Modification Form for Permit BIO-LRCC-0022

Permit Holder: Ann Chambers

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

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	E. coli	
Approved Cells	Human (established): 21T series (21PT, 21NT, 21MT-1) mammary epithelial, breast cancer lines: MDA MB 231, MDA MB 231 BRMS1, MDA MB 435, MDA MB 435 BRMS1, MDA MB 435 HAL, MDA MB 468,	MDA-MB-231Br MDA-MB-231Br Her2 LN-M2 MDA-MB-231-Inc-D3H2LN
Approved Use of Human Source Material	blood (whole): plasma. Human tissues (unpreserved): lymph node and spleen	
Approved GMO	EIA (293 VSV cell), pREVITRE, pcDNA3, pREV-tet-on	
Approved use of Animals	mouse	
Approved Toxin(s)		

* *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.*

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca.

Signature of Permit Holder: _____

Classification: 2

Date of Last Biohazardous Agents Registry Form: Mar 31, 2009

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____

Additional Information of new agents:

MDA-MB-231BR & MDA-MB-231BR-HER2

- received from NIH/NCI (shipping information attached)
- Pathogen test results are attached
- Flow charts of planned experiments are attached

LN-M2

- derived from 2 rounds of lymph node metastases of MDA-MB-468LN cells in nude mice
- will be used as a model to study lymphatic metastasis
- RADIL test results are attached
- Flow charts of planned experiments are attached

MDA-MB-231-luc-D3H2LN

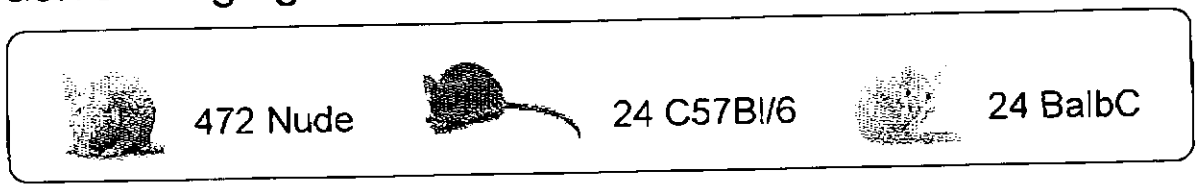
- purchased from Caliper Life Sciences as a model of lymphatic metastasis (company information is attached)
- will be used as a model of experimental and spontaneous lymphatic metastasis
- RADIL test results are attached
- Flow charts of planned experiments are attached

RECEIVED 09-04-'09 16:37 FROM- 5196858646 TO- UWO-HR-Occ. Health P005/021

09/04/09 FRI 16:36 FAX 519 685 8646 CANCER RESEARCH/LRCC 005

Chambers – Flow Chart

Non-Invasive Imaging of Metastasis: Detection, Monitoring and Intervention



1a. Characterization of Biological & Cellular Differences Between Cell Lines - MRI

- 14 Nude: MDA-MB- 231
- 14 Nude: MDA-MB- 231BR

1b. Detection of Tumour Growth Over Time by MRI

- 30 Nude: MDA-MB- 231BR

1c. Detection of Single Cells Over Time by MRI

- 30 Nude: MDA-MB- 231BR

1d. Detection of Arrest & Growth In Other Organs by MRI

- 8 C57BL6: B16F10 – mesenteric vein
- 8 C57BL6: B16F10 – tail vein
- 8 C57BL6: B16F10 – left ventricle
- 8 BalbC: 4T1 – mesenteric vein
- 8 BalbC: 4T1 – tail vein
- 8 BalbC: 4T1 – left ventricle

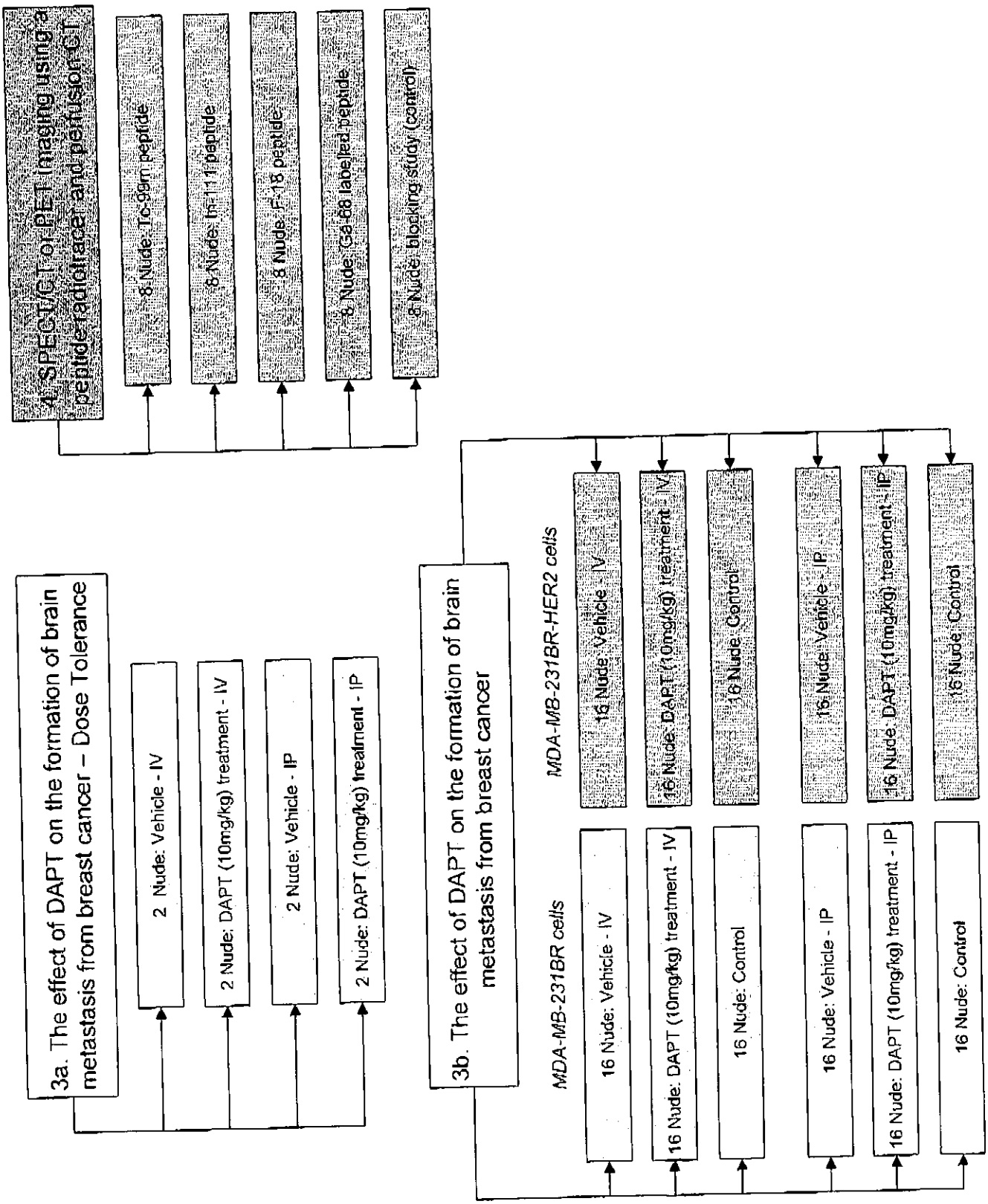
2. Cell Sorting

MDA-MB-231BR cells

- 24 Nude: CD44hi/CD24lo
- 24 Nude: CD44lo/CD24hi
- 24 Nude: Unsorted

MDA-MB-231 parental cells

- 24 Nude: CD44hi/CD24lo
- 24 Nude: CD44lo/CD24hi
- 24 Nude: Unsorted


4) SPECT/CT or PET imaging using a peptide-radiotracer and perfusion CT
8 Nude: Tc-99m peptide
8 Nude: In-111 peptide
8 Nude: F-18 peptide
8 Nude: Ga-68 labelled peptide
8 Nude: blocking study (control)
3a. The effect of DAPT on the formation of brain metastasis from breast cancer – Dose Tolerance
2 Nude: Vehicle - IV
2 Nude: DAPT (10mg/kg) treatment - IV
2 Nude: Vehicle - IP
2 Nude: DAPT (10mg/kg) treatment - IP
3b. The effect of DAPT on the formation of brain metastasis from breast cancer
MDA-MB-231BR cells
16 Nude: Vehicle - IV
16 Nude: DAPT (10mg/kg) treatment - IV
16 Nude: Control
16 Nude: Vehicle - IP
16 Nude: DAPT (10mg/kg) treatment - IP
16 Nude: Control
MDA-MB-231BR-HER2 cells
16 Nude: Vehicle - IV
16 Nude: DAPT (10mg/kg) treatment - IV
16 Nude: Control
16 Nude: Vehicle - IP
16 Nude: DAPT (10mg/kg) treatment - IP
16 Nude: Control

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09/04/09 FRI 16:38 FAX 519 685 8646 CANCER RESEARCH/LRCC 007

Chambers Tuck Lewis MacDonald – Flow Chart
Steps of Breast Cancer Metastasis:
Experimental Models and Identification of Targets for Intervention.

595 Nude

88 C57Bl/6

1a Determine the Efficiency of Cell Recovery from Lymph Nodes

- 8 Nude: MDA-MB-231-luc-D3H2LN

1b. Experimental Lymph Node Metastasis Model Development – Breast Cancer

- 12 Nude: MDA-MB-231-luc-D3H2LN
- 12 Nude: MDA-MB-231-luc-D3H2LN + tdTomato
- 12 Nude: MDA-MB-231-luc-D3H2LN + FUCCI
- 12 Nude: LN-M2
- 12 Nude: MDA-MB-435

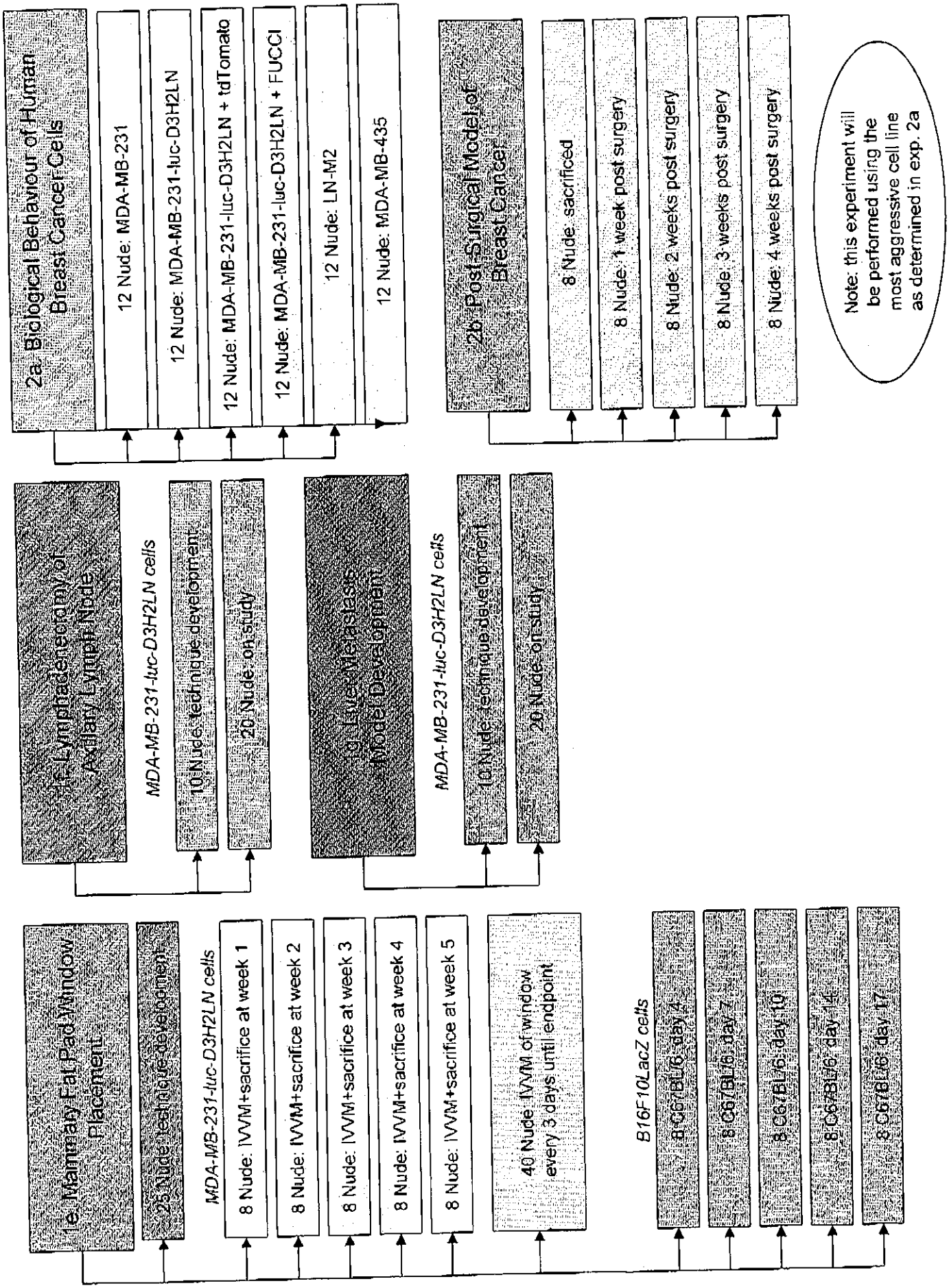
1c. Metastasis Inefficiency Experiment

- 12 Nude: 1 week post injection
- 12 Nude: 2 weeks post injection
- 12 Nude: 3 weeks post injection
- 12 Nude: 4 weeks post injection
- 12 Nude: 5 weeks post injection

Note: this experiment will be performed using the most aggressive cell line as determined in exp. 1b

1d. Experimental Lymph Node Metastasis Model Development – Melanoma

- 8 C57Bl/6: media control
- 8 C57Bl/6: killed cells control
- 8 C57Bl/6: B16F10LacZ cells remove site of injection
- 8 C57Bl/6: B16F10LacZ cells 8 day endpoint
- 8 C57Bl/6: B16F10LacZ cells 10 day endpoint
- 8 C57Bl/6: B16F10LacZ cells 14 day endpoint


2a: Biological Behaviour of Human Breast Cancer Cells
12 Nude: MDA-MB-231
12 Nude: MDA-MB-231-luc-D3H2LN
12 Nude: MDA-MB-231-luc-D3H2LN + tdTomato
12 Nude: MDA-MB-231-luc-D3H2LN + FUCCI
12 Nude: LN-M2
12 Nude: MDA-MB-435
2b: Post-Surgical Model of Breast Cancer
8 Nude: sacrificed
8 Nude: 1 week post surgery
8 Nude: 2 weeks post surgery
8 Nude: 3 weeks post surgery
8 Nude: 4 weeks post surgery
Note: this experiment will be performed using the most aggressive cell line as determined in exp. 2a
3a: Lymphadenectomy of Axillary Lymph Node
MDA-MB-231-luc-D3H2LN cells
10 Nude: technique development
20 Nude: on study
3b: Luc+ Metastasis Model Development
MDA-MB-231-luc-D3H2LN cells
10 Nude: technique development
20 Nude: on study
1a: Mammary Fat Pad Window-Placement
25 Nude: technique development
MDA-MB-231-luc-D3H2LN cells
8 Nude: IVVM+sacrifice at week 1
8 Nude: IVVM+sacrifice at week 2
8 Nude: IVVM+sacrifice at week 3
8 Nude: IVVM+sacrifice at week 4
8 Nude: IVVM+sacrifice at week 5
40 Nude: IVVM of window every 3 days until endpoint
B16F10LacZ cells
8 C67BL/6: day 4
8 C67BL/6: day 7
8 C67BL/6: day 10
8 C67BL/6: day 14
8 C67BL/6: day 17

3c. Genes of interest in 468-derived Human Breast Cancer Cells
Cells are injected directly into the lymph node
8 Nude : MDA-MB-468LN
8 Nude : MDA-MB-468GFP
8 Nude : MDA-MB-468GFP+gene of interest
Cells are injected into the thoracic mammary fat pad
8 Nude : MDA-MB-468LN
8 Nude : MDA-MB-468GFP
8 Nude : MDA-MB-468GFP+gene of interest
Note: the above experiments will be repeated for each gene of interest. There will be 3 genes of interest for a total of 288 mice.
3a. Mouse lymph nodal tissue for in vitro co-culture experiments
6 Nude : Naive
Mice are sacrificed for lymph node harvest to create 3D in vitro model
3b. Genes of Interest in 21T series Human Breast Epithelial Cells
12 Nude : 21MT-1 cells (control)
12 Nude : 21NT cells
12 Nude : 21NT+gene of interest
12 Nude : 21MT-1 cells (control)
12 Nude : 21PT cells
12 Nude : 21PT +gene of interest
Note: the above experiments will be repeated for each gene of interest. There will be 3 genes of interest for each cell line (21PT cells and 21NT cells) for a total of 432 mice.
2c. Spontaneous Lymph Node Metastasis Model Development
8 Nude - MDA-MB-231-luc-D3H2LN
8 Nude - MDA-MB-231
8 Nude - MDA-MB-231-luc-D3H2LNGFP
8 Nude - MDA-MB-231GFP
8 Nude - MDA-MB-231-luc-D3H2LN + gene of interest 1
8 Nude - MDA-MB-231 + gene of interest 1
8 Nude - MDA-MB-231-luc-D3H2LN + gene of interest 2
8 Nude - MDA-MB-231 + gene of interest 2

Printable

Subject: Her2 cells
From: "Bronder, Julie (NIH/NCI)" <bronderj@mail.nih.gov>
Date: Wednesday, November 2, 2005 10:27 am
To: dwdales@uwo.ca
Cc: Ann.Chambers@lrcc.on.ca

Dear David,

I am shipping one frozen vial of 231BR-EGFP vector (pc1) cells and one vial of 231BR-EGFP Her-2 overexpressing cells (Her2 1.1). The cell lines should reach you by late tomorrow/Friday.

I am also sending 1 ml of zeocin as well at 100 mg/mL (use it in solution at 0.375 mg/mL; the Her-2 cells were selected in the presence of zeocin). I would suggest not putting zeocin in the media for the first few days to allow the cells to recover from the thaw. After a day or two, the growth media is DMEM + 10% FCS + 1X HEPES (0.02 M) + 0.375 mg/mL zeocin (Invitrogen).

To thaw:
Quickly thaw cells in 37 degree water bath, resuspend in 10 mLs DMEM (Invitrogen # 11965-092)+ 10% FCS, and pellet at low speed for 3-4 mins. Aspirate the supernatant and resuspend the cells in 10 mL DMEM + 10% FCS and put in 100 mm dish. After 1-2 days of letting the cells sit down on a 100 mm dish, then change to fresh media. These cells grow rapidly, so I split them twice a week at ~ 1:6. To freeze cells, I use 90% FBS + 10% DMSO.

These cells have been deemed mycoplasma free, free of human pathogens, and MAP tested negative. I have electronic files of the mycoplasma and human pathogen test results (attached); for the MAP test results, it is best that I simply fax those to you. Could you please let me know the fax number to send these to?

Please do not hesitate to contact me if you have any questions.

Julie

Julie L. Bronder, Ph.D.
Women's Cancers Section
Laboratory of Molecular Pharmacology
National Cancer Institute
National Institutes of Health
Building 37, Room 1126, MSC 4264
Bethesda, MD 20892
Tel: 301-451-6445
Fax: 301-402-8910

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An Employee-Owned Company

MEMORANDUM

DATE: September 7, 2005

TO: Dr. Julie L. Bronder, Eleazar Vega-Valle
NCI Laboratory of Molecular Pharmacology
Building 37/1126
Bethesda, Md. 20892
Phone: 301- 451-6445, 301-846-7233
bronderj@mail.nih.gov
evegavalle@ncifcrf.gov

FROM: Kristen Pike
Senior Research Associate
Laboratory of Molecular Technology
915 Tollhouse Avenue Suite 211, Room 113
Frederick, Md. 21701
Phone: 301-846-6897
Fax: 301-846-6100
E-mail: pike@mail.ncifcrf.gov

SUBJECT: VIRAL PCR ANALYSIS FOR CELL LINES pc1, pc2F5, 2D11-ErbB2, 3H10-ErbB2, ErbB2-1.1, and ErbB2-1.12

We received the above human cell line pellets on Tuesday September 6, 2005. DNA was extracted, quantitated, and standardized. It was first tested for its integrity utilizing the glyceraldehyde phosphate dehydrogenase PCR. Then it was tested for presence of CMV, EBV, HBV, HCV, HTLV 1, HTLV 2, HIV 1, HIV 2, JCV, and MoMuLV DNA sequences. Viral DNA controls were used, mixed with human DNA to monitor inhibition. The PCR reactions were then analyzed on our Agilent 2100 Bioanalyzer chip instrument.

These samples tested negative for all of the above viruses by our assay.

The chip images and data chart are attached. Please feel free to contact me if you have any questions.

Attachments
cc: Martin White

_rep002_myc **Results for Luminescence Mycoplasma Test (Cambrex MycoAlert)** 9/26/2005

Animal Molecular Diagnostic Laboratory

Requestor Name: **BRONDER, J**
Address: **SAIC**
SAIC-NCI
Bldg: **0560** Room:
Frederick, MD 21702

Request Date: **09/23/05** Group No: **05-0071M**
Yellow Task #: Reviewed By:
Center #: **200531408193** Charge: **Y**
Phone #: **301-846-7233**
Fax #:
Email:

Do the samples contain biohazardous material? **N**
If yes, please specify the pathogen:
(Please contact the Animal Molecular Diagnostics Laboratory for information on special handling and packaging of biohazardous material.)

Comment: **Samples from Eleazar Vega-Valle**

Procedure	Sample #	# Vials	Sample Description	Result	Comment
Mycoplasma Lum 5+ samps	05-0071-1	1	Pc1	NEG	
Mycoplasma Lum 5+ samps	05-0071-2	1	2D11	NEG	
Mycoplasma Lum 5+ samps	05-0071-3	1	1.1 ErbB2	NEG	
Mycoplasma Lum 5+ samps	05-0071-4	1	Media	NEG	

Animal Molecular Diagnostics Laboratory, NCI-Frederick, P.O. Box B, Frederick, MD 21702.
Contact: Rhonda Anderson; Telephone: 301-846-1053, Fax: 301-846-6225, Email: randerson@ncifcrf.gov

For information about the MycoAlert (TM) assay, visit the manufacturer's web site: http://www.cambrex.com/default.asp.

RADIL Case 14788-2009

RADIL

FINAL REPORT OF LABORATORY EXAMINATION
Research Animal Diagnostic Laboratory
4011 Discovery Drive, Columbia MO 65201
1-800-669-0825 1-573-882-5983
radil@missouri.edu www.radil.missouri.edu

CASE NUMBER: 14788-2009

RECEIVED ON: 6/30/2009
COMPLETED ON: 7/7/2009

SUBMITTED BY:

Dave Dales
London Regional Cancer-Programme
790 Commissioners Rd E
London ON N6A 4L6
Canada
(519) 685-8600 x53271
[519] 685=8646 (fax)

SPECIMEN DESCRIPTION:

SPECIES: Human
DESCRIPTION: human cells (tumor) recovered from mouse
NUMBER OF SPECIMENS: 1

PURCHASE ORDER #: VH-412077

ID
MDA MB 468-LN-M2

SERVICES/TESTS PERFORMED IMPACT III PCR Profile
PCR evaluation for: Ectromelia, EDIM, LCMV, LDEV, MHV, MNV, MPV, MVM, *Mycoplasma* sp., Polyoma, PVM, REO3, Sendal, TMEV GDVII

GENERAL COMMENTS: cultured $2x10^6$ cells/vial frozen in media + 10% FBS + 10% DMSO

SUMMARY: All test results were negative.

If you have questions, please call our toll free number at 1-800-669-0825 or e-mail us at radil@missouri.edu.

Case Number: 14788-2009
Page 2

PCR EVALUATION:

Specimen: cells	MDA MB 468-LN-M2
Ectromelia	-
EDIM	-
LCMV	-
LDEV	-
MHV	-
MNV	-
MPV	-
MVM	-
Mycoplasma sp.	-
Polyoma	-
PVM	-
REO3	-
Sendai	-
TMEV GDVII	-

(LEGEND: + = positive - = negative id:id = pooled sample blank = test not performed equ = equivocal NT or blank = no test performed sus = suspect wps = weak positive)

COPY

RADIL

FINAL REPORT OF LABORATORY EXAMINATION
MU Research Animal Diagnostic Laboratory
1600 East Rollins, Columbia MO 65211 1-800-669-0825 1-573-882-5983
radil@missouri.edu http://www.radil.missouri.edu

CASE NUMBER: 6374-2008

RECEIVED ON: 1/31/2008
COMPLETED ON: 2/8/2008

CONFIDENTIAL

SUBMITTED BY:

Amanda Ibarra
Xenogen Corporation
2061 Challenger Drive
Alameda, CA 94501
510-291-6173

SPECIMEN DESCRIPTION:

SPECIES: Human
DESCRIPTION: MB231-D3H2LN (P8)
NUMBER OF SPECIMENS: 1

PURCHASE ORDER #: 29266

ID
MB231-D3H2LN (P8)

PROFILE/EXAM REQUESTED: IMPACT I PCR Profile

PCR evaluation for: Ectromelia, EDIM, Hantaan, K virus, LCMV, LDEV, MAD, mCMV, MHV, MNV, MPV, MTV, MVM, *Mycoplasma* sp., Polyoma, PVM, REO3, Sendai, TMEV GDVII

GENERAL COMMENTS: Human Mammary

SUMMARY: All test results were negative.

If you have questions, please call our toll free number at 1-800-669-0825 or e-mail us at radil@missouri.edu.

Case Number: 6374-2008
Page 2

PCR EVALUATION:

Specimen: cells	MB231-D3H2LN (P8)
Ectromelia	-
EDIM	-
Hantaan	-
K virus	-
LCMV	-
LDEV	-
MAD	-
mCMV	-
MHV	-
MNV	-
MPV	-
MTV	-
MVM	-
Mycoplasma sp.	-
Polyoma	-
PVM	-
REO3	-
Sendai	-
TMEV GDVII	-

LEGEND: + = positive - = negative id:id = pooled sample blank = test not performed equ = equivocal NT or blank = no test performed sus = suspect wps = weak positive)

CELL LINES

Bioware Cell Line MDA-MB-231-luc-D3H2LN

Designation:	MDA-MB-231-luc-D3H2LN
Tissue	Human: adenocarcinoma; mammary gland; pleura effusion
Parental Line Source:	American Type Culture Collection (#HTB-26)
Co-Trasnfection Plasmids:	1) pGL3 control red (SV40-luc) (Promega/C. Contag, Stanford University) 2) pSV40/Zeo (Invitrogen)
Transfection Method:	Lipofectamine/Plus Reagent (Invitrogen)
Bioluminescence *In Vitro*:	Approximately 189-208 photons/second/cell, subject to imaging and culturing conditions
Passage:	MDA-MB-231-D3H2LN cells are derived from an orthotopic MB-231-luc-D3H1 tumor.

The Features

Caliper Life Sciences Bioware Cell Line Models Offer the Ability to:

- Monitor early tumor development
- Monitor tumor growth and metastases *in vivo*
- Quantify tumor burden in the whole animal
- Follow responses to therapeutic treatments non-invasively in longitudinal studies using the same cohorts of mice.

Murine Pathogen Free

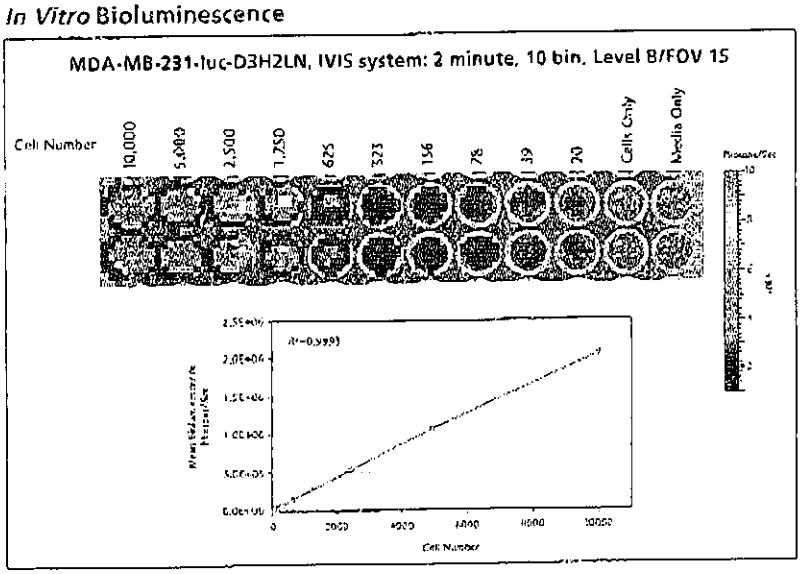
All Caliper Life Sciences cell lines are confirmed to be pathogen free by the IMPACT Profile I (PCR) at the University of Missouri Research Animal Diagnostic and Investigative Laboratory.

Model Description

MDA-MB-231-luc-D3H2LN is a luciferase expressing cell line that was derived from MDA-MB-231 human adenocarcinoma cells by stable transfection for the North American Firefly Luciferase gene expressed from the SV40 promoter. MDA-MB-231-luc-D3H2LN cells are derived from a primary orthotopic tumor of MDA-MB-231-luc-D3H2LN cells. This cell line can be used *in vivo* to establish:

- Experimental Metastasis model (intravenous) and Intracardiac
- Orthotopic mammary fat pad model with metastasis

In Vitro Bioluminescence

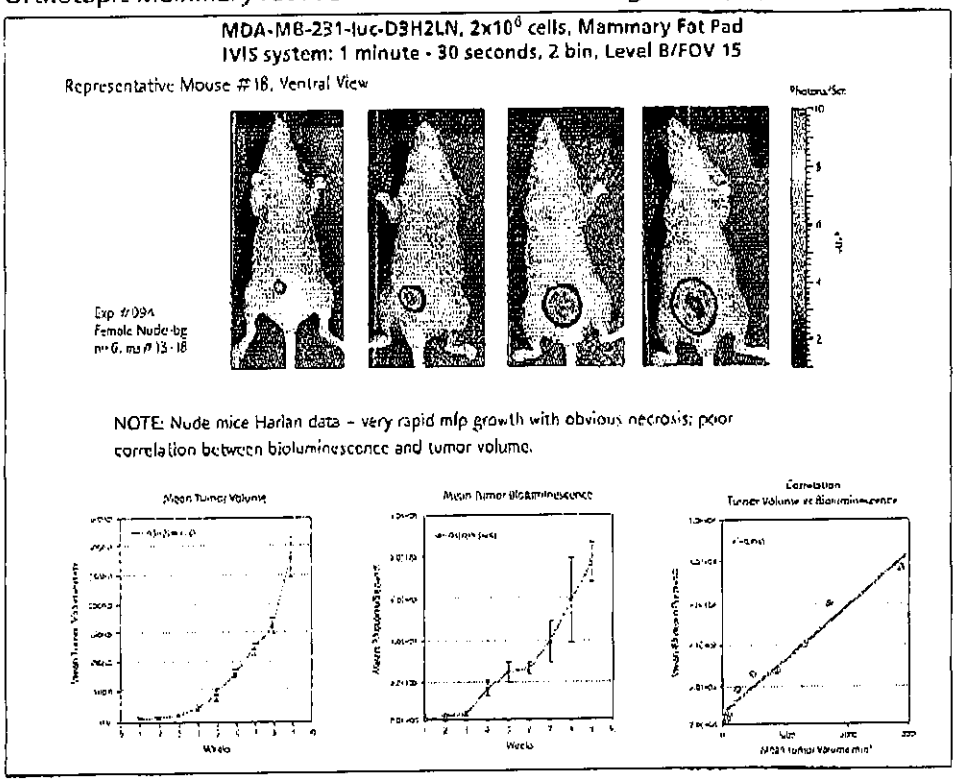


Protocols:
MDA-MB-231-luc-D3H2LN cells in 100 µL media were seeded into a 96 well plate by 1:2 serial dilution from 10,000 cells (well#1) to 20 cells (well#10). The plate was imaged using the IVIS system (2 min, 10 bin, level B/FOV 15) approximately 2-3 minutes after addition of 100 µL 2X luciferin. Wells #11 and #12 served as negative controls.

Conclusions:
Approximately 20 cells were detectable in vitro in this experiment. A strong correlation between cell number and bioluminescence (R2 = 0.999) was also demonstrated.

CELL LINE MDA-MB-231-LUC-D3H2LN

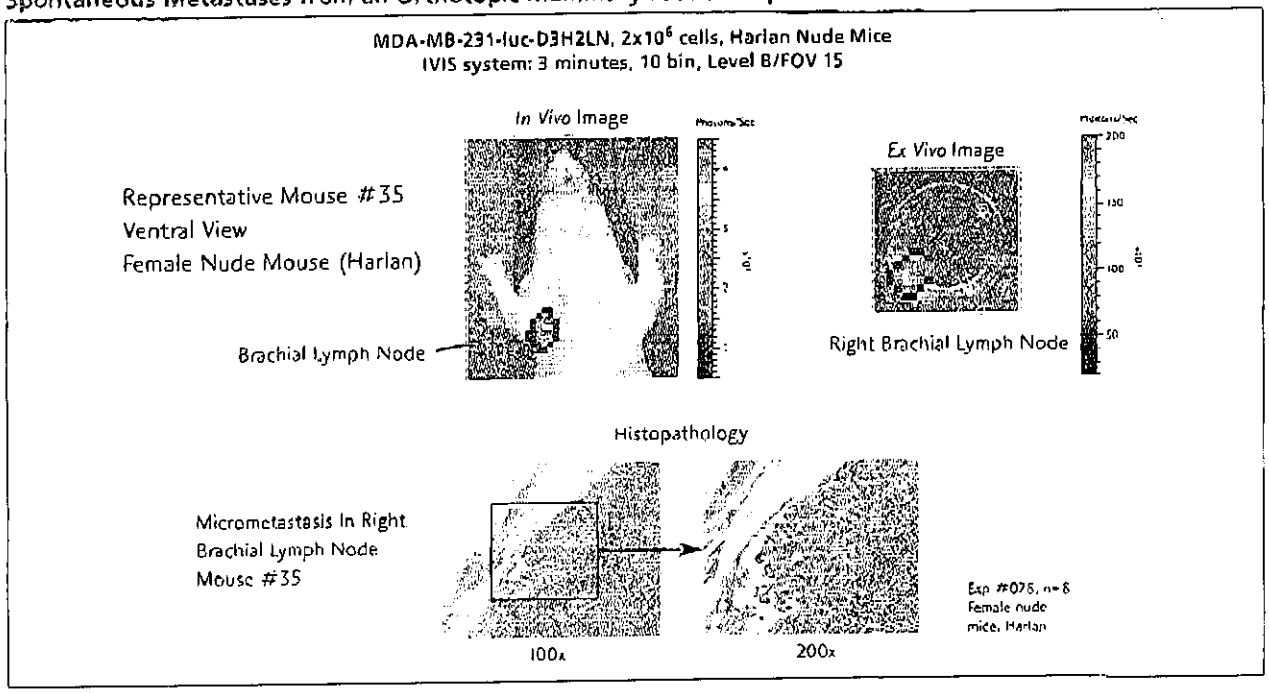
Orthotopic Mammary Fat Pad Tumor Growth-Nude beige mice (CR)



Protocols:
MDA-MB-231-luc-D3H2LN cells ($2x10^6$) are injected into the mammary fat pad of female nude beige mice (Charles River). Mice are imaged weekly for 9 weeks to monitor tumor growth.

Conclusions:
In vivo imaging demonstrates the progression of MDA-MB-231-luc tumors in the mammary fat pad of female nude beige mice (Charles River). Correlation of mean tumor volume to mean bioluminscence is R2=0.958

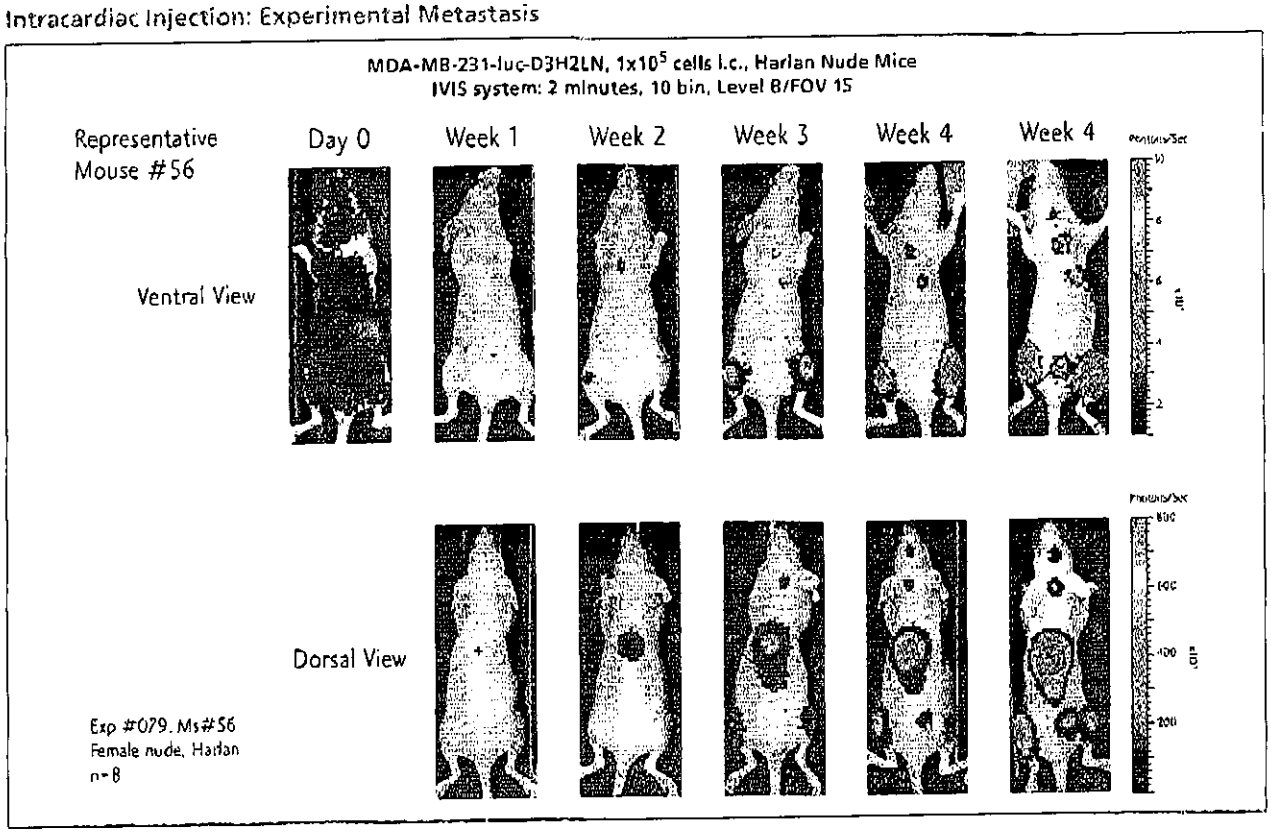
Spontaneous Metastases from an Orthotopic Mammary Fat Pad Implant



Protocols:
MDA-MB-231-luc-D3H2LN cells ($2x10^6$) are injected orthotopically into the abdominal mammary fat pad of female nude mice (Harlan) (n=8). Mice are imaged weekly for five weeks from the ventral view. Primary tumors are shielded in order to detect low signals from secondary metastases. Selected tissues are analyzed by *ex vivo* imaging and processed for subsequent histology.

Conclusions:
Metastatic signals begin to appear after 3-4 weeks. By week 4, lymph node metastases were detected *in vivo* in 100% (8/8) of mice. Subsequent histopathology confirmed metastases in 5/8 lymph nodes.

Intracardiac Injection: Experimental Metastasis



Protocols:
MDA-MB-231-luc-D3H2LN cells (1x10^5) were injected into the left ventricle of female nude mice (n=8). Mice were imaged weekly from dorsal and ventral views for 5 weeks. Selected tissues were imaged *ex vivo* to confirm *in vivo* signals.

Conclusions:
Metastatic signals begin to appear after 2 weeks. By week 3, metastases were detected *in vivo* in 100% of mice (8/8) to multiple sites.

References

1. Contag CH, Jenkins DE, Contag PR, Negrin R. Use of Reporter Genes for Optical Measurements of Neoplastic Disease *In Vivo*. *Neoplasia* 2000 Jan-Apr;2(1-2):41-52.
2. Edinger M, Cao YA, Hornig YS, Jenkins DE, Verneris MR, Bachmann MH, Negrin RS, Contag CH. Advancing animal models of neoplasia through *in vivo* bioluminescence imaging. *Eur J Cancer* 2002 Nov;38(16):2128-36.
3. Jenkins DE, Oei Y, Hornig Y, Yu SF, Dusich J, Purchio T, Contag PR. Bioluminescent Imaging (BLI) to Improve and Refine Traditional Murine Models of Tumor Growth and Metastasis. *Clin Exp Metastasis* 2003;20(8):733-44.
4. Jenkins DE, Yu SF, Hornig YS, Purchio T, Contag PR. In Vivo Monitoring of Tumor Relapse and Metastasis Using Bioluminescent PC-3M-luc-C6 cells in Murine Models of Human Prostate Cancer. *Clin Exp Metastasis* 2003;20(8):745-56.
5. Murray LJ, Abrams TJ, Long KR, Ngai TJ, Olson LM, Hong W, Keast PK, Brassard JA, O'Farrell AM, Cherrington JM, Pryer NK. SU11248 inhibits tumor growth and CSF-1R-dependent osteolysis in an experimental breast cancer bone metastasis model. *Clin Exp Metastasis* 2003;20(8):757-66.
6. Mendel DB et al. In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. *Clin Cancer Res*. 2003 Jan; 9(1):327-37.
7. Scatena CD, Hepner MA, Oei YA, Dusich JM, Yu SF, Purchio T, Contag PR, Jenkins DE. Imaging of Bioluminescent LNCaP-luc-M6 Tumors: A New Animal Model for the Study of Metastatic Human Prostate Cancer. *Prostate* 2004 May 15;59(3): 292-303 .
8. Jenkins DE, Hornig Y, Oei Y, Dusich J, Purchio T. Bioluminescent human breast cancer cell lines that permit rapid and sensitive *in vivo* detection of mammary tumors and multiple metastases in immune deficient mice. *Breast Cancer Research* 2005, 7:R444-R454 (8

April 2005)

Credits

Bioware cell line information compiled by Yvette Hornig, Joan Dusich and Darlene Jenkins. Edited by Joycelyn Bishop.

CELL LINE MDA-MB-231-LUC-D3H2LN

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Buyer acknowledges that the wild-type and mutant recombinant Coleoptera luciferase nucleic acid and protein ("UC Patented Material") is the subject of U.S. Patent Nos. 5,583,024, 5,674,713 and 5,700,673, assigned to The Regents of The University of California.

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(2) transfer the Materials to any third party or to any place other than Buyer's premises;

(3) attempt to alter, modify or re-engineer the Materials in any way, or extract or transfer any genetic material from the product to another organism; or

(4) sublicense the rights granted herein.

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  (i) does not use the animal model for any purpose other than breeding for the benefit of Buyer,

  (ii) destroys or returns the animals upon conclusion of the breeding services, and

  (iii) is otherwise legally bound by the terms of this label license.
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- In the event of termination hereof, Buyer shall destroy or return all Materials to Caliper Life Sciences.

Contact Information:

If you have any questions regarding these cell lines, please contact Caliper at 508-497-6592 or e-mail: reagents@caliperls.com

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BIO-SS-33 Oct 08

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 Ann F. Chambers / Dr. Alan Tuck (Co-PI)
SIGNATURE
DEPARTMENT Cancer Research Labs
ADDRESS 790 Commissioners Rd. E.
PHONE NUMBER (519) 685-8652
EMAIL ann.chambers@lhsc.on.ca

Location of experimental work to be carried out: Building(s) LRCP Room(s) A4-822, 903, 925
*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) Clincal and Experimental Studies of Osteopontin and Breast Cancer: A Translational Program to Examine its Functional Contribution to Malignancy and its potential as a Marker of Progression and as a Therapeutic Target
2) Steps of Breast Cancer Metastasis: Experimental Models and Identification of Targets for Intervention

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 1) Ontario Cancer Research Network (OCRN)
2) Canadian Breast Cancer Research Alliance (CBCRA # 016506)

Names of all personnel working under Principal Investigators supervision in this location:
i) Waleed Al-Katib, PhD
ii) Pieter Anborgh, PhD
iii) Laura Caria
iv) David Dales
v) Brigitte Goulet, PhD
vi) Wendy Kennette
vii) Carl Postenka
viii) Terlika Sharma, PhD
ix) Lesley Souter

* 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	Yes No	Yes No	Yes No	1 L
	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)? Commercial Source (e.g. 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	lymph node & splenic tissue from organ donors
Rodent	Yes No	lymph node & tissue of mice, Balb-c
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)	Supplier / Source
Human	Yes No	see attached sheet	see attached sheet
Rodent	Yes No	see attached sheet	see attached sheet
Non-human primate	Yes No		
Other (specify)	Yes No		

2.4 For above named cell types(s) circle HC or CFIA containment level required 1 2 3
Cell lines are containment level 1 other than those marked "*" on the attached sheet which are containment level 2.

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

Established Cells that will be grown in culture.

Cell Line		Source
Human		
21 T series (21PT, 21NT, 21MT-1) mammary epithelial		Dana Farber Res. Inst.
Breast cancer lines*	MDA MB 231	ATCC
	MDA MB 231 BRMS1	Chambers' laboratory
	MDA MB 435	ATCC
	MDA MB 435 BRMS1	Chambers' laboratory
	MDA MB 435 HAL	Chambers' laboratory
	MDA MB 468	ATCC
	MDA MB 468 GFP	Chambers' laboratory
	MDA MB 468 LN	Chambers' laboratory
* 293vsv		Dr. A. Nepveu, McGill University
Rodent		
4T1		ATCC
66cl4		Dr. F. Miller, Michigan University
67NR		Dr. F. Miller, Michigan University
168 FARN		Dr. F. Miller, Michigan University
B16-F1		ATCC
B16-F10		ATCC
D2A1		Chambers' laboratory
D2OR		Chambers' laboratory
PAP2		Chambers' laboratory
Hybridoma mAb53		Chambers' laboratory
Hybridoma mAb87-B		Chambers' laboratory

Additional Information for Established Cell Lines:

1) All cell lines are mycoplasma free and are routinely tested for mycoplasma.

2) Rodent derived cell lines have been tested for pathogens at University of Missouri's Research Animal Diagnostic Laboratory (RADIL) by means of IMPACT PCR Profile. No pathogens were detected.

3) Cell containment is level 1 other than *293vsv which is level 2.

4) A recombinant defective retrovirus (MMLV) will be used to transduce a gene of interest into cell lines. During the initial stage of transduction the cells have to be treated as Level 2 containment. The transduced gene of interest will be integrated into the host genome and propagated as an endogenous gene. After several passages with a selection agent to establish a stable transduced cell line, no viable recombinant retrovirus will be present in the cell line and therefore the cell line can be handled as Level 1 containment.

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 plasma
- 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 lymph node & splean

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 6.0

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 _____ EIA (293 VSV cell)
- Known oncogenes (YES) NO — If YES specify oncogenes based on results of gene profile

4.4 Will a live vector(s) (viral or bacterial) be used for gene transduction (YES) NO
If YES name virus recombinant moloney murine leukemia

4.5 List specific vector(s) to be used: pREV-tet-on, pREVTRE, pcDNA 3

4.6 Will virus be replication defective (YES) NO

4.7 Will virus be infectious to humans or animals (YES) NO

4.8 Will this be expected to increase the Containment Level required YES (NO)

* 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? N/A

5.4 Please give the Health Care Facility where the clinical trial will be conducted: N/A

5.5 Has human ethics approval been obtained? ☐ YES ☐ NO (N/A)

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 mouse

6.3 AUS protocol # 2007-042-06, 2005-057-09, 2005-059-03, 2005-062-09

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

8.2 If YES, please name the toxin _____

8.3 What is the LD_{50} (specify species) of the toxin _____

• 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 _____

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 # _____

10.0 Training Requirements for Personnel named on Form

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 _____ / _____ (Co-PI)

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: ~~[illegible]~~ APRIL 3, 2008 AR

12.0 Approvals

UWO Biohazard Subcommittee

Signature _____ Date 1.25.08.

Safety Officer for Institution where experiments will take place

Signature _____ Date April 3, 2008

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

Signature _____ Date Mar 31/08

* Work is Level 2 unless researcher can show that virus particles are not shed (by PCR shows) If PCR shows that virus is not shed, work is Level 1.

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