, Hs 578T, Hs 578 BST, cos-7	
Approved Use of Human Source Material		
Approved GMO	SV 40 LARGE T antigen (cos-7), Adeno E1A gene, pEGFP, pEYFP, pcDNA3, pRS	
Approved use of Animals		· nude mice (immune compromised) w/ ACVS (UWO) # 2008-086-06 (Barrier facility) with MDA-MB-231 Knock down (shRNA) β-arrestin 1 and/or 2 constructs or NT (stable cell lines) - cell lines independently tested (Radil) for mycoplasma/contaminants.

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

Classification: 2

Date of last Biohazardous Agents Registry Form: Mar 28, 2008

Signature of Permit Holder:

BioSafety Officer(s):

Chair,Biohazards Subcommittee:

Modification Form for Permit BIO-UWO-0122

Permit Holder: Moshmi Bhattacharya

Approved Toxin(s) Pertussis, Cholera

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Classification: 2

Date of last Biohazardous Agents Registry Form: Mar 28, 2008

Signature of Permit Holder:

BioSafety Officer(s):

Chair,Biohazards Subcommittee:

-------- Original Message --------
Subject: Bhattacharya BIO-UWO-0122
Date: Tue, 18 Aug 2009 10:08:26 -0400
From:cynthia Pape <cynthia.pape@schulich.uwo.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>

Jennifer,
I've included more info for you to add to our permit regarding the stable B-arrestin cell lines generated:

re: M.Bhattacharya BIO-UWO-0122
Initial investigation in our lab showed elevated endogenous levels of B-arrestin2 mRNA in human breast carcinoma cell lines.
Transient transfection with shRNA constructs (commercially available) to B-arrestin1 (shBarr1) and/or B-arrestin2 (shBarr2) to knock-down (k/d) but not eliminate B-arrestin signaling showed a marked decrease in
the motility of the cells in response to stimulation with either 10uM LPA (lysophosphatidic acid) or to serum, however the efficiency of transfection was very low. Stable cell lines were generated using MDA-MB-231 parent cells electroporated with shBarr1 and/or shBarr2. A control 'scrambled' line was also generated. These cell lines require the use of antibiotic (puromycin) to provide selective pressure for the cells to maintain the transfected shRNA construct (pRS vector).
The stable cell lines also show significant reduction in cell motility (2D/migration assay) as well as a decrease in the formation of stellate structures (3D/invasion assay) as compared to the parent MDA-MB-231 cells.

This information was included in a recently published paper from this lab: Mol Cancer Res 2009;7(7):1064-77
{beta}-Arrestin/Ral Signaling Regulates Lysophosphatidic Acid-Mediated Migration and Invasion of Human Breast Tumor Cells
Timothy T. Li, et.al.

The stable MDA-MB-231 shBarr cell lines have further been used for in vivo studies using nude mice in UWO Barrier facility (i.e. contained within micro-isolator cages). Immune compromised (nu/nu mice) animals must be used for the cell line to grow. All animal material (bedding/carcasses) is incinerated.
UWO-ACVS protocol # 2008-086-06.

Hope that helps,
Cindy

Cynthia Pape
Lab Technician
MSB 231; Dr. M. Bhattacharya lab

UWO Biosafety Permit # BIO-UWO-0122

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Revised Biohazards Subcommittee: January, 2007

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario where the use of biohazardous infectious agents are described in the experimental work proposed. The form must also be completed if animal work is proposed involving the use of biohazardous agents or animal carrying zoonotic agents infectious to humans. Containment Levels will be required in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Health Canada (HC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety (Stevenson-Lawson Building, Room 60) for forward to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Coordinator at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies) modifications must be completed and sent to Occupational Health and Safety. See website: www.uwo.ca/humanresources

PRINCIPAL INVESTIGATOR Moshmi Bhattacharya
SIGNATURE _____
DEPARTMENT Physiology & Pharmacology
ADDRESS MSB 329 (office)
PHONE NUMBER x 82970
EMAIL Moshmi.Bhattacharya@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) MSB Room(s) 224 (TC Room); 231 (main lab)
*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to it being sent to Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Centre, Child and Parent Research Institute or Robarts Research Institute, University Biosafety Committee members can also sign as the Safety Officer.

TITLE OF GRANT(S):
1. Molecular regulation of lysophosphatidic acid receptor desensitization and endocytosis (NSERC)
2. β-arrestins and LPA receptor signaling in breast cancer migration and invasion (CIHR)

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK, SUCH A THE RESEARCH GRANT SUMMARY(S) THAT EXPLAINS THE BIOHAZARDS USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

FUNDING AGENCY/AGENCIES NSERC / CIHR

Names of all personnel working under Principal Investigators supervision in this location:
i) Cynthia Pape (technician)
ii) Timothy Li (MSc. student)
iii) Adel Aziziyeh (MSc student)
iv) Mistre Alemayeh (MSc. student)
v) _____

* DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED*

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? ☒ YES ☐ NO
If no, please proceed to Section 2.0

1.2 Please complete the table below:

Name of Biological agent(s)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time?
E. coli - DH5α	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	500 mls
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	

1.3 For above named organism(s) or biological agent(s) circle HC or CFIA Containment Level required. (1) 2 3

1.4 Source of microrganism(s) or biological agent(s)? Invitrogen

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☒ YES ☐ NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (ie. derived from fresh tissue) that will be grown in culture in the table below

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue
Human	☐ Yes ☒ No	
Rodent	☐ Yes ☒ No	
Non-human primate	☐ Yes ☒ No	
Other (specify)		

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s) level	Supplier / Source
Human	☒ Yes ☐ No	HEK293 ✓ (2) MDA-MB-231 ✓ (2) MDA-MB-435S ✓ (2) MDA-MB-468 ✓ (2) MCF-7 ✓ (1) MCF-10A ✓ (2) MCF-12A ✓ (2) SK-BR-3 ✓ (2) Hs 578T ✓ (1) Hs 578Bst ✓ (1)	ATCC
Rodent	☐ Yes ☒ No		
Non-human primate	☒ Yes ☐ No	COS-7 ✓ (2)	ATCC
Other (specify)	☐ Yes ☒ No		

2.4 For above named cell types(s) circle HC or CFIA containment level required 1 (2) 3

* DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED*

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? ☐YES ☒NO
If no, please proceed to Section 4.0

3.2 Indicate if the following will be used in the laboratory

- Human blood (whole) or other bodily fluids ☐ YES ☐ NO If YES, Specify _____
- Human blood (fraction) or other bodily fluids ☐ YES ☐ NO If YES, Specify _____
- Human organs (unpreserved) ☐ YES ☐ NO If YES, Specify_____
- Human tissues (unpreserved) ☐ YES ☐ NO If YES, Specify_____

3.3 Is human source known to be infected with and infectious agent ☐ YES ☐ NO
If YES , please name infectious agent _____

3.4 For above named materials circle HC or CFIA containment level required. 1 2 3

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents or cells described in Sections 1.0 and 2.0 ? ☒ YES ☐ NO
If no, please proceed to Section 5.0

4.2 Will genetic sequences from the following be involved:

- HIV ☐ YES ☒ NO
if YES specify_____
- HTLV 1 or 2 or genes from any CDC class 1 pathogens ☐ YES ☒ NO
if YES specify_____
- Other human or animal pathogen and or their toxins ☐ YES ☒ NO
if YES specify_____

4.3 Will intact genetic sequences be used from

- SV 40 Large T antigen ☒ YES ☐ NO If YES specify present in COS-7 cells
- Known oncogenes ☒ YES ☐ NO If YES specify Adeno E1A gene in HEK 293 cells

4.4 Will a live vector(s) (viral or bacterial) be used for gene transduction ☒ YES ☐ NO
If YES name virus not applicable.

4.5 List specific vector(s) to be used: pEGFP; pEYFP; pcDNA3; pRS

4.6 Will virus be replication defective ☐ YES ☐ NO N/a

4.7 Will virus be infectious to humans or animals ☐ YES ☐ NO N/a

4.8 Will this be expected to increase the Containment Level required ☐ YES ☐ NO N/a

* DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED*

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials using the viral vector in 4.0 be conducted? ☐ YES ☒ NO
If no, please proceed to Section 6.0
If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? ☐ YES ☐ NO

5.3 How will the virus be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted:_____

5.5 Has human ethics approval been obtained? ☐ YES ☐ NO

6.0 Animal Experiments

6.1 Will any of the agents listed be used in live animals? ☐ YES ☒ NO
If no, please proceed to section 7.0

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

6.4 If using murine cell lines, have they been tested for murine pathogens? ☐ YES ☐ NO

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other bodily fluids including blood be used:

- Pound source dogs ☐ YES ☒ NO
- Pound source cats ☐ YES ☒ NO
- Sheep or goats ☐ YES ☒ NO
- Non- Human Primates ☐ YES ☒ NO If YES specify species _____
- Wild caught animals ☐ YES ☒ NO If YES specify species _____
  colony # _____

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? ☒ YES ☐ NO
If no, please proceed to Section 9.0

- NB: Keep it locked up (i.e. locked box and/or locked fridge)
- Logbook

8.2 If YES, please name the toxin Pertussis toxin ; Cholera toxin

8.3 What is the LD_{50} (specify species) of the toxin Rat 114 µg/kg, Mouse 127 µg/kg — Pertussis toxin IV.

Mouse 260 µg/kg - Cholera toxin IV.

- Cholera toxin used in MCF10 media to maintain cells
- pertussis toxin used in signaling inhibition experiments

* DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED*

9.0 Import Requirements

9.1 Will the agent be imported? * ☒ YES ☐ NO
If no, please proceed to Section 10.0
If yes, country of origin USA

9.2 Has an Import Permit been obtained from HC for human pathogens? ☐ YES ☒ NO

9.3 Has an import permit been obtained from CFIA for animal pathogens? ☐ YES ☒ NO

9.4 Has the import permit been sent to OHS? ☐ YES ☒ NO
If yes, Permit # (expired)

* Cedarlane is the Canadian distributor for ATCC & they now apply for import permits.

10.0 Training Requirements for Personnel named on Form

Sigma Aldrich supply both toxins used in this lab, and Sigma USA & Sigma Canada apply for the appropriate export/import permits to deliver the substances

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE [signature]

11.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please circle the highest HC or CFIA Containment Level required. 1 (2) 3

11.2 Has the facility been certified by OHS for this level of containment? ☒ YES ☐ NO

11.3 If yes, please give the date and permit number: BIO-UWO-0122
(last inspection 20 June 2007).

12.0 Approvals

UWO Biohazard Subcommittee.

Signature G.M. Kidder Date 28 Mar. '08

Safety Officer for Institution where experiments will take place

Signature J. Stanley, UWO Date March 28/08

Safety Officer for University of Western Ontario (if different than above)

Signature _____ Date _____

* DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED*