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 immunization, 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 & splaen

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
- Known oncogenes (YES) NO If YES specify oncogenes based on results of gene profile

EIA (293 VSV cells)

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 (circled: 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 [signature] / [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 (circled: 2)

11.2 Has the facility been certified by OHS for this level of containment? ☐ YES ☐ NO (circled: YES)

11.3 If yes, please give the date and permit number: ~~[struck-out entry]~~ AR APRIL 3, 2008 AR

12.0 Approvals

UWO Biohazard Subcommittee

Signature [signature] Date 1.25.08.

Safety Officer for Institution where experiments will take place

Signature [signature] Date April 3, 2008

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*

Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.

Customers in Europe, Australia, Canada, China, Hong Kong, India, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

Cell Biology

ATCC® Number: HTB-26™ Order this Item

Designations: MDA-MB-231

Biosafety Level: 1

Medium & Serum: See Propagation

Organism: Homo sapiens (human)

Price: $244.00

Depositors: R Cailleau

Shipped: frozen

Growth Properties: adherent

Morphology: epithelial

Photo

Source:
Organ: mammary gland; breast
Disease: adenocarcinoma
Derived from metastatic site: pleural effusion
Cell Type: epithelial

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

Applications: transfection host(technology from amaxa
Roche FuGENE® Transfection Reagents)

Receptors: epidermal growth factor (EGF), expressed
transforming growth factor alpha (TGF alpha), expressed

Tumorigenic: Y

DNA Profile (STR):
Amelogenin: X
CSF1PO: 12,13
D13S317: 13
D16S539: 12
D5S818: 12
D7S820: 8,9
THO1: 7,9.3
TPOX: 8,9
vWA: 15,18

Cytogenetic Analysis: The cell line is aneuploid female (modal number = 64, range = 52 to 68), with chromosome counts in the near-triploid range. Normal chromosomes N8 and N15 were absent. Eleven stable rearranged marker chromosomes are noted as well as unassignable chromosomes in addition to the majority of autosomes that are trisomic. Many of the marker chromosomes are identical to those shown in the karyotype reported by K.L. Satya-Prakash, et al.

Isoenzymes:
AK-1, 1
ES-D, 1
G6PD, B
GLO-I, 2
Me-2, 1-2
PGM1, 1-2
PGM3, 1

Age: 51 years adult

Gender: female

Re: Allan Lab - MDA-MB-435

Subject: Re: Allan Lab - MDA-MB-435
From: Jean-Nicolas Gagnon <jean-nicolas_gagnon@phac-aspc.gc.ca>
Date: Wed, 12 Mar 2008 13:45:40 -0400
To: Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer:

According to the info provided we would consider this cell line a RG1.

I hope this helps

Cheers!

Jean-Nic

Jennifer Stanley
<jstanle2@uwo.ca>
2008-03-12 01:43 PM

To: Jean-Nicolas Gagnon <jean-nicolas_gagnon@phac-aspc.gc.ca>
cc:
Subject: Re: Allan Lab - MDA-MB-435

Jean-Nic

The researcher uses the MDA-MB-435 parental line - this cell line is no longer sold by ATCC, however a similar one is MDA-MB-435S line is available

Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.

Customers in Europe, Australia, Canada, China, Hong Kong, India, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

Cell Biology

ATCC® Number: HTB-132™ Order this Item

Price: $244.00

Designations: MDA-MB-468

Depositors: R Cailleau

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Morphology: epithelial

Organism: Homo sapiens (human)

Source: **Organ:** mammary gland; breast
Disease: adenocarcinoma

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

Isolation: **Isolation date:** 1977

Applications: transfection host(technology from amaxa Roche FuGENE® Transfection Reagents)

Receptors: epidermal growth factor (EGF)
transforming growth factor alpha (TGF alpha)

Tumorigenic: Y

Antigen Expression: Blood Type AB; HLA Aw23, Aw30, B27, Bw35, Cw2, Cw4 (patient)

DNA Profile (STR): Amelogenin: X
CSF1PO: 12
D13S317: 12
D16S539: 9
D5S818: 12
D7S820: 8
THO1: 7
TPOX: 8,9
vWA: 18

Cytogenetic Analysis: modal number = 64; range = 60 to 67.
The cell line is aneuploid human, presumably female (X, abnormal X) with most chromosome counts in the hypotriploid range.; Normal chromosomes X, N2, N3, N7, N8, N10, and N22 are clearly under-represented due to their involvement in the formation of the many marker (19) chromosomes present in this cell line.; A normal chromosome N1 (or two) is identified in each karyotype, but, in addition, regions of chromosome N1 are also present in five different marker chromosomes.; Variation is evident in the normal and marker chromosome copy number from karyotype to karyotype.

Isoenzymes: AK-1, 1
ES-D, 1
G6PD, A
GLO-I, 1-2
Me-2, 1-2
PGM1, 1
PGM3, 2

Age: 51 years

-------- Original Message --------
Subject: Biohazardous Agents Registry Form: Chambers
Date: Fri, 04 Apr 2008 17:10:39 -0400
From: Jennifer Stanley <jstanle2@uwo.ca>
To: Ann F Chambers <ann.chambers@lhsc.on.ca>
CC: David Dales <dwdales@uwo.ca>
References: <fbf1f32a1c54.47e8a6f0@uwo.ca>

Hello Dr. Chambers and David:

I received the Biohazardous Agents Registry Form yesterday. I have a couple of questions/comments:

1. Please provide information, such as a technical data sheet, on the 21 T series cell lines (21PT, 21NT, 21MT-1) from the Dana Farber Research Institute.
2. Please describe what modifications have been done to the MDA-MB-231 cell line to create the "MDA-MB-231 BRMS1" cell line
3. Please describe what modifications have been done to the MDA-MB-435 cell line to create the "MDA-MB-435 BRMS1" and the "MDA-MB-435 HAL" cell lines?
4. Please describe what modifications have been done to the MDA-MB-468 cell line to create the "MDA-MB-468 GFP" and the "MDA-MB-468 LN" cell lines?

Thanks!
Jennifer

Hi Jennifer:

In response to some questions you had for Dr. A. Chambers' Biohazardous Agents Registry Form.

The 21T series cells are three established mammary epithelial cell lines (21T series) derived (by clonal isolation and serum selection) from the same patient with metastatic breast cancer (Band, V. et al. Tumor progression in four mammary epithelieal cell lines derived from the same patient. Cancer Res, 50: 7351-7357, 1990). 21PT cells are immortal but nontumorigenic in the nude mouse; 21NT are weakly tumorigenic, but non-metastatic; and 21MT-1 are tumorigenic, weakly metastatic. As announced by V. Band (e-mail sent to you Dec 7, 2005 from Lisa Mackenzie) these cells are non-pathogenic to humans and contain no virus. The paper refers to four cell lines of which we have 3 but do not have the 4th (21MT-2).

For the MDA MB-435 HAL (435 HAL) cell line, MDA MB-435 breast carcinoma cell line was chemically transfected with Green Flourescent Protein (GFP) cDNA. After multiple in vivo passages the 435 HAL was selected for its increased primary tumor growth and pulmonary metastasis relative to MDA MB-435 (Schmidt CM et al. Characterization of spontaneous metastasis in an aggressive breast carcinoma model using flow cytometry. Clin Exp Metastasis 1999; 15:537-544). These cells have been tested and are clean of any mouse pathogens.

The 468 GFP cell line is a stable transfectant in which MDA MB-468 (ATCC) was chemically transfected with the Green Flourescent Protein (GFP) cDNA. These were injected (mammary fat pad) into a nude mouse and metastases (tumor) cells harvested from lung. Plating the harvested cells in G418 selective media allowed the establishment of the MDA MB-468 LN (468 LN) cell line. The 468 LN cells are highly metastatic in the lymphatics in relation to parental MDA MB-468 (Vantyghem S et al. A New Model for Lymphatic Metastasis: Development of a Variant of the MDA-MB-468 Human Breast Cancer Cell Line that Aggressively Metastasizes to Lymph Nodes. Clin Exp Metastasis 2005; 22:351-361). The 468 LN cells have been tested (Radil IMPACT profile) for mouse pathogens and are clean.

For both of the BRMS1 cell lines, they are stable transfectants of the parental ATCC cell lines MDA MB-231 and MDA MB-435. The plasmid pcDNA 3 containing the cDNA for Breast Cancer Metastasis Suppressor 1 gene (BRMS1) was transfected by electoporaion into the parental cells and stable transfectants select with the use of the agent G418 (Seraj MJ et al. Functional Evidence for a Novel Human Breast Carcinoma Metastasis Suppressor, BRMS1, encoded at chromosome 11q13. Cancer Res. 2000; 60:2764-2769). The BRMS1 gene makes the cells less malignant relative to the parental cell lines.

Dr. Chambers believes these questions had been answered in previous (2005 and earlier) Biohazard Registry Forms.

David Dales
Senior Tech for Dr. A. Chambers

[redacted]

SCIENTIFIC SUMMARY

Clinical 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

RATIONALE. Metastasis is responsible for most breast cancer deaths. Unmet clinical needs that could improve care and survival for women with breast cancer, include the needs for reliable prognostic factors, ways to monitor for recurrence and treatment response, and less toxic treatments. We will assess the role of the protein osteopontin (OPN) in breast cancer. We have studied OPN in the context of breast cancer for many years. Our data suggest that it plays an important role in breast cancer progression and has potential to address these three clinical needs. HYPOTHESES. 1. OPN can contribute functionally to the malignancy of breast cancer, and knowledge of OPN's mechanism of action will reveal potential clinical targets for breast cancer treatment. 2. Measurement of OPN levels in patients' tumors or serial blood samples will provide information about prognosis and progression, which will be useful in clinical management of breast cancer. SPECIFIC AIMS. 1. To identify molecular mechanisms by which OPN induces malignant behavior in breast cancer cells and identify therapeutic targets based on blocking the effects of OPN on breast cancer cells. 2. To determine the utility of OPN measurement, in blood plasma or tumors, in providing information useful in prognosis and management of patients with early stage or metastatic breast cancer. STUDY DESIGN. We will use a combined experimental and clinical approach. Experimentally, we will focus on mechanisms of OPN's effects on breast cancer cells, and on identifying therapeutic opportunities created by this information. Clinically, we will assess OPN levels in tumors and in serial blood samples, from women with breast cancer, using samples from a series of local and national clinical trials. We also will adapt our current plasma-based ELISA for use in serum samples, which will facilitate future participation in collaborative trials. RELEVANCE. Our multidisciplinary research team (scientists, medical oncologists, pathologist, epidemiologist), coupled with our use of samples and data from already funded, ongoing clinical trials, provide a translational and cost-effective approach to understanding the role of OPN in breast cancer progression. These studies will help us to learn if OPN may have use as a prognostic marker, a means to monitor for breast cancer recurrence, and a possible therapeutic target, for women with breast cancer.

PROBLEM: ...cancer remains a leading cause of death in women. Most of these deaths will be due to metastasis. Our program has 4 projects that address specific areas where models for the study of breast cancer are especially needed. These models will be used to characterize molecular and biological mechanisms of breast cancer progression and metastasis, and to identify and assess novel treatment strategies in clinically relevant models. Our focus is on two areas of metastasis research that are understudied but clinically very important: ***Lymphatic metastasis and detection and treatment of occult metastasis.***

PROJECT #1: I. MacDonald, PI; A. Groom Co-PI. *Intravital Videomicroscopy of Lymphatic Metastasis.* This Project will use in vivo videomicroscopy procedures pioneered by the applicants for studying metastasis via the blood circulation, and will extend these procedures to in vivo videomicroscopy analyses of the steps of breast cancer metastasis via lymphatics.

PROJECT #2: A. Chambers, PI; S. Vantyghem, D. Rodenhiser, Co-PIs. *Cell and Animal Models for Lymphatic Metastasis and Post-surgical Therapy of Breast Cancer.* This Project will characterize a newly developed breast cancer cell line that aggressively metastasizes via lymphatics, to identify molecular changes important in lymphatic metastasis, and will develop and characterize post-surgical treatment models for testing new therapeutic strategies pre-clinically in a clinically relevant, metastatic setting.

PROJECT #3: A. Allan, PI; M. Keeney, Co-PI. *Detection and Analysis of Rare Metastatic Events in Mouse Models of Breast Cancer.* This Project will develop and use techniques to detect rare disseminated tumor cells in models of breast cancer metastasis, using 3 complementary technologies: flow cytometry, laser scanning cytometry, and real time PCR, and use these to monitor kinetics of early steps in breast cancer metastasis, to quantify circulating tumor cells and rare metastatic events in tissues (including lymph node), and to characterize treatment responses.

PROJECT #4: A. Tuck, PI; D. Rodenhiser, Co-PI. *Modeling of Critical Steps in Mammary Tumor Progression in 3D Culture and In Vivo.* This Project will develop and use 3D models to study two important stages in breast cancer progression and metastasis – the early stages from an intraductal to an invasive phenotype, and regulation of growth of cancer cells in a lymph node environment. These 3D models will provide rapid assays to complement slower in vivo models.

GOALS AND SIGNIFICANCE: Our program goals are to develop novel models to address understudied aspects of breast cancer metastasis, and to use these models to characterize the biology and molecular mechanisms of breast cancer metastasis. These models will enable more rapid progress to be made in preventing and treating metastatic breast cancer.

New Approaches To Metastatic Disease In Breast Cancer Research

New Approaches to Metastatic Disease

Individual Component

9. DETAILED SCIENTIFIC ABSTRACT

Project # 1

MacDonald, I.C. (P.I.) and Alan C. Groom
Department of Medical Biophysics, University of Western Ontario

Intravital Videomicroscopy of Lymphatic Metastasis: Animal models for direct observation of cancer cell – host tissue interactions during the metastatic process.

Keywords/Technical Terms: Intravital Videomicroscopy (IVVM), lymphatic metastasis models, lymphatic endothelium, lymphatic flow, metastatic potential, cell interactions, lymphangiogenesis, fluorescent labels, quantitative morphology, tumor cell survival.

THE PROBLEM: Death from cancer generally results from the growth of metastases rather than the primary tumor. In breast cancer, lymphatic metastasis is a strong predictor of tumor growth at other, vital sites. If the lymphatic system is a major pathway for metastasis, therapies are needed to block this route. Given the multi-step nature of the metastatic process, there should be several opportunities for halting the lymphatic spread of breast cancer. We need new models to: 1) advance our knowledge of the dynamics of tumor cell transport, survival and growth within the lymphatic system, 2) identify specific steps in the metastatic process that can serve as targets for therapeutic intervention, and 3) assess tumor cell progression and the effects of therapy. We now have well established IVVM models for studying hematogenous metastasis. However, the thin walls, sluggish flow and absence of red blood cells in lymphatic vessels have made their identification a much greater challenge. Our preliminary studies show that by using IVVM, excellent views of lymphatic vessels and lymph nodes are obtainable, particularly in combination with the use of fluorescent markers. What still remains is the development of lymphatic metastasis models using IVVM to provide quantitative information on lymphatic morphology and flow, as well as the movement of breast cancer cells, their survival at sequential stages of the metastatic process, their growth within the lymph nodes and subsequent spread to other organs.

SPECIFIC AIMS: (1) To develop IVVM models for monitoring lymphatic metastasis. (2) To characterize tumor cell progression through sequential stages of lymphatic metastasis, either from primary tumors or via direct injection of cells into lymph vessels. (3) To determine the timing, location and effects of therapeutic intervention on lymphatic metastasis of breast cancer cells.

STUDY DESIGN: Our overall objective is to create IVVM models that can be used to develop and assess novel therapeutic strategies.(1) Based on our established techniques for IVVM of the blood circulation and preliminary successes using IVVM of the lymphatic system in mice, we will developed IVVM models for quantifying the morphology and fluid dynamics of lymphatic tissues and the movement of breast cancer cells within them. Initial development will include surgical techniques, lighting, new fluorescent markers, camera characteristics, digital image processing and enhancement, and correlation with histological assessment. (2) We will develop models using breast cancer cells of high and low metastatic efficiency to identify stages in metastasis that are critical to tumor development. Direct injection of cells, microspheres and "Quantum Dot" nanoparticles will be used to quantify lymphatic transport and the fate of tumor cells, and to identify potential therapeutic targets. (3) Using our newly developed IVVM models, we will assess therapeutic strategies, chosen for their relevance to this project and the other projects in this program, to determine the timing and location of their effectiveness.

RELEVANCE: Effective cures for breast cancer metastasis will depend on the appropriate choice of therapy and optimizing the timing and delivery of treatment. By developing IVVM models that will allow us to observe directly the progression of breast cancer metastasis within the complex environment of the host lymphatic system, we will be able to identify the most promising strategies for therapeutic intervention and assess their effectiveness.

New Approaches to Metastatic Disease

Individual Component

9. DETAILED SCIENTIFIC ABSTRACT

Responses must be limited to one page. Refer to the *2004 New Approaches to Metastatic Disease Grant Application Guide* for specific instructions regarding the format to be used for this section.

Project #2

Chambers, Ann F. (PI); Vantyghem, Sharon A.; and Rodenhiser, David I.
London Regional Cancer Program, Lawson Health Research Institute

Cell and Animal Models for Lymphatic Metastasis and Post-surgical Therapy of Breast Cancer

Keywords/Technical Terms: lymphatic metastasis, animal models, novel cell lines, methylation and gene expression analyses, post-surgical adjuvant treatment models for breast cancer

THE PROBLEM: This project will address two major problems in breast cancer metastasis, namely the needs for better models *1)* with which to study lymphatic metastasis and *2)* for assessing therapies to prevent outgrowth of tumor cells seeded to distant organs prior to breast cancer surgery. Often the first sites of metastasis are lymph nodes near the primary tumor. Lymph node metastasis is an important prognostic factor, which guides treatment decisions, but may also provide a reservoir for subsequent distant metastasis. Despite its clinical importance, the biology of lymphatic metastasis is poorly understood, partly due to lack of good experimental models. This project will address this first need, by characterizing a new cell model of human breast cancer that aggressively spreads via lymphatics in mice. Second, post-surgical adjuvant therapy may be important for women whose tumors are suspected to have shed potentially metastatic cells prior to surgery, to prevent later metastatic outgrowth (often after long periods of dormancy). Reliable animal models are needed to model this situation. This project will address this second need, by developing and characterizing clinically relevant animal models for anti-metastatic therapy in an adjuvant, post-surgical setting.

SPECIFIC AIMS: *1)* To characterize ***new models for lymphatic metastasis of breast cancer,*** starting with a cell line we have selected for aggressive lymph node metastasis, and to identify genetic and functional changes important to lymph node metastasis. *2)* To develop and characterize ***post-surgical treatment models for metastatic breast cancer and dormant, micrometastatic disease,*** for use in assessing therapies to prevent metastatic outgrowth of micro-metastases or dormant solitary cancer cells.

STUDY DESIGN: *1)* We derived the novel cell line 468LN, from human MDA-MB-468 breast cancer cells, that aggressively spreads via lymphatics from mammary fat pad tumors in mice. We will characterize: *a)* biological behavior of 468LN cells in vivo (metastatic properties; dormancy patterns from intravenous, intracardiac and mammary fat pad injections) and in vitro, *b)* gene expression differences of 468LN vs. parental cells (candidate genes, expression array profiles, gene validation; methylation patterns), *c)* functional characterization of the role of these genes in lymphatic spread (transfection to up- or down-regulate [eg siRNA]; demethylating agents; gene-specific inhibitors). *2)* New therapeutic strategies are needed to prevent outgrowth of cancer cells shed prior to cancer surgery. It is thus important that models be developed for testing in this setting. We will develop and characterize in vivo models in which primary mammary fat pad tumors are surgically removed at various times, and metastatic burden (micro- and macroscopic metastases, solitary dormant cells) quantified. Knowledge of the kinetics of metastasis for cells of differing metastatic abilities, will provide models for assessing anti-metastatic effects of new therapies (e.g. cytotoxic chemotherapy, anti-angiogenic, vascular targeting agents, dietary interventions, molecularly targeted agents) in an adjuvant setting.

RELEVANCE: This project will develop and characterize models that will address two important but unmet clinical needs in breast cancer metastasis, namely the need for models of lymphatic metastasis and models with which to assess new therapies in a clinically relevant, post-surgical adjuvant setting. These models are needed for better understanding of the biology of breast cancer metastasis and for testing of new strategies for breast cancer treatment.

New Approaches to Metastatic Disease
Individual Component

9. DETAILED SCIENTIFIC ABSTRACT

Allan, Alison L. (PI)[1,2] and Keeney, Michael (Co-investigator)[2,3] Project #3
London Regional Cancer Program[1]; Lawson Health Research Institute[2]; London Health Sciences Centre[3]; London, ON, CANADA

Detection and Analysis of Rare Metastatic Events in Mouse Models of Breast Cancer Using Flow Cytometry, Laser Scanning Cytometry, and Real-Time PCR

Keywords/Technical Terms: breast cancer metastasis, mouse models, rare event detection, circulating tumor cells, lymph node, flow cytometry, laser scanning cytometry, real-time PCR

THE PROBLEM: Given the multi-step nature of the metastatic cascade, there should be several opportunities for early therapeutic targeting of metastatic cells before they become a clinical problem for the patient. However, imperfect techniques to detect, quantify, and analyze single cells or even small numbers of cells in the bloodstream or distant organs of post-surgical breast cancer patients has been a significant obstacle to accurate prognosis, proper adjuvant treatment decisions, assessment of treatment efficacy, and long-term monitoring of disease recurrence. Similar challenges in detecting and quantifying rare metastatic cells in experimental mouse models of breast cancer have hindered the ability to quantify early steps in metastasis and to determine the timing and location of metastatic spread of cells.

SPECIFIC AIMS: *1)* To develop, optimize, and validate three distinct but complementary approaches for detection, quantification, and analysis of rare metastatic events in mouse models of breast cancer using flow cytometry, laser scanning cytometry (LSC), and real-time PCR; *2)* To use these techniques to quantify early steps, timing, and location of metastatic spread in experimental and spontaneous breast cancer models; and *3)* To apply these techniques for characterizing the new post-surgical and lymph node metastasis models described in Projects 1, 2, and 4, and for characterizing tumor cell response to therapy.

STUDY DESIGN: *1)* Initial technique development will be done by "spiking" samples of mouse blood, bone marrow aspirates, and tissue homogenates with set numbers of human breast cancer cells prior to analysis in order to allow for optimization and preparation of standard curves for sensitivity. For flow cytometry and LSC, human breast cancer cells will be differentiated from mouse cells based on their larger size, positive staining with a human-specific antibody, negative staining with a mouse-specific antibody, and nuclear staining for aneuploid DNA content. With LSC, we will have the added ability to assess cytomorphology and molecular characteristics of individual cells in suspension as well as tumor cells within the microenvironmental architecture of various organs (i.e. the lymph node). For real-time PCR, human breast cancer cells will be differentiated from mouse cells using amplification of the human-specific *Alu* repeat sequence. *2)* Early steps in metastasis and timing and location of metastatic spread will be quantified using a combination of the three techniques to most appropriately analyze tumor cells in blood, bone marrow, lymph node, and/or distant organs at various time points following mammary fat pad, tail vein, or intracardiac injection of human breast cancer cells into immunocompromised mice. *3)* The post-surgical and lymph node metastasis models described in Projects 1, 2, and 4 will be characterized for *a)* timing and occurrence of tumor cells shed from the primary tumor and correlation with post-surgical metastatic burden; *b)* molecular/cellular characterization of *in vivo* protein expression and interactions between disseminated tumor cells and the lymph node microenvironment; and *c)* molecular response to therapy.

RELEVANCE: Optimization and integration of three distinct but complementary techniques for rare event analysis in pre-clinical mouse models of metastatic breast cancer will provide the opportunity to elucidate the mechanistic details of early steps in metastasis and determine how these steps relate to the development, monitoring, and treatment of metastatic disease.

New Approaches to Metastatic Disease

Individual Component

9. DETAILED SCIENTIFIC ABSTRACT

Responses must be limited to one page. Refer to the *2004 New Approaches to Metastatic Disease Grant Application Guide* for specific instructions regarding the format to be used for this section.

Project # 4

Tuck, Alan (PI)[1,2] and Rodenhiser, David[2]

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Modeling of critical steps in mammary tumor progression in 3D culture and in vivo

Keywords/Technical Terms: breast, 3D models, gene expression profile, early progression, atypical ductal hyperplasia, ductal carcinoma *in situ*, invasive carcinoma, lymph node metastasis

THE PROBLEM: Two critical stages in breast cancer metastasis are the progression from an intraductal to an invasive process and involvement of the locoregional lymph nodes. In addition to *in vivo* studies in nude mice, which are time consuming and not readily manipulated, *in vitro* assays are desirable to closely mimic a "tissue-like" environment, in order to more rapidly identify and test molecules potentially regulating these key steps in progression.

SPECIFIC AIMS: 1) To develop an *in vitro* model of the early stages of tumor progression in the breast, in order to allow the identification of genes important in transition between stages of pre-malignant growth, and to provide a system for testing the ability of interventions to block progression through these specific stages. 2) To develop an *in vitro* model of the lymph node (LN) environment, in order to allow the identification of genes important in determining the ability of breast cancer cells to survive and grow in this environment, and to provide a system for testing the ability of interventions to block these processes.

STUDY DESIGN: 1) Three cell lines, all initially derived from the same patient with metastatic breast cancer (21T series), will be grown in 3D Matrigel, to model 3 distinct phases of progression at the primary site. In studies in nude mice, we have found 21PT to be stably non-tumorigenic, 21NT tumorigenic, non-metastatic and 21MT-1 tumorigenic and weakly metastatic. Mammary fat pads (mfp) injected with 21PT reproducibly show no malignancy on histology, but can show an atypical ductal hyperplasia-like morphology. 21NT form tumors that show the morphology of ductal carcinoma *in situ* (DCIS) on histology. In contrast, 21MT-1 cells show tumors that are comprised of both an *in situ* and an invasive component. Preliminary work has shown that growth of these three cell lines in 3D Matrigel reflects the *in vivo* morphology. This system will then be used, in conjunction with in vivo studies, to identify differences in gene expression profile at these different stages of progression, how they may be regulated (in terms of DNA methlyation), how they may function, and how their manipulation may control transitions between these stages of progression (eg. block DCIS from evolving to invasive cancer). 2) Dissociated human LN or nude mouse mfp LN tissue, suspended in 3D Matrigel, will be used to grow breast cancer cells in LN tissue-like environment *in vitro*. Cell lines of known, differing propensity for lymphatic metastasis, grown in this environment, will be tested for differences in gene expression profile. Candidate genes identified in this manner will be assessed as above, in terms of how they may be regulated (i.e. DNA methylation), how they may function, and how their manipulation may alter ability to survive, grow and invade in a LN environment. In this way, we expect to identify key regulators of the ability to establish progressive growth in the LN, representing a crucial step in the metastatic dissemination of breast carcinoma.

RELEVANCE: Once characterized, these models can be used to identify and test key molecules involved in early progression in the breast and in the ability to establish lymph node metastases. This information would be of potential use clinically in determining which lesions are more likely to progress, as well as in providing prime therapeutic targets.

From: Jennifer Stanley <jstanle2@uwo.ca>
To: Alan Tuck <Alan.Tuck@lhsc.on.ca>
Date: 6/20/08 3:43 PM
Subject: Biohazardous Agents Registry Form will expire soon - Tuck
Attachments: Chambers_A.pdf

Hello Dr. Tuck
I am afraid that, given that you have your own grants and your own staff/students, you will need your own biohazard approval number.
If you want, add your name to the Chambers one as a PI (attached) - be sure to sign the first page and the last page of the form beside Dr. Chamber's signatures.
Thanks
Jennifer

Ann Chambers wrote:
> Jennifer -
>
> Why not label Dr. Tuck as "Clinical Collaborator" on my protocol. He is not doing anything in the lab that is separate from what is already in my protocol.
>
> Ann
>
>
>>>> Alan Tuck 5/22/2008 8:26 AM >>>
>>>>
> What I am is a clinician (pathologist), co-PI with Ann Chambers on a number of grants. I am not a worker in Ann's lab, but each and every one of the students and post-docs that is working with biohazardous agents on any of the protocols IS a worker in Ann Chambers lab, co-supervised by Ann and myself.
>
> Alan T.
>
> ----------
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>

... as you have requested...
signed x2
Alan T.