Modification Form for Permit BIO-LRCC-0021

Permit Holder: Alison Allan

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

Lori Lowes

~~Michel Beausoliel~~

~~George Ormond~~

~~Erika Schulze~~

Alysha Croker

David Goodale

Ben Hedley

Additional Personnel
(Please list additional personnel here)

Jenny Chu.

Irene Ma.

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms		
Approved Cells	Human (primary): breast tissue, Human (established): MCF 10A. SUM149PT, SUM159PT, SUM1315M02 (all human breast cancer, immortilized)	MCF-7 - human breast cancer cells - source ATCC mDA-mB-468 " " " " " " mDA-mB-231 " " " " " " mDA-mB-435 " " " " " " 468 LN - human breast cancer cells derived from 468 in our lab
Approved Use of Human Source Material	Core biopsies from hospital patients	
Approved GMO		

* *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: Sep 30, 2008

Signature of Permit Holder: [signature]

BioSafety Officer(s): [signature] July 28/09

Chair,Biohazards Subcommittee: _____

Modification Form for Permit BIO-LRCC-0021

Permit Holder: Alison Allan

Approved use of Animals		
Approved Toxin(s)	Cholera toxin	

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*** PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

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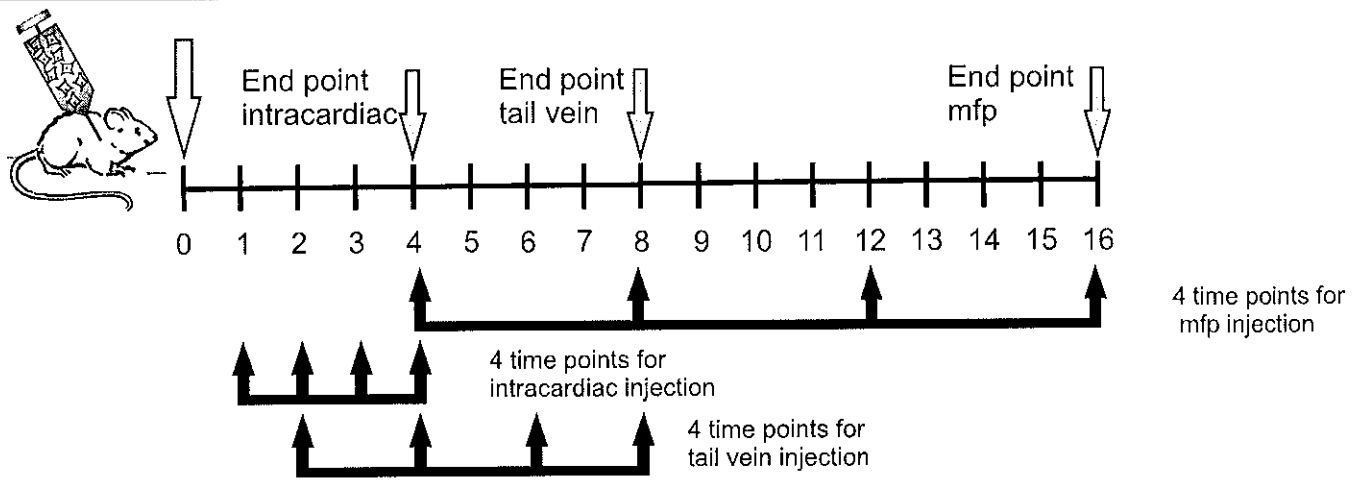
Chair,Biohazards Subcommittee:

Nude Mice Group Timeline: Experimental Group 2A,B,C:

Determining early steps in metastasis and timing and location of metastatic spread in spontaneous and experimental models of breast cancer

Injection of breast cancer cells
into Nude mice via one of three routes:
1. Mammary fat pad (mfp), **2.** Tail vein, **3.** Intracardiac



Mammary fat pad injection of 4 cell line

1) MDA-MB-468 (n=32)
2) MDA-MB-468 LN (n+32)
3) MDA-MB-435 n=32)
4)MDA-MB-231 (n=32)

Tail vein injection of 4 cell line

1) MDA-MB-468 (n=32)
2) MDA-MB-468 LN (n+32)
3) MDA-MB-435 n=32)
4)MDA-MB-231 (n=32)

Intracardiac injection of 4 cell line

1) MDA-MB-468 (n=32)
2) MDA-MB-468 LN (n+32)
3) MDA-MB-435 n=32)
4)MDA-MB-231 (n=32)=32)

Three different techniques (real-time PCR, flow cytometry, and LSC) will be used at each time point to quantify tumor cell dissemination

Euthanasia by CO_2 asphyxiation
Blood will be collected by terminal cardiac puncture
Tissue collection of: spleen, liver, kidneys, ovaries, pancreas, lungs, lymph nodes, and brain.

contaminating mouse cells. Spontaneous metastases in distant organs (lung, liver, etc.) will be assessed following tissue collection and processing. Tissues will be snap-frozen (for immunofluorescence [IF] and GUSB staining) or formalin-fixed for H&E staining. Slides will be evaluated in a blinded fashion by a pathologist to determine micrometastatic involvement and comparison/correlation with MRI data. $ALDH^{hi}CD44^{+}$ cells in metastatic lesions will be quantified by IF and laser scanning cytometry (LSC) using antibodies against human CD44 (BD Biosciences) and ALDH1 (a gift from Dr. Norman Sladek, University of Minnesota)[76].

(1b) Comparison of tumor cell ***growth*** in common sites of breast cancer metastasis (lung, liver, bone, brain, lymph node). Next, we will use MPSVII mice for assessment of direct metastatic growth in lung, liver, bone, and brain. However, because lymphoid organs in these mice are extremely small[61,62], they may not be suitable for assessing growth in the lymph node (LN). We will instead use athymic nude (*nu/nu*) mice, since we have previously observed that human breast cancer cells grow well in the LNs of these mice[73,77]. $ALDH^{hi}CD44^{+}$ and $ALDH^{low}CD44^{-}$ subsets will be isolated from 468, 231, 159, and/or 149 cell lines by FACS. Using methods familiar to our research group, sorted and unsorted populations will be injected via the tail vein (t.v.; delivery to lung), mesenteric vein (m.v.; delivery to liver), intrafemorally (i.f.; delivery to bone), left ventricle (l.v.; delivery to brain) or directly into the thoracic LN (*Publications 1 & 4* and[77-80]) using 2 dilutions per injection route (1×10^5 and 1×10^4 in 100 µl for t.v., m.v., and l.v.; 1×10^4 and 1×10^3 in 25 µl for i.f. and LN; n=4 mice/group). To control for tissue damage caused by direct injections, we will also include vehicle only groups (4 mice/injection site). Because of the large number of mice in these experiments, the high cost of imaging, and the fact that we expect metastatic growth to be limited to sites of injection, MRI will not be used. However, we will still track any possible systemic dissemination by assessing

CTCs weekly via blood draws as described above. Endpoint will be determined by morbidity associated with distant metastases. Tissues will be collected and quantified for GUSB staining, metastatic involvement, and ALDH1/CD44 population distribution as described above. Anticipated Outcome of **1a** and **1b**: Our use of imaging for tracking stem-like breast cancer cells *in vivo* represented a highly innovative application of cellular MRI[81], and combined with our novel CTC assay, will provide us with the capacity to assess dynamic, real-time differences in metastasis of $ALDH^{hi}CD44^{+}$ versus $ALDH^{low}CD44^{-}$ cells. Relative to $ALDH^{low}CD44^{-}$ cells, we expect $ALDH^{hi}CD44^{+}$ cells will show enhanced dissemination, seeding, and growth in all target organs. Furthermore, we predict $ALDH^{hi}CD44^{+}$ cells may grow particularly well in the bone marrow, since it is a key site of breast cancer metastasis and represents a rich stem cell niche[9,34,40].

Alternative Strategies & Knowledge Gained: Although we have previously observed that MPIO labeling does not affect the ability of MDA-MB-231 cells to proliferate or metastasize (*Publication 3*), it is possible that MPIO labeling of sorted $ALDH^{hi}CD44^{+}$ and $ALDH^{low}CD44^{-}$ subsets could alter their functional behavior. Thus, we will carry preliminary testing of labeled versus unlabeled cells to confirm this, and if necessary, utilize smaller alternative iron labels such as superparamagnetic iron oxide (SPIO) nanoparticles[82]. Overall, these experiments will allow us to determine if $ALDH^{hi}CD44^{+}$ tumor cells isolated from human breast cancer cell lines display differential seeding and growth in common sites of breast cancer metastasis. The results of these experiments will lay the groundwork for future clinical studies aimed at investigating whether increased $ALDH^{hi}CD44^{+}$ cells in the primary tumor may pre-dispose some patients to metastatic disease in specific organs, and if so, whether directed monitoring of these organs (by MRI, CT, or ultrasound) may be beneficial for early detection of metastatic lesions.

SPECIFIC AIM 2: **To investigate the molecular mechanisms underlying the metastatic capacity of stem-like ALDH^{hi}CD44^{+} cells.** The first Specific Aim will test whether ALDH^{hi}CD44^{+} cells exhibit specific functional characteristics relating to metastatic seeding/ growth. *However, it is our hypothesis that ALDH1 and CD44 are not simply markers for highly aggressive stem-like cancer cells, but instead that these molecules play important functional and mechanistic roles in regulating malignant/metastatic behavior.* Previous studies suggest that individually, ALDH1 and CD44 are important for mediating key properties of both stem cells and metastatic cancer cells. For example, ALDH1 is responsible for the metabolism of retinol to retinoic acid, and has a critical protective function in stem cells and cancer cells through its ability to detoxify aldehydes and chemotherapeutics such as cyclophosphamide[76,83,84]. In a parallel role, ALDH1 has recently been shown to regulate hematopoietic stem cell differentiation, proliferation, and lineage commitment[85,86], as well as being highly expressed in undifferentiated human mammary stem/progenitor cells[54]. In its own right, CD44 has been implicated in normal stem cell mobilization and homing[87], and also has a well-established role in cell adhesion, migration, and metastasis of cancer cells[44,88]. However, the functional contribution and molecular mechanisms of these two molecules, alone or in combination, has yet to be investigated in stem-like breast cancer cells.

Proposed Experiments: (2a) Generation of breast cancer cells with modified expression of ALDH1 and/or CD44. Moderately metastatic human breast cancer cell lines containing a sizable population of ALDH^{hi}CD44^{+} cells (231 and 149 cells; *Publication 1* and *Fig. 4*) will be engineered for stable knockdown of ALDH1 and/or CD44 using viral siRNA constructs targeting ALDH1 (from Jan Moreb, *see Letter 4*)[84] and/or CD44 (from Eva Turley, *see Letter 5*) and standard transduction methods familiar to our research group[84]. Control ("con") cells will be

transduced with a non-specific siRNA vector. This will generate the following engineered cell lines: 231con, 231ALDH$^{low/-}$, 231CD44$^{low/-}$, 231ALDH$^{low/-}$CD44$^{low/-}$; 149con, 149ALDH$^{low/-}$, 149CD44$^{low/-}$, and 149ALDH$^{low/-}$CD44$^{low/-}$. In addition, non-metastatic breast cancer cells with a known ALDH^{low}CD44^{-} phenotype (MCF-7; *Publication 1*) will be transfected to stably overexpress ALDH1 and/or CD44 using pcDNA3.1 expression constructs for ALDH1 (cDNA from Open Biosystems) and/or CD44S (standard isoform, from Dr. Turley) and transfection methods already in use in our lab[73,75]. Control cells will be transfected with empty vector. This will generate the following engineered cell lines: MCF-7con, MCF-7ALDH^{+}, MCF-7CD44^{+}, and MCF-7ALDH^{+}CD44^{+}. Engineered cell lines will be screened for ALDH1 and CD44 by FACS, and the highest/lowest expressing cells will be selected by cell sorting.

(2b) *In vitro* characterization of breast cancer cell lines with modified ALDH1/CD44. To investigate the functional role of ALDH1 and CD44, cell lines generated in **2a** will be compared for differential cell behavior *in vitro* using standard assays for growth, colony formation, adhesion, migration, and invasion as described in *Publication 1*. For assessment of behavior related to differentiation, we will investigate differences in 3D morphogenesis/growth in Matrigel as described[89]. For experiments utilizing siRNA knockdown of ALDH1/CD44 in 231 and 149 cells, we will also compare the functional behavior of these engineered cells with FACS-sorted populations of parental cell lines and patient pleural effusions (i.e. ALDH^{hi}CD44^{+}, ALDH^{hi}CD44^{-}, ALDH^{low}CD44^{+}, and ALDH^{low}CD44^{-}). If significant differences are observed between cell populations, we will begin to investigate the unique signaling pathways that correspond to the functional role of these proteins. In collaboration with Eva Turley, we will investigate the differential activity of CD44-related signaling molecules via immunoblotting (RhoA, Rac1, ERK-1,2; total/phosphorylated protein)[90,91]. We will also assess the activity of

transcription factors associated with ALDH1 activity (HOX11, retinoic receptor α, CEBPβ)[86,92] using gel-shift and transcriptional reporter assays in use in our lab[93]. Finally, we will investigate the differential expression of molecules linked to the transition between an undifferentiated/mesenchymal phenotype and a more differentiated/epithelial phenotype (vimentin, E-cadherin, smooth muscle actin, cytokeratins 8/14/18/19)[94,95] using RT-PCR and immunoblotting. Anticipated Outcome of 2a and 2b: These experiments will allow us to determine if ALDH1 and CD44 contribute functionally to regulation of malignant behavior, as opposed to simply being markers of highly aggressive breast cancer cells. Assessment of siRNA engineered cells compared to FACS-sorted populations will also allow us to investigate the relative hierarchy of cell phenotypes from most malignant to least malignant, and the functional association of this hierarchy with ALDH1/CD44 expression. We anticipate that knockdown of CD44 and ALDH1 alone or in combination will result in a cumulative reduction in malignant behavior. We expect these two molecules will influence different behaviors that collaboratively contribute to the metastatic phenotype. For example, knockdown of CD44 may be particularly influential in reducing adhesion, migration, and invasion[44,88]; whereas knockdown of ALDH1 may be more important for reducing cell growth and regulating a more differentiated phenotype[85,86]. In contrast, we anticipate that overexpression of ALDH1/CD44 will cumulatively enhance the malignant behavior of breast cancer cells in a similar but opposing manner.

Alternative Strategies & Knowledge Gained: Because the ALDEFLUOR® assay is optimized for specific interaction with human ALDH1[47,49], we have proposed to investigate the functional contribution of this ALDH isozyme. This rationale is also supported by previous studies demonstrating that ALDH1 is a poor prognosis marker for breast cancer patients[54]. However, both ALDH1 and ALDH3 isozymes are enzymatically active on the BAAA

substrate[47,49], so it is possible that ALDH3 is also important. Dr. Moreb has offered us siRNA vectors targeting ALDH3 as well as ALDH1 (*see Letter 4*), so we will have the capacity to investigate this. Similarly, we have proposed to investigate CD44S, but will also have the ability to examine other CD44 isoforms (*see Letter 5*). Overall, the results of these experiments will provide us with a greater understanding of the functional and molecular mechanisms underlying the metastatic capacity of stem-like $ALDH^{hi}CD44^{+}$ cells. Together with the *in vivo* experiments described below, this data will lay the groundwork for the identification of novel signaling pathways that could serve as new therapeutic targets for cancer drug development.

SPECIFIC AIM 3: To determine the response of $ALDH^{+}CD44^{+}$ breast cancer cells to therapy. Another characteristic shared by normal stem cells and metastatic cells is *an increased capacity for self-protection against cellular insult from toxins or drugs*. Our preliminary studies indicate that, relative to $ALDH^{low}CD44^{-}$ cells, $ALDH^{hi}CD44^{+}$ cells show enhanced expression of multi-drug resistance transporter 1 (MDR1) and glutathione S-transferase P1 (GSTP1) (*Fig. 6*). Interestingly, both MDR1 and GSTP1 have been shown to be expressed by normal stem cells[96]. MDR1 has been implicated in resistance against chemotherapeutics such as taxanes and vinblastine[96,97], whereas GSTP1 is involved in resistance to taxanes and cyclophosphamide (CP)[98,99]. In addition, clinical studies have demonstrated that breast cancer patients with high expression of ALDH1 are more resistant to alkylating agents such as CP[76]. This suggests that, as well as being more metastatic, stem-like $ALDH^{hi}CD44^{+}$ cells may also be more resistant to treatment. Intriguingly, high activity of ALDH1 in these stem-like cells represents a potentially "druggable" target. All-trans retinoic acid (ATRA) is a clinically approved differentiation agent that is used in combination with chemotherapy to treat patients with acute promyelocytic leukemia[100], and has been tested in Phase I/II clinical trials in breast

cancer[101,102]. In experimental *in vitro* studies, ATRA has been shown to downregulate the expression of ALDH1 in lung cancer cell lines and increase sensitivity to chemotherapeutic agents[84,103]. However, its role in influencing treatment response of stem-like breast cancer cells with high ALDH1 expression has not been investigated.

Proposed Experiments: (3a) Determination of *in vitro* response to therapy. Docetaxel (a taxane) and CP (an alkylating agent) are chemotherapeutics commonly used to treat breast cancer[104]. Because CP is a pro-drug, *in vitro* studies require the use of 4-hydroperoxycyclophosphamide (4-HC), an active metabolite of CP[104]. Based on the results of Specific Aim **2b**, we will utilize the ALDH1/CD44 engineered cell lines that show the greatest difference in functional behavior (growth, adhesion, migration, invasion, morphogenesis) relative to their paired controls. As discussed above, we expect these to be 231con and 231ALDH$^{low/-}$CD44$^{low/-}$; 149con and 149ALDH$^{low/-}$CD44$^{low/-}$; and MCF-7con and MCF-7ALDH^{+}CD44^{+}. We will assess the differential *in vitro* response of these paired cell lines to chemotherapy, ATRA, or a combination of both. Using previously established dose ranges[57,84,105,106], cells will be treated with vehicle, docetaxel (5-25 μM) or 4-HC (20-100 μM) alone or in combination with ATRA (1-2 μM) for 48 hr. Using standard assays[107,108], cells will first be compared for dose-dependent differences in cell proliferation (MTT assay) and apoptosis (Annexin V/PI staining/flow cytometry). If significant differences are observed between cell populations, we will begin to investigate possible mechanisms of treatment response via assessment of cell cycle kinetics (PI staining/flow cytometry), functional drug resistance (flow cytometry analysis of ALDH1 activity and immunoblotting for MDR1/GSTP1 expression), and activation of DNA damage response (alkaline comet assays, immunoblotting analysis of DNA checkpoint proteins [Rad17, Chk1, Chk2; total/phosphorylated protein], or flow cytometry

analysis of H2AX phosphorylation)[37,109,110]. For experiments utilizing siRNA knockdown of ALDH1/CD44 in 231 and 149 cells, we will also compare the treatment response of these engineered cells with FACS-sorted populations of the parental cell lines and patient pleural effusion samples ($ALDH^{hi}CD44^{+}$ and $ALDH^{low}CD44^{-}$ subsets).

(3b) Determination of *in vivo* response to therapy. Based on the results of **3a**, one of the siRNA engineered cell line pairs (i.e. 231con and $231ALDH^{low/-}CD44^{low/-}$ or 149con and $149ALDH^{low/-}CD44^{low/-}$) and the MCF-7con and $MCF\text{-}7ALDH^{+}CD44^{+}$ cell lines will be used for *in vivo* experiments. FACs-sorted cells will not be used in these experiments, since (as discussed in Specific Aim 1), we expect that purified $ALDH^{hi}CD44^{+}$ cells will generate tumors with a heterogeneous mix of both $ALDH^{hi}CD44^{+}$ and $ALDH^{low}CD44^{-}$ cells, thus making it difficult to specifically test *in vivo* differences in response to therapy between sorted populations. Cells will be injected into the m.f.p. of MPSVII mice (8 mice/group). For MCF-7 cell lines (estrogen receptor positive)[111], 17ß-estradiol pellets will be implanted interscapularly into mice 24 h prior to cell injection. Once tumors have grown to a mean volume of 200 mm^3, mice will be randomized into groups and treated with vehicle, docetaxel (i.v., 5 mg/kg)[105,112], or CP (i.p., 100 mg/kg)[57,113] 1 x weekly for 6 weeks, alone or in combination with ATRA (implanted time-release pellets, 160 mg/kg/day)[114]. Doses will be adjusted accordingly if toxicity occurs. Changes in ALDH1/CD44 population distribution in the primary tumor will be assessed by flow cytometry at 3 weeks (4 mice/group) and 6 weeks (4 mice/group) after start of therapy. Distant organs will be collected and assessed for GUSB staining and metastatic involvement as described above. Anticipated Outcome of **3a** and **3b**: Relative to $ALDH^{low/-}CD44^{-}$ cells, we expect that $ALDH^{+}CD44^{+}$ cells will demonstrate enhanced resistance to chemotherapy *in vitro* and *in vivo*, and that targeting ALDH1 with ATRA will reduce/eliminate this resistance.

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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

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Key words: breast cancer, lymph node metastasis, orthotopic model

Abstract

Breast cancer often spreads from the primary tumor to regional lymph nodes. Lymph node status provides clinically important information for making treatment decisions. Spread via lymphatics is also important for the biology of breast cancer, as tumor cells in lymph nodes may provide a reservoir of cells leading to distant, lethal metastases. Improved understanding of the biology of lymphatic spread thus is important for improved breast cancer survival. Advances towards understanding the interactions between tumors cells and lymphatic vessels have in part been limited by the lack of suitable cell lines and experimental models. We have addressed this need by developing a new model of lymphatic metastasis. Here we describe the establishment of 468LN cells, a variant of the MDA-MB-468 human breast adenocarcinoma cell line, which produces extensive lymph node metastasis following orthotopic injection of nude mice. 468LN cells are also more aggressive *in vitro*, produce more osteopontin and express different surface integrins compared to the parent line. The dramatic *in vitro* and *in vivo* phenotypic and molecular differences of 468LN and parental 468GFP cells make this pair of cell lines a unique model for the specific study of lymph node metastasis of breast cancer.

Abbreviations: ECM – extracellular matrix; EGFR – epidermal growth factor receptor; GFP – green fluorescent protein; mfp – mammary fat pad; OPN – osteopontin; VCAM-1 – vascular cell adhesion molecule

Introduction

For women with breast cancer, perhaps the most important question that affects their course of treatment and long-term survival is whether the cancer has spread from the primary tumor to locoregional lymph nodes [1, 2]. Of the women with invasive breast carcinoma, as many as one-third will also present with lymph node involvement at diagnosis and surgery [3–5]. Metastatic spread is the leading cause of breast cancer deaths and spread to the lymph nodes is considered one of the earliest clinical indications that a breast tumor has a propensity for metastasis [6, 7]. The presence of lymph node metastasis has long been regarded as one of the most important factors in evaluating the prognosis of breast cancer patients and has been shown to correlate with disease-free and overall survival better than any other prognostic factor [8, 9].

The process of metastasis depends on the ability of tumor cells to successfully complete a series of events that include breaking away from the primary tumor, accessing the vasculature, surviving in the circulation, arresting in a new site and colonizing that site to form a metastatic tumor [10, 11]. Tumor cells that are capable of spreading from their primary site do so by invading directly into the stroma, by infiltrating adjacent organs or body cavities, or by invading local lymphatic and/or blood vessels and entering the systemic circulation [10, 12]. In the case of breast cancer, tumor-associated lymphatics have long been believed to be the main route for early spread of tumor cells [7, 13, 14].

The lymphatic system is composed of a network of thin-walled, low-pressure capillaries that extend throughout the body collecting interstitial fluid, macromolecules

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of 5.0×10^4 cells/35 mm plate ($n = 3$ for each time point) and maintained in regular growth media. Every 24 h for 8 days, triplicate cultures were trypsinized, stained with trypan blue and counted for viable cells using a hemocytometer. The doubling time of each cell population was estimated during the exponential growth phase according to the equation $T_d = 0.693t/\ln(N_t/N_0)$, where t is time (in h), N_t is the cell number at time t, and N_0 is the cell number at initial time. An inverted microscope equipped with fluorescence was used to directly observe cell morphology, and representative digital photographs were taken.

In vivo *characterization of tumorigenicity and metastatic behavior*

For *in vivo* injection, cells were cultured as described above and harvested at ~70% confluency (log phase of growth), washed twice by centrifugation with sterile phosphate buffered saline (PBS), counted by hemocytometer, resuspended at a concentration of 1×10^7 cells/ml PBS, and kept on ice until injection.

For tumorigenicity and spontaneous metastasis assays, 1×10^6 tumor cells in a volume of 100 μl were orthotopically injected into the second thoracic mammary fat pad of 7–8 week old female nude mice (Harlan, Indianapolis, IN) as described elsewhere [27]. Primary tumor growth was evaluated weekly by measurement with calipers in two perpendicular dimensions and the tumor volume was estimated using the following formula [volume = $0.52 \times (\text{width})^2 \times (\text{length})$], for approximating the volume (mm^3) of a ellipsoid. Mice injected with 468GFP cells ($n = 10$) were autopsied 12 weeks post injection, when the mean tumor volume was ~1200 mm^3. Mice injected with 468LN cells ($n = 20$) were autopsied at two end-points; when the mean tumor volume reached ~1200 mm^3 (~8 weeks post injection) and 12 weeks post injection.

For experimental lung metastasis assays, 7–8 week old female nude mice were injected intravenously with 1×10^6 tumor cells in a volume of 100 μl via the lateral tail vein. Both the 468LN group ($n = 8$) and the 468GFP group ($n = 8$) were autopsied 16 weeks post injection.

For the duration of all experiments, animals were maintained under specific pathogen-free conditions in microisolator cages in an isolated colony where sterilized food and water were provided *ad libitum*. Animals were monitored regularly for evidence of morbidity related to the primary tumor or metastatic burden. Animal care and surgical procedures were conducted in accordance with the standards of the Canadian Council on Animal Care, under an approved protocol of the University of Western Ontario Council on Animal Care.

At end-point, animals were euthanized and autopsies performed. Organs (including lungs, lymph nodes, liver, spleen, pancreas, kidneys, ovaries, and any other tissues of abnormal appearance) were examined superficially for evidence of metastases using a dissecting microscope equipped with fluorescence. The incidence of macroscopically detectable metastases was determined, and representative digital photographs were taken before organs were processed for histology.

Histopathological and immunohistochemical analysis

Primary tumors and organs harvested at autopsy were formalin-fixed, paraffin-embedded and thin-sectioned (4 μm). Standard hematoxylin and eosin (H&E) staining procedures were used to observe histopathological characteristics and to determine metastatic involvement. An immunohistochemical approach was developed to aid in the detection of micrometastases and to confirm their human origin. The monoclonal antibody, Mitochondria Ab-2 (1:100 dilution; Clone MT02; NeoMarkers, MediCorp, Montreal, QC) reacts specifically with a 60 kDa non-glycosylated protein component of human mitochondria. The resulting staining detects mitochondria of both normal and malignant human cells (but not mouse) and we found that it provided an effective means of identifying small nests of human tumor cells in mouse tissue.

Tissue sections were deparaffinized with xylene and rehydrated in graded ethanol. Antigen retrieval was performed by incubating tissue sections in 10 mM sodium-citrate buffer (pH 6.0) at 98 °C for 10 min using a microwave; sections were then left to cool for 20 min at room temperature. Sections were rinsed in dH_2O (5 min), endogenous peroxidase quenched with 3.0% H_2O_2 in methanol (5 min), slides rinsed in dH_2O (5 min), and incubated for 20 min in 5 mM Tris-HCl-buffered saline (TBS, pH 7.8). To prevent nonspecific staining due to reactivity of the secondary anti-mouse IgG antibodies with the surrounding murine tissue, the primary antibody (diluted 1:100 in Antibody Diluting Buffer; DakoCytomation Inc., Mississauga, ON) was reacted with a biotinylated secondary antibody and blocking reagent before the antibody was applied to the tissue sections, according to the manufacturer's instructions (DAKO ARK™; DakoCytomation Inc.). The prepared biotinylated primary antibody was applied to the tissue sections for 30 min. Sections were then rinsed with TBS (5 min) before treatment with streptavidin-peroxidase (15 min) and stained with the DAB chromogen (5 min). Lastly, sections were rinsed in dH_2O and counter-stained with Meyer's hematoxylin and examined by light microscopy. Positively-stained human cells had brown, granular cytoplasm. The positive control tissue was human liver and negative controls included normal mouse lung tissue, as well as human liver incubated without primary antibody.

Western blot analysis

Conditioned media (CM) was prepared by seeding 5×10^5 cells/100 mm plate in regular growth media and incubating for 18 h at 37 °C, 5% CO_2. Media was removed, plates were washed 1× with sterile PBS, media

that it took 468GFP tumors (Figure 2b). Overall, primary 468LN tumors grew at an accelerated rate relative to the 468GFP tumors, producing significantly larger tumor volumes from week 2 through to week 12 ($P \leq 0.05$; Figure 2b). Due to this enhanced growth rate, mice in the 468LN group were autopsied at 2 end-points in order to directly compare metastatic burden with the 468GFP group, both in terms of the time post injection (12 weeks) and status at a given tumor size (1300 mm^3). Histological examination revealed that both the 468LN and 468GFP primary tumors were essentially the same in appearance and that their histopathology corresponded to a poorly differentiated (high grade, no special type) adenocarcinoma (data not shown).

On gross examination at 12 weeks, vigorous metastatic spread of 468LN cells to locoregional lymph nodes was apparent (Figure 3a). Autopsy revealed widespread lymph node involvement in both the thoracic and abdominal cavities including: axillary, cervical, peri-scapular, peri-tracheal, peri-aortic, inguinal mammary fat pad, peri-renal and pelvic lymph nodes (Figure 3b–e). Gross metastases were not seen in other organs. Histological investigation of the enlarged lymph nodes showed the destruction of nodal architecture by infiltrating tumor cells that in many cases extended beyond the node capsule (Figure 3f). Standard H&E staining was used to further assess the incidence of metastatic involvement (Figure 3g) and immunohisto-

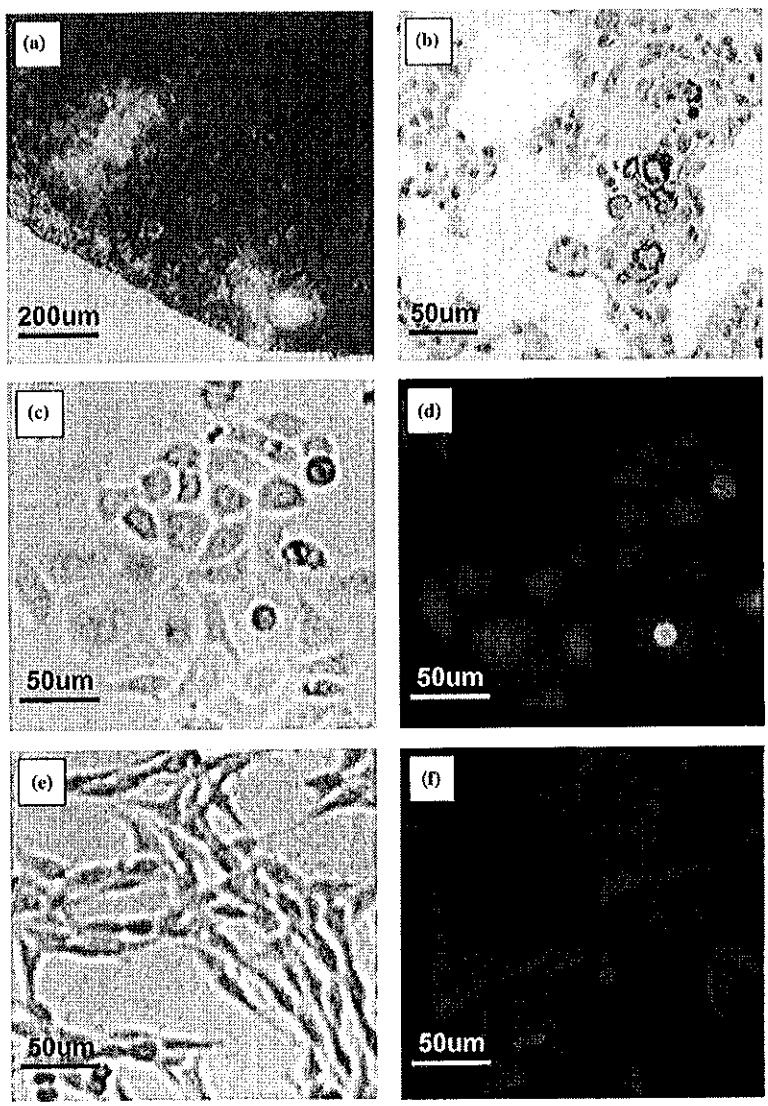

Figure 1. Selection of the metastasis-derived 468LN cell line and *in vitro* phenotypic comparison with the parental 468GFP cell line. (a) Lung metastases were detected by fluorescence in 1 of 8 nude mice sixteen weeks after injection of 1×10^6 468GFP cells into the mammary fat pad (original magnification 10×). Metastatic tumor cells were recovered from this lobe of lung tissue and established as a new line 468LN. (b) Lung tissue was further examined for metastatic burden by immuno-staining with a monoclonal antibody to a 60 kDa protein component of human mitochondria; the resulting cytoplasmic staining readily distinguished single human tumor cells from mouse cells (original magnification 60×). 468GFP metastases were again only identified in 1 of 8 mice. (c–f) *In vitro* morphology and GFP expression of parental 468GFP cells (c and d) and metastasis-derived 468LN cells (e and f) by phase contrast (c and e) and fluorescent (d and f) microscopy.

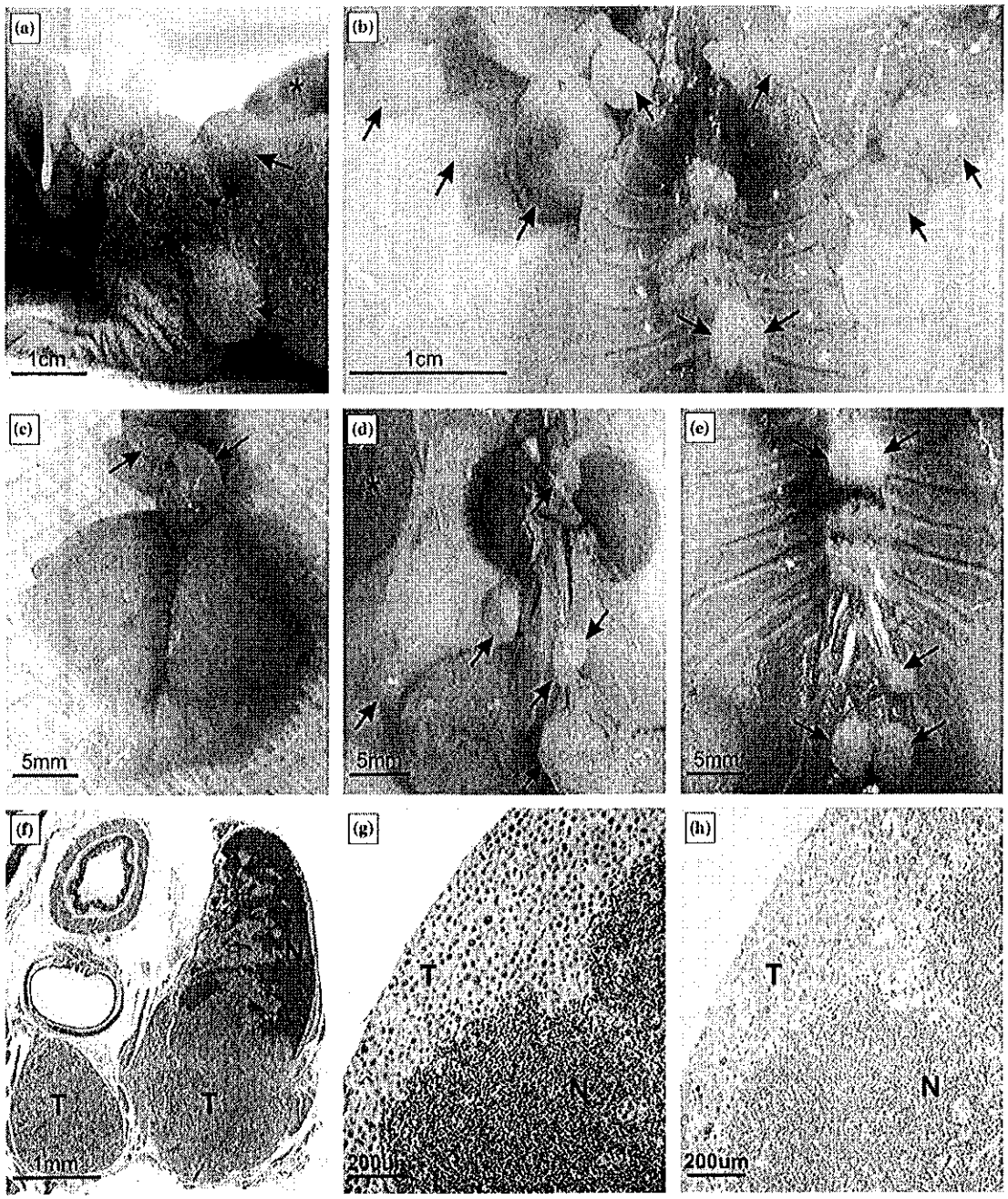

Figure 3. In vivo metastatic potential of 468LN cells. (a–e) Representative photographs of gross lymphatic metastasis of 468LN cells in nude mice twelve weeks after injection of 1×10^6 468LN cells into the right thoracic mammary fat pad (asterisk). Metastatic nodules (arrows) are grossly visible in the peri-scapular, cervical and left axilla regions (a). Autopsy revealed evidence of extensive lymph node involvement (arrows) in both the thoracic and abdominal cavities including: axillary (b), cervical (b), peri-scapular (b), peri-tracheal (c), peri-aortic (b, d, e), right inguinal mammary fat pad (d), peri-renal (d) and pelvic (d, e) regions. (f) Histological examination of grossly enlarged peri-tracheal lymph nodes (from c) shows destruction of normal nodal architecture (N) and tumor (T) expansion beyond node capsule. (g) Standard H&E staining was used to assess lymph nodes (N) for metastases (T) and immunohistochemical staining with a human mitochondrial-specific antibody confirmed the human origin of the lesions (h).

stood [30]. Advances towards understanding these interactions have in part been limited by the lack of suitable cell lines and experimental models. We have addressed this need here.

The availability of reliable, reproducible experimental models is a fundamental requirement for the successful study of cancer progression and the metastatic process. The isolation of cell populations that differ from the parental line in metastatic ability has proven to be a valuable method for studying the intrinsic properties that distinguish metastatic cells from nonmetastatic cells [21, 31–33]. The availability of such variants has

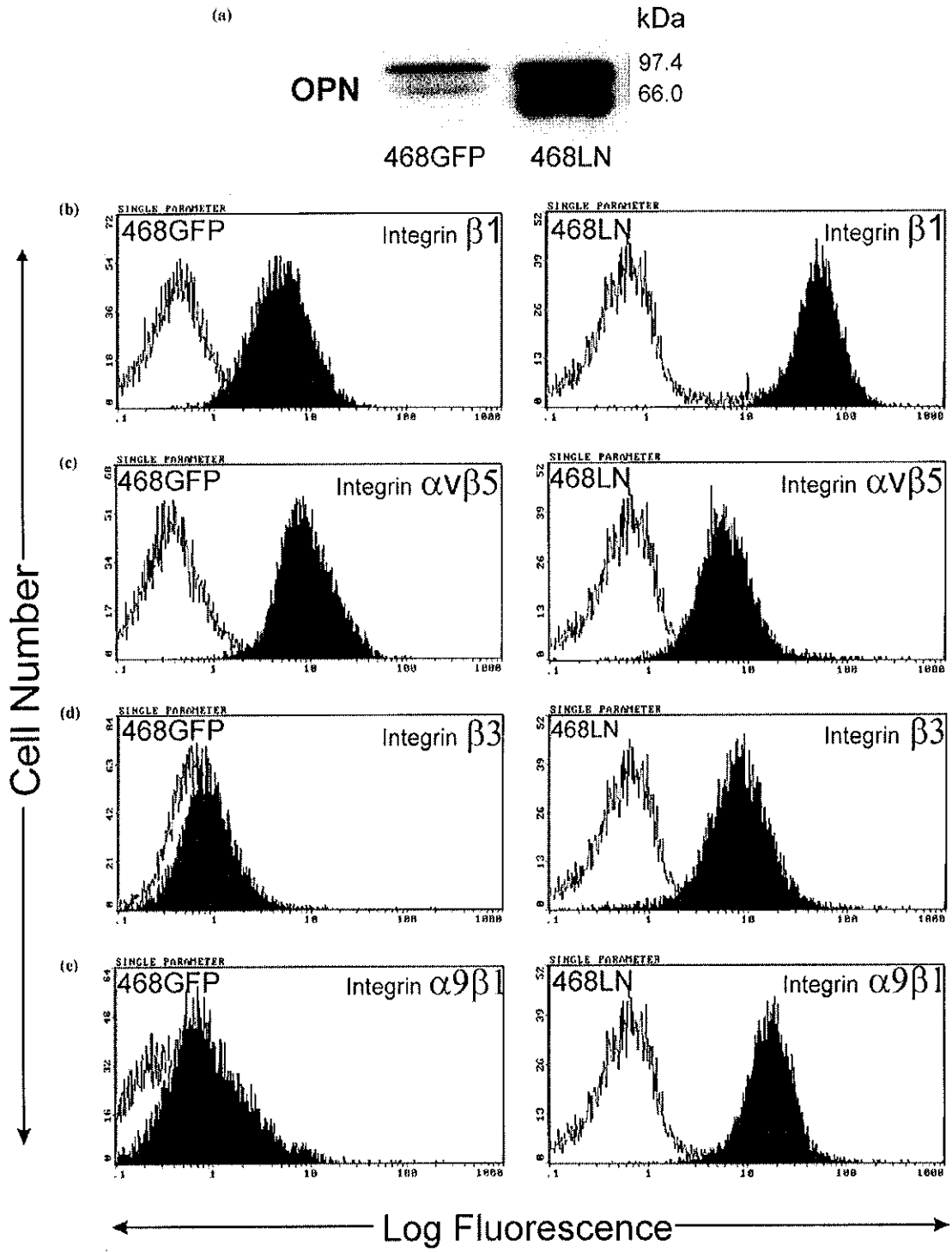

Figure 4. Osteopontin (OPN) and integrin expression by cultured 468GFP and 468LN cells. (a) Detection of OPN by Western blot analysis of conditioned media from 5×10^5 cultured 468GFP and 468LN cells. Two typical forms of OPN were detected: high molecular weight (~97 kDa) and lower molecular weight (66 kDa band). (b–e) Flow cytometry analysis of the integrin expression profiles of 468GFP cells (histograms, left) and 468LN cells (histograms, right). Cells were incubated with anti-integrin antibodies (filled profiles) or with a nonspecific isotype control primary IgG antibody (open profiles), as described in "Materials and Methods". (b) Surface expression of β_1 integrin (filled). (c) Surface expression of $\alpha v \beta_5$ integrin (filled). (d) Surface expression of β_3 integrin (filled). (e) Surface expression of $\alpha_9 \beta_1$ integrin (filled).

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Cell Biology

ATCC® Number:	**HTB-22™** Order this Item	**Price:**	**$264.0**
Designations:	MCF7	**Depositors:**	CM McC
Biosafety Level:	1	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	adheren
Organism:	*Homo sapiens* (human)	**Morphology:**	epithelia

PHOTO

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

Cellular Products: insulin-like growth factor binding proteins (IGFBP) BP-2; BP-4; BP-5

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

Related Ce

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

Receptors: estrogen receptor, expressed

Antigen Expression: Blood Type O; Rh+

DNA Profile (STR):
Amelogenin: X
CSF1PO: 10
D13S317: 11
D16S539: 11,12
D5S818: 11,12
D7S820: 8,9
THO1: 6
TPOX: 9,12
vWA: 14,15

Cytogenetic Analysis: modal number = 82; range = 66 to 87.
The stemline chromosome numbers ranged from hypertriploidy to hypotetraploidy, wi occurring at 1%. There were 29 to 34 marker chromosomes per S metaphase; 24 to 28 r least 30% of cells, and generally one large submetacentric (M1) and 3 large subtelocen markers were recognizable in over 80% of metaphases. No DM were detected. Chromosc and X was disomic.

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

Age: 69 years adult

Gender: female

Ethnicity: Caucasian

Comments: The MCF7 line retains several characteristics of differentiated mammary epithelium inclu estradiol via cytoplasmic estrogen receptors and the capability of forming domes.The cel oncogene [PubMed: 8168088].
Growth of MCF7 cells is inhibited by tumor necrosis factor alpha (TNF alpha).
Secretion of IGFBP's can be modulated by treatment with anti-estrogens.

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formul Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the to the base medium: 0.01 mg/ml bovine insulin; fetal bovine serum to a final concentratio
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

Subculturing: **Protocol:** Volumes used in this protocol are for 75 sq cm flasks; proportionally reduce dissociation medium for culture vessels of other sizes.
Note: if floating cells are present, it is recommended that they be transferred at the first described below. It is not necessary to transfer floating cells for subsequent subcultures.

1. Remove culture medium to a centrifuge tube.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution t serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inv cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask whi to detach. Cells that are difficult to detach may be placed at 37C to facilitate disper
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting
5. Transfer the cell suspension to the centrifuge tube with the medium and cells from at approximately 125 xg for 5 to 10 minutes. Discard the supernatant.
6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the culture vessels.
7. Incubate cultures at 37C.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: 2 to 3 times per week

Preservation: **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Doubling Time: 29 hrs

Related Products: Recommended medium (without the additional supplements or serum described under AT 2003
recommended serum:ATCC 30-2020
purified DNA:ATCC HTB-22D
purified RNA:ATCC HTB-22R
0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101
Cell culture tested DMSO:ATCC 4-X

References:

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Cell Biology

ATCC® Number:	**HTB-132™** Order this Item	**Price:**	**$264.(**
Designations:	MDA-MB-468	**Depositors:**	R Caill
Biosafety Level:	1	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	adhere
Organism:	*Homo sapiens* (human)	**Morphology:**	epithel

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

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

Related Ce

Isolation: **Isolation date:** 1977

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

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

Tumorigenic: Yes

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 chrom

hypotriploid range.; Normal chromosomes X, N2, N3, N7, N8, N10, and N22 are clearly u to their involvement in the formation of the many marker (19) chromosomes present in tl chromosome N1 (or two) is identified in each karyotype, but, in addition, regions of chr present in five different marker chromosomes.; Variation is evident in the normal and mai 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

Gender: female

Ethnicity: Black

Comments: The MDA-MB-468 cell line was isolated in 1977 by R. Cailleau, et al., from a pleural effi Black female patient with metastatic adenocarcinoma of the breast. Although the tissue dc for the G6PD alleles, the cell line consistently showed only the G6PD A phenotype.There is codon 273 of the p53 gene resulting in an Arg -> His substitution.EGF receptor is present

Propagation:
ATCC complete growth medium: The base medium for this cell line is ATCC-formu Medium, Catalog No. 30-2008. To make the complete growth medium, add the followi base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 100%
Temperature: 37.0°C

Subculturing:
Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution tc serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inv cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask whi to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispe
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipettinç
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:4 is recommended
Medium Renewal: 2 to 3 times per week

Preservation:
Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:
0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101
Cell culture tested DMSO:ATCC 4-X
recommended serum:ATCC 30-2020
Recommended medium (without the additional supplements or serum described under AT 2008

References:
1206: Brinkley BR, et al. Variations in cell form and cytoskeleton in human breast ca Cancer Res. 40: 3118-3129, 1980. PubMed: 7000337
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carcinoma. Cancer Res. 57: 5386-5390, 1997. PubMed: 9393764
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Cell Biology

ATCC® Number:	**HTB-26™** Order this Item	**Price:**	**$256.(**
Designations:	MDA-MB-231	**Depositors:**	R Caill
Biosafety Level:	1	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	adhere
Organism:	*Homo sapiens* (human)	**Morphology:**	epitheli

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 re of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for o Please click here for information regarding the specific requirements for shipment to your l

Related Ce

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

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

Tumorigenic: Yes

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 chromosom

triploid range. Normal chromosomes N8 and N15 were absent. Eleven stable rearranged are noted as well as unassignable chromosomes in addition to the majority of autosor Many of the marker chromosomes are identical to those shown in the karyotype rep 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

Ethnicity: Caucasian

Comments: The cells express the WNT7B oncogene [PubMed: 8168088].

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formu Medium, Catalog No. 30-2008. To make the complete growth medium, add the followi base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 100%
Temperature: 37.0°C

Subculturing: **Protocol:**

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution tc serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inv cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask whi to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispe
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C without CO2.

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:4 is recommended
Medium Renewal: 2 to 3 times per week

Preservation: **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products: purified DNA:ATCC 45519
purified DNA:ATCC HTB-26D
purified RNA:ATCC HTB-26R
purified DNA:ATCC 45518
Recommended medium (without the additional supplements or serum described under AT 2008
recommended serum:ATCC 30-2020

References:
1206: Brinkley BR, et al. Variations in cell form and cytoskeleton in human breast ca Cancer Res. 40: 3118-3129, 1980. PubMed: 7000337
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ATCC® Number:	**HTB-129™** Order this Item	**Price:**	**$264.0**
Designations:	MDA-MB-435S		
Biosafety Level:	1	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	adheren
Organism:	*Homo sapiens* (human)	**Morphology:**	spindle :

PHOTO

Source: **Organ:** previously described as: mammary gland; breast
Disease: previously described as ductal carcinoma
Derived from metastatic site: pleural effusion

Cellular Products: tubulin; actin

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Related Ce

Isolation: **Isolation date:** 1976

Tumorigenic: No

DNA Profile (STR):
Amelogenin: X
CSF1PO: 11
D13S317: 12
D16S539: 13
D5S818: 12
D7S820: 8,10
THO1: 6,7
TPOX: 8,11
vWA: 16,18

Cytogenetic Analysis: modal number = 56; range = 55 to 62
The cell line is aneuploid human female (XX), with most chromosome counts in the 55 chromosomes N6, N11, and N22 were absent, while chromosomes N7, N13, N18 and N2

the remainder of normal chromosomes were usually paired, but chromosome N2 was tr chromosomes were identified, with most of them formed from structural alterations of the normal chromosomes. Six of these markers involve regions of chromosome N7, while th derivatives of chromosome N6. Regions of a third copy of the normal and paired chromc N20 are noted in markers M1, M2, M15, and M5, respectively.

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

Age: 31 years adult

Gender: female

Ethnicity: Caucasian

Comments:T his cell line was originally described as a spindle shaped variant of the parental MDA-MB 1976 by R. Cailleau, et al. from the pleural effusion of a 31 year old female wi adenocarcinoma of the breast. However, recent studies have generated questions about tl cell line, MDA-MB-435, and by extension HTB-129. Gene expression analysis of the cells in which MDA-MB-435 clustered with cell lines of melanoma origin instead of breast [P PubMed ID: 15150101, PubMed ID: 15679052]. Additional studies have since corroborate of MDA-MB-435, to which ATCC has responded by pursuing its own investigation into tl line. The cell line to which MDA-MB-435 is reported to have been cross-contaminated with line [PubMed ID: 12354931 and PubMed ID: 17004106].

Derivatives of HTB-129 with identities in question:
M4A4, ATCC ® CRL-2914
M4A4 GFP, ATCC ® CRL-2915
M4A4 LM3-2 GFP, ATCC ® CRL-2916
M4A4 LM3-4 CL 16 GFP, ATCC ® CRL-2917
NM2C5, ATCC ® CRL-2918
NM2C5 GFP, ATCC ® CRL-2919

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formu Medium, Catalog No. 30-2008. To make the complete growth medium, add the followi base medium: 0.01 mg/ml insulin; fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 100%
Temperature: 37.0°C

Subculturing: **Protocol:** Remove medium, add fresh 0.25%trypsin - 0.53 mM EDTA, rinse and remov temperature (or incubated at 37C) for approximately 10 minutes or until the cells detac aspirate and dispense into new flasks.
Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: 2 to 3 times per week

Preservation: **Freeze medium:** Culture medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Related Products: Recommended medium (without the additional supplements or serum described under AT 2008
recommended serum:ATCC 30-2020
purified DNA:ATCC HTB-129D
purified RNA:ATCC HTB-129R

References:
1206: Brinkley BR, et al. Variations in cell form and cytoskeleton in human breast ca Cancer Res. 40: 3118-3129, 1980. PubMed: 7000337
22429: Siciliano MJ, et al. Mutually exclusive genetic signatures of human breast tu common chromosomal marker. Cancer Res. 39: 919-922, 1979. PubMed: 427779
22656: Cailleau R, et al. Long-term human breast carcinoma cell lines of metastat characterization. In Vitro 14: 911-915, 1978. PubMed: 730202
32341: Sheng S, et al. Maspin acts at the cell membrane to inhibit invasion and mot prostatic cancer cells. Proc. Natl. Acad. Sci. USA 93: 11669-11674, 1996. PubMed: 887619
32925: Zhu X, et al. Cell cycle-dependent modulation of telomerase activity in tumor cells USA 93: 6091-6095, 1996. PubMed: 8650224
49803: Ross DT, et al. Systematic variation in gene expression patterns in human ca Genetics 24: 227-235, 2000. PubMed: 10700174
89918: Ellison G, et al. Further evidence to support the melanocytic origin of MDA-MB 294-299, 2002. PubMed: 12354931

90826: Sellappan s, et al. Lineage infidelity of MDA-MB-435 cells: expression of melanocy cancer cell line. Cancer Res. 64: 3479-3485, 2004. PubMed: 15150101
90828: Rae JM, et al. Common origins of MDA-MB-435 cells from various sources with melanoma properties. Clin. Exp. Metastasis 21: 543-552, 2004. PubMed: 15679052
16173093: Rae JM, et al., MDA-MB-435 cells are derived from M14 Melanoma cells - a l but a boon for melanoma research. Breast Cancer Res. Treat. 104:13-19, 2007. PubMed:

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Subject: Allan Lab - MDA-MB-435 cell line
From: Jennifer Stanley <jstanle2@uwo.ca>
Date: Wed, 12 Mar 2008 14:14:57 -0400
To: Alison Allan <alison.allan@lhsc.on.ca>

Dr. Allan
Health Canada has deemed your cell line as Level 1 - you may wish to keep this information for your records in case you need it in the future. I will keep a record of it as well!
Jennifer

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

Hi Jennifer:

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

Hope this helps

Cheers!

Jean-Nic

Jennifer Stanley <jstanle2@uwo.ca> — 2008-03-12 09:43 PM
To: Jean-Nicolas Gagnon <jean-nicolas_gagnon@phac-aspc.gc.ca>
Subject: Re: Allan Lab - MDA-MB-435

Jean-Nic

The researcher used 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 on the website.

http://www.atcc.org/common/catalog/numSearch/numResults.cfm?atccNum=HTB-129

Jennifer

Jean-Nicolas Gagnon wrote:

>Hi Jennifer:
>
>From the info provided here I would consider this cell line a RC'. I could
>not access ATCC's catalog this morning (website seems to be down) did it
>mention that the cells contained viruses? If so it could change the RC
>classification of the line.
>
>I hope this helps. let me know if you need more info.
>
>Cheers!
>Jean-Nic
>
>Jean-Nicolas Gagnon
>A/Head, Importation and Biosafety Programs / Chef intérimaire Importation
>et Services de biosécurité
>Office of Laboratory Security / Bureau de la Sécurité des laboratoires
>Center for Emergency Preparedness and Response / Centre de mesures et
>d'interventions d'urgence
>Tel: (613) 946-6962
>Fax: (613) 941-0596
>
>Public Health Agency of Canada / Agence de la santé publique du Canada
>100 Colonnade Road
>Ottawa, ON
>K1A 0K9
>A/L: 6207A
>
>
>
>Jennifer Stanley <jstanle2@uwo.ca> — 2008-03-10 09:53 AM
>To: Jean-Nicolas Gagnon <jean-nicolas_gagnon@phac-aspc.gc.ca>
>Subject: Allan Lab - MDA-MB-435
>
>
>
>
>
>
>
>
>
>
>Jean-Nic:
>
>Do you have any idea about the containment level for this cell line, MDA-MB-435?
>
>I have attached the information I received from the investigator.
>
>Jennifer
>
>-------- Original Message --------
>
>Subject: Re: Allan Lab - MDA-MB-435
>

Allan

------- Original Message --------
Subject: Re: 468-LN cell line: containment level request
Date: Wed, 29 Jul 2009 09:18:50 -0400
From: Geneviève Lacroix <genevieve lacroix@phac-aspc.gc.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer,

I agree with you risk assessment, if the cell line does not contain any pathogens then it can remain a risk group 1.

Regards

Genevieve Lacroix, M.Sc.
A/Head, Importation and Biosafety Programs
Chef intérimaire/Importation et servuce de biosécurité
Office of Laboratory Security / Bureau de la sécurité des laboratoires
Public Health Agency of Canada / Agence de la santé publique du Canada
100 ch. Colonnade Rd. AL: 6201A, Ottawa, Ontario, Canada, K1A 0K9
Tel: (613) 946-6982
Fax: (613)941-0596
genevieve lacroix@phac-aspc.gc.ca
http://www.phac-aspc.gc.ca/ols-bsl/index.html

Jennifer Stanley <jstanle2@uwo.ca>
2009-07-29 09:16 AM

To
genevieve lacroix@phac-aspc.gc.ca
cc

Subject
468-LN cell line: containment level request

Hello there Genevieve:

The 468-LN cell line was derived
from the ATCC cell line MDA-MB-468 (from ATCC) in the following manner:
MDA-MB-468
cells were injected into a nude mouse and subsequently developed into
lung metastases. These lung tumors were isolated in a sterile manner,
dissagregated into single cells, and grown in sterile culture to
generate the derivative 468-LN cell line.

I would imagine that it should
still be Level 1 because it has not been virally transformed, exposed
to
any pathogens etc.

Let me know your thoughts on this,
Jennifer

Modification Form for Permit BIO-LRCC-0021

Permit Holder: Alison Allan

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

Michel Beausoliel

Alysha Croker

David Goodale

Ben Hedley

Additional Personnel
(Please list additional personnel here)

Lori Lowes

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms		
Approved Cells	Human (primary): breast tissue, Human (established): MCF 10A	SUM149PT, SUM159PT, SUM1315MO2 } all human breast cancer, immortalized
Approved Use of Human Source Material	Core biopsies from hospital patients	
Approved GMO		
Approved use of Animals		

* *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: Sep 30, 2008

Signature of Permit Holder:

BioSafety Officer(s): Dec. 17, 2008

Chair, Biohazards Subcommittee: Dec 12/08

Modification Form for Permit BIO-LRCC-0021

Permit Holder: Alison Allan

Approved Toxin(s)	Cholera toxin	

* *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: Sep 30, 2008

Signature of Permit Holder:

BioSafety Officer(s): Gail Ryder Dec. 17, 2008

Chair,Biohazards Subcommittee: G.M. Kidder

allan

Subject: [Fwd: Containment Level request for cell lines (SUM). Allan Lab]
From: Jennifer Stanley <jstanle2@uwo.ca>
Date: Mon, 22 Dec 2008 14:52:38 -0500
To: Jennifer stanley <jstanle2@uwo.ca>

-------- Original Message --------
Subject: Containment Level request for cell lines (SUM), Allan Lab
Date: Mon, 22 Dec 2008 14:52:02 -0500
From: Jennifer Stanley <jstanle2@uwo.ca>
To: Alison Allan <Alison.Allan@lhsc.on.ca>

Dr. Allan
Here are comments from the Public Health Agency of Canada regarding your new cell lines.
Happy Holidays
Jennifer

-------- Original Message --------
Subject: Re: Containment Level request for cell lines (SUM)
Date: Mon, 22 Dec 2008 00:35:20 -0500
From: Geneviève Lacroix <genevieve_lacroix@phac-aspc.gc.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>

Dear Ms. Stanley,

From the available literature and the product description, these cell lines look like naturally immortalized cell line. I could not find any indication of immortalization with a virus or any other contaminants. I would classify these cells at containment level 1. However, please note that because they are human cell lines, I would pay particular attention to avoid aerosols of the cells or the use of sharp objects (such as needles). In such cases, I would strongly recommend a CL2.

If you need further assistance, do not hesitate to contact me.
Regards

Geneviève Lacroix
A/Head, Importation and Biosafety Program/
Chef intérimaire, Importation et Services de biosécurité
Office of Laboratory Security / Bureau de la sécurité des laboratoires
Public Health Agency of Canada / Agence de la santé publique du Canada
[illegible] Promenade P.L. AL: 0603A, Ottawa, Ontario, Canada, K1A 0K9
Tel: 613 [illegible]
Fax: 613 [illegible]
genevieve_lacroix@phac-aspc.gc.ca
http://www.phac-aspc.gc.ca/ols-bsl/index.html

[illegible] <jstanle2@uwo.ca> [illegible]

[illegible] genevieve_lacroix@phac-aspc.gc.ca>

Containment level request for cell lines (SUM

Hello there
I am wondering if you could give us your advice on the containment level for these cells for in vitro work.
Thanks
Jennifer

Cell lines SUM....pdf **Content-Type:** application/octet-stream
Content-Encoding: base64

New Approaches to Metastatic Disease
Individual Component

9. DETAILED SCIENTIFIC ABSTRACT

Allan, Alison L. (PI)[1,2] and Keeney, Michael (Co-investigator)[2,3]
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.

Western

Researcher: Dr. Alison Allan

Biosafety Approval Number: BIO-LRCC-0021

February 20, 2008

Dear Dr. Allan:

Please note your biosafety approval number listed above. This number is very useful to you as a researcher working with biohazards. It is a requirement for your research grants, purchasing of biohazardous materials and Level 2 inspections.

Research Grants:

- This number is required information for any research grants involving biohazards. Please provide this number to Research Services when requested.

Purchasing Materials:

- This number must be included on purchase orders for Level 1 or Level 2 biohazards. When you order biohazardous material, use the on-line purchase ordering system (www.uwo.ca/finance/people/). In the "Comments to Purchasing" tab, include your name as the Researcher and your biosafety approval number.

Annual Inspections:

- If you have a Level 2 laboratory on campus, you are inspected every year. This is your permit number to allow you to work with Level 2 biohazards.

To maintain your Biosafety Approval, you need to:

- Ensure that you update your Biohazardous Agents Registry Form at least every three years, or when there are changes to the biohazards you are working with.
- Ensure that the people working in your laboratory are trained in Biosafety.
- Ensure that your laboratory follows the University of Western Ontario Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories.
- For more information, please see: www.uwo.ca/humanresources/biosafety.

Please let me know if you have questions or comments.

Regards,

Jennifer Stanley

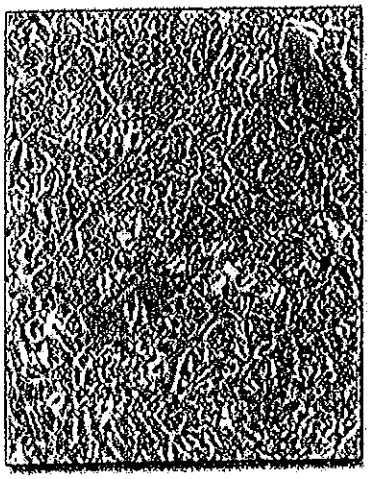
Jennifer Stanley
Biosafety Coordinator for Western
Stevenson Lawson Building Room 295G
Phone: 519-661-2111 X81135
Fax: 519-661-3420

SUM149
P75
3/12/2007
Vial 5
CS5
Breast Cancer Line

Go

SUM-149PT

Cell Lines
Human Serum
Primary Cells
RNA
How to Order
FAQ



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Price
$7,000.00
Academic Price:
$500.00
Your price:
$500.00

Qty: 1

Add to Lab Cart

All of the cell lines available from Asterand were developed in conjunction with different academic institutions. As a result of our agreements with these institutions, we offer academic and governmental researchers discounted prices.

Description **Additional Information**

Cell Source	Invasive Ductal Carcinoma, Inflammatory
ER/PR Status	ER-/PR-
Culture Media	Ham's F-12 with 5% Fetal Bovine Serum, Insulin and Hydrocortisone added.
Oncogene amplification	None
TGF-beta response	Very sensitive
ERBB receptor status	EGFR ++ (activated) ERBB-2,3 +ve (not activated) ERBB-4 -ve

Browse for more products in the same category as this item:

Cell Lines > Human Breast Cancer
Cell Lines

Cell Line: SUM149PT

Product Description: The SUM149 cell line was developed from Invasive Ductal Carcinoma from a patient with ER negative and PR negative, inflammatory breast cancer. The cell line is immortal and expresses luminal cytokeratins 8, 18, and 19 consistent with their origin from luminal breast epithelial cells. SUM149 has been known to form tumors in nude mice.

Quality control: The cells are grown in antibiotics free medium and monitored for bacterial contamination. The cell cultures are also tested for mycoplasma contamination. One test vial from each lot is thawed and recultured to test for contamination and growth.

Contents and Storage: One vial of 1×10^6 cells in freezing media.

Handling: Upon receiving the frozen cells from Asterand, it is essential that the user consult the enclosed data CD for technical advice regarding cell culture and obtain the necessary growth media and supplements before propagating the cells.

Place frozen cells in liquid nitrogen until you are ready to thaw and propagate them. We strongly recommend that you propagate the cells, using the provided procedure, as soon as possible. This will ensure the best cell viability.

The cells are shipped frozen on dry ice. If you notice any damage to the package or the cells are not frozen, please contact us immediately and if possible send us images of the damage or thawed cells. In this case we will replace the cells free of charge.

If any help is needed to grow the cells please call Asterand's customer service at 313-263-0960 and our scientists will help you over the phone to insure the successful growth of your cells.

Required Cell Culture Media:

Component	Stock Concentrations	Final Concentrations	Amount added to 500ml Medium
Ham's F-12	-	-	500ml bottle
Insulin	1mg/ml	5ug/ml	2.5ml
Hydrocortisone	1mg/ml	1ug/ml	500ul
HEPES	1M	10mM	5ml
Fetal Bovine Serum	-	-	25mls

Maintaining the cells:

Reviving the Frozen Cells:

1. Bring the temperature of the culture medium to 37°C
2. Thaw the cells fast (until a small crystal of ice is left) using 37°C water bath, and transfer the cells to a sterile T-25 culture flask containing the above growth medium, and incubate at 37°C humidified tissue culture incubator with 5% CO2 gas supply.
3. Feed the cells three times per week.

Maintain the Cell Culture:

1. Completely change the culture medium the day after initiation and every Monday, Wednesday and Friday thereafter.
2. Subculture the cells when they are 90% confluent. (See Figure 2 below) ~
3. Passage the SUM149 cells at a 1:3 split ratio for the first passage and 1:6 thereafter.

Freezing the cells:

1. Harvest the cells at about 90% confluent
2. Determine cell number and viability by using Trypan blue staining.
3. Centrifuge the cells at 1500 rpm for 5 mins at room temperature and resuspend in 1ml of freezing medium (recommend CryoStore CS 5 (Biolife Solutions) per 1x10^6 cells).
4. Using a Mr. Frosty or similar freezing device, the cell vials are placed into a 80°C freezer overnight and then stored long term in vapor phase liquid nitrogen.

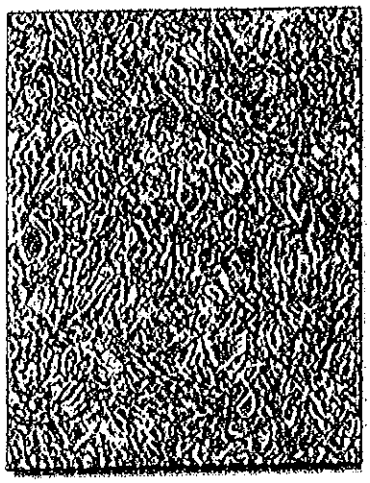
Additional Information:

SUM149 cells should not be grown to 100% confluency, as they will start to die off and likely not recover.

SUM159
P38
3/5/2007
Vial 10
CS5
Breast Cancer line

SUM-159PT

Cell Lines
Human Serum
Primary Cells
RNA
How to Order
FAQ



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Academic Price: **$500.00**
Your price: **$500.00**

Qty: 1

Add to Lab Cart

All of the cell lines available from Asterand were developed in conjunction with different academic institutions. As a result of our agreements with these institutions, we offer academic and governmental researchers discounted prices.

Description **Additional Information**

Cell Source	Anaplastic Carcinoma
ER/PR Status	ER-/PR-
Culture Media	Ham's F-12 with 5% Fetal Bovine Serum, Insulin & Hydrocortisone added
Oncogene amplification	C-MYC
TGF-beta response	Very sensitive
ERBB receptor status	EGFR +ve, ERBB-2 +ve

Browse for more products in the same category as this item:

Cell Lines > Human Breast Cancer
Cell Lines

Maintaining the cells:

Reviving the Frozen Cells:

1. Bring the temperature of the culture medium to 37°C
2. Thaw the cells fast (until a small crystal of ice is left) using 37°C water bath, and transfer the cells to a sterile T-25 culture flask containing the above growth medium, and incubate at 37°C humidified tissue culture incubator with 5% CO2 gas supply.
3. Feed the cells three times per week.

Maintain the Cell Culture:

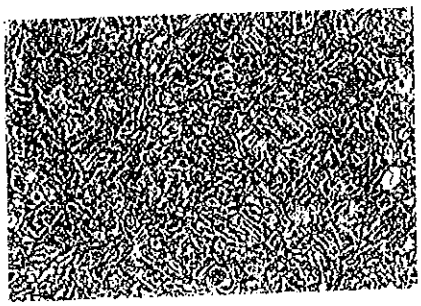
1. Completely change the culture medium the day after initiation and every Monday, Wednesday and Friday thereafter.
2. Subculture the cells when they are 95% confluent.
3. Passage the SUM159 cells at a 1:3 split ratio for the first passage and 1:10 thereafter.

Freezing the cells:

1. Harvest the cells at about 95% confluent
2. Determine cell number and viability by using Trypan blue staining.
3. Centrifuge the cells at 1500 rpm for 5 mins at room temperature and resuspend in 1ml of freezing medium (recommend CryoStore CS 5 (Biolife Solutions) per 1x10^6 cells).
4. Using a Mr. Frosty or similar freezing device, the cell vials are placed into a 80°C freezer overnight and then stored long term in vapor phase liquid nitrogen.

Additional Information:

The SUM159 lines recover easily from freeze/thaw process. They resemble fibroblasts, and tend to reach confluency in 3-5 days.



SUM159 cells in culture

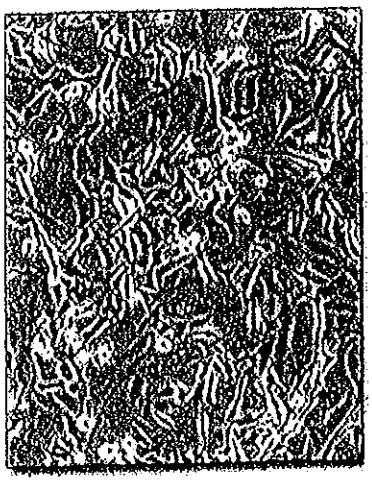
SUM1315 P37 5/1/2006 Vial 5 Cryostor Breast Cancer 1st vial

SUM1315 6/13/2006 39 Vial 15 2nd vial received 19 Aug 08

Go

SUM-1315MO2

Cell Lines
Human Serum
Primary Cells
RNA
How to Order
FAQ



LARGER PHOTO

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Price $7,000.00
Academic Price: $500.00
Your price: $500.00

Qty: 1

Add to Lab Cart

All of the cell lines available from Asterand were developed in conjunction with different academic institutions. As a result of our agreements with these institutions, we offer academic and governmental researchers discounted prices.

Description **Additional Information**

Cell Source	Skin Metastasis ductal carcinoma
ER/PR Status	ER-/PR-
Culture Media	Ham's F-12 with 5% Fetal Bovine Serum, Insulin & Epidermal Growth Factor added
Oncogene amplification	None
TGF-beta response	Moderately sensitive
ERBB receptor status	EGFR +ve. Others not determined

Browse for more products in the same category as this item:

Cell Lines > Human Breast Cancer
Cell Lines

Cell Line: SUM1315MO2

Product Description: The SUM1315 cell line was developed from a xenografted metastatic nodule of a patient with invasive infiltrating ductal carcinoma. The cells are immortal and are negative for estrogen and progesterone receptors and express high levels of Her2 and EGF receptor. They have also been shown to form lung and bone metastases after injection into nude mice.

Quality control: The cells are grown in antibiotics free medium and monitored for bacterial contamination. The cell cultures are also tested for mycoplasma contamination. One test vial from each lot is thawed and recultured to test for contamination.

Contents and Storage: One vial of 1×10^6 cells in freezing media.

Handling: Upon receiving the frozen cells from Asterand, it is essential that the user consult the enclosed data CD for technical advice regarding cell culture before propagating the cells.

Place frozen cells in liquid nitrogen until you are ready to thaw and propagate them. We strongly recommend that you propagate the cells, using the provided procedure, as soon as possible. This will ensure the best cell viability.

The cells are shipped frozen on dry ice. If you notice any damage to the package or the cells are not frozen, please contact us immediately and if possible send us images of the damage or thawed cells. In this case we will replace the cells free of charge.

If any help is needed to grow the cells please call Asterand's customer service at 313-263-0960 and our scientists will help you over the phone to insure the successful growth of your cells.

Required Cell Culture Media:

Component	Stock Concentrations	Final Concentrations	Amount added to 500ml Medium
Ham's F-12	-	-	500ml bottle
Insulin	1mg/ml	5ug/ml	2.5ml
EGF	10ug/ml	10ng/ml	500ul
HEPES	1M	10mM	5ml
Fetal Bovine Serum	-	-	25mls

Maintaining the cells:

Reviving the Frozen Cells:

1. Bring the temperature of the culture medium to 37°C
2. Thaw the cells fast (until a small crystal of ice is left) using 37°C water bath, and transfer the cells to a sterile T-25 culture flask containing the above growth medium, and incubate at 37°C humidified tissue culture incubator with 5% CO2 gas supply.
3. Feed the cells three times per week.

Maintain the Cell Culture:

1. Completely change the culture medium the day after initiation and every Monday, Wednesday and Friday thereafter.
2. Subculture the cells when they are 90% confluent. (See Figure 2 below)
3. Passage the SUM1315 cells at a 1:3 split ratio.

Freezing the cells:

1. Harvest the cells at about 90% confluent
2. Determine cell number and viability by using Trypan blue staining.
3. Centrifuge the cells at 1500 rpm for 5 mins at room temperature and resuspend in 1ml of freezing medium (recommend CryoStore CS 5 (Biolife Solutions) per 1x10^6 cells).
4. Using a Mr. Frosty or similar freezing device, the cell vials are placed into a 80°C freezer overnight and then stored long term in vapor phase liquid nitrogen.

Additional Information: The **SUM1315** cells MUST BE grown in 5% FBS media with insulin and epidermal growth factor as described above. The cells may take two or more passages to recover from the thawing process. Because of the size of the cell, about 1.5x10^6 cells can be expected per 1 T-75 flask.

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Revised Biohazards Subcommittee: April, 2008
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents are described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans. This form must also be updated at least every 3 years or when there are changes to the biohazards being used.

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, OHS (Stevenson-Lawson Building, Room 295) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies), modifications must be submitted to Occupational Health and Safety. See website: www.uwo.ca/humanresources/biosafety/

PRINCIPAL INVESTIGATOR Dr Alison Allan
SIGNATURE
DEPARTMENT Cancer Research
ADDRESS 790 Commissioners Rd East
PHONE NUMBER 519-685-8600 ext 55134
EMAIL alison.allan@lhsc.on.ca

Location of experimental work to be carried out: Building(s) LRCP/VRL Room(s) A4-114

*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 its being sent to Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Program, Child and Parent Research Institute, or Robarts Research Institute, a University Biosafety Committee member can also sign as the Safety Officer for the Institution.

FUNDING AGENCY/AGENCIES: Canadian Breast Cancer Research Alliance (CBCRA)-#016506
GRANT TITLE(S): Detection and analysis of rare metastatic events in mouse models of breast cancer using flow cytometry, laser scanning cytometry and real-time PCR

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

Names of all personnel working under Principal Investigators supervision in this location:
David Goodale
Ben Hedley
Alysha Croker
Michel Beaudoin

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)? O 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? (in Litres)	Source/ Supplier	Health Canada or CFIA Containment Level
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

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

2.2 Please indicate the type of primary cells (i.e. 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	AUS Protocol Number
Human	⊘Yes O No	Human Breast Tissue	Not applicable
Rodent	O Yes ⊘No		
Non-human primate	O Yes ⊘No		
Other (specify)	O Yes ⊘No		

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 O No	MCF 10A	ATCC
Rodent	O Yes O No		
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate HC or CFIA containment level required O1 ⊘2 O3

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 in the table below the Human Source Material to be used.

Human Source Material	Source/Supplier /Company Name	Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO	Name of Infectious Agent (If applicable)	HC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)	Uterine biopsies from hospital patients	O Yes ✗ No ✓		O 1 ✗2 ✓ O 3
Human Organs or Tissues (preserved)		O Yes O No		O 1 O 2 O 3

per conversation with Dr Allan Sept 29/08

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? O YES ✓NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? O YES, complete table below O NO

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results

* Please attach a Material Data Sheet or equivalent if available.

4.3 Will genetic modification(s) involving viral vectors be done? O YES, complete table below O NO

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ O NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ O NO
- SV 40 Large T antigen O YES O NO
- E1A oncogene O YES O NO
- Known oncogenes O YES, please specify _____ O NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ O NO

4.5 Will virus be replication defective? O YES O NO

4.6 Will virus be infectious to humans or animals? O YES O NO

4.7 Will this be expected to increase the containment level required? O YES O 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 be conducted using the viral vector in 4.0? O 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? O YES O 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? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? O 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 Will any of the agents listed be used in live animals O YES, specify: _____ O 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 body fluids including blood be used?

- Pound source dogs O YES ☑ NO
- Pound source cats O YES ☑ NO
- Cattle, sheep or goats O YES ☑ NO
- Non- Human Primates O YES, please specify species _____ ☑ NO
- Wild caught animals O YES, please specify species & colony # _____ ☑ NO
- Birds O YES ☑ NO
- Others (wild or domestic) O YES, please specify _____ ☑ NO

8.0 Biological Toxins

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

8.2 If YES, please name the toxin(s) Cholera Toxin
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin 250 µg/kg in mice

9.0 Import Requirements

9.1 Will the agent be imported? O YES, please give country of origin _____ ☑ NO
If no, please proceed to Section 10.0

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

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

9.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

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

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
- Employee Health and Safety Orientation

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 _____

11.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please indicate 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, permit # if on-campus BIO-LRCC-0021

○ NO

○ NOT REQUIRED

OAH-114 certified level 2 on March 5, 2008 by Gail Ryder

12.0 Procedures to be Followed

12.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories. I will ensure that workers have an up-to-date Position Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE _____ Date: 22 August 2008.

13.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: G.M. Fidler
Date: 30 Sept. 2008

Safety Officer for Institution where experiments will take place: SIGNATURE: Gail Ryder
Date: September 5, 2008

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: A Stanley
Date: Sept 30/08

Approval Number: BIO-LHRI-0051 LRCC-0021 ✓ Expiry Date (3 years from Approval): Sept 30, 2011

Special Conditions of Approval: re: cholera toxin
- follows recommended biosecurity requirements (see attached)
www.uwo.ca/human resources/faculty staff/h. and s/biosafety biosecurity.htm

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

Western

Biosecurity Requirements for Facilities Using Biological Agents

(1) Biological agents protected by a lock. For example, biological agents in a freezer, fridge, laboratories or other type of container must be locked after-hours/if no one present.

(2) The supervisor must ensure that each person has the qualifications and training to do the work without supervision.

(3) Visitors must be accompanied.

(4) The supervisor must keep a current inventory and a list of the location(s) where the biological agent(s) are stored and handled.

(5) Labelling to identify samples and the container in which they are stored.

(6) Notify the biosafety officer if a sample is lost, stolen, or otherwise misused.

(7) Notify Campus Community Police Services of suspicious behaviour.

There are two additional requirements for Facilities Using or Storing Biological Toxins:

(8) Do not keep on hand more than the amounts regulated by the United States Select Agents regulation: www.selectagents.gov/index.htm/

(9) For best practices, it is recommended to use or handle less than one human dose at any given time.

New Approaches to Metastatic Disease
Individual Component

9. DETAILED SCIENTIFIC ABSTRACT

Allan, Alison L. (PI)[1,2] and Keeney, Michael (Co-investigator)[2,3]
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.

Modification Form for Permit BIO-LRCC-0021

Permit Holder: Alison Allan

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

Lori Lowes
~~Michel Beausoliol~~
~~George Ormond~~
~~Erika Schulze~~
Alysha Croker
David Goodale
Ben Hedley

Additional Personnel
(Please list additional personnel here)

Jenny Chu
Irene Ma.

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms		
Approved Cells	Human (primary): breast tissue, Human (established): MCF 10A, SUM149PT, SUM159PT, SUM1315M02 (all human breast cancer, immortilized)	MCF-7 - human breast cancer cells - Source ATCC mDA-mB-468 " " " " " " mDA-mB-231 " " " " " " mDA-mB-435 " " " " " " 468 LN - human breast cancer cells - derived from 468 in our lab
Approved Use of Human Source Material	Core biopsies from hospital patients	
Approved GMO		

* *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: Sep 30, 2008

Signature of Permit Holder: _____

BioSafety Officer(s): _____ July 28/09

Chair,Biohazards Subcommittee: _____

Modification Form for Permit BIO-LRCC-0021

Permit Holder: Alison Allan

Approved use of Animals		
Approved Toxin(s)	Cholera toxin	

* *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: Sep 30, 2008

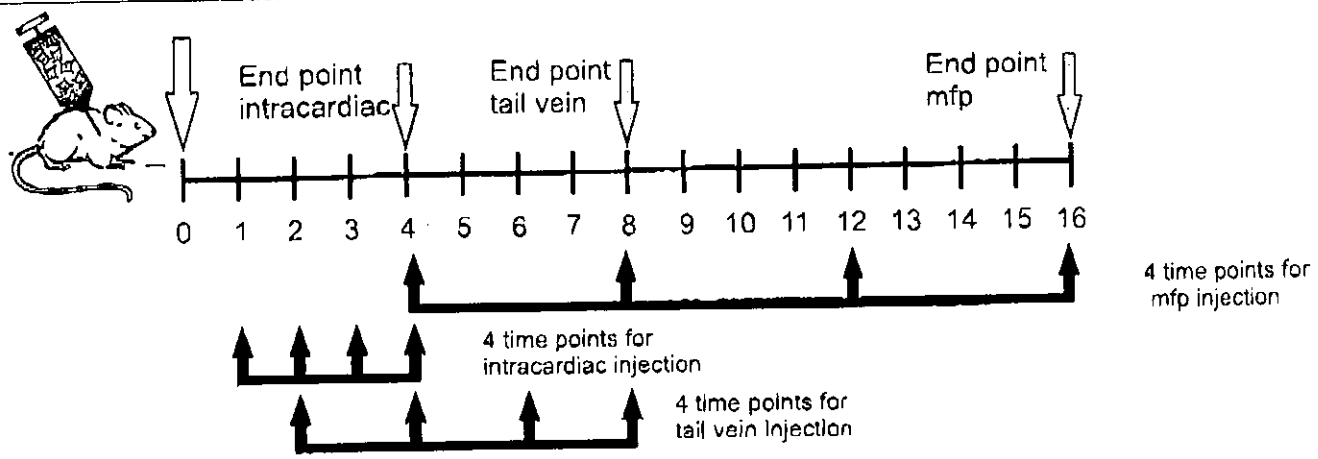
Signature of Permit Holder:

BioSafety Officer(s): June 28/09

Chair,Biohazards Subcommittee:

Nude Mice Group Timeline: Experimental Group 2A,B,C:
Determining early steps in metastasis and timing and location of metastatic spread in spontaneous and experimental models of breast cancer

Injection of breast cancer cells
into Nude mice via one of three routes:
1. Mammary fat pad (mfp), **2.** Tail vein, **3.** Intracardiac



Mammary fat pad injection of 4 cell line

1) MDA-MB-468 (n=32)
2) MDA-MB-468 LN (n+32)
3) MDA-MB-435 n=32)
4)MDA-MB-231 (n=32)

Tail vein injection of 4 cell line

1) MDA-MB-468 (n=32)
2) MDA-MB-468 LN (n+32)
3) MDA-MB-435 n=32)
4)MDA-MB-231 (n=32)

Intracardiac injection of 4 cell line

1) MDA-MB-468 (n=32)
2) MDA-MB-468 LN (n+32)
3) MDA-MB-435 n=32)
4)MDA-MB-231 (n=32)=32)

Three different techniques (real-time PCR, flow cytometry, and LSC) will be used at each time point to quantify tumor cell dissemination

Euthanasia by CO_2 asphyxiation
Blood will be collected by terminal cardiac puncture
Tissue collection of: spleen, liver, kidneys, ovaries, pancreas, lungs, lymph nodes, and brain.

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Cell Biology

ATCC® Number: HTB-22™ Order this Item

Designations: MCF7

Biosafety Level: 1

Medium & Serum: See Propagation

Organism: *Homo sapiens* (human)

Price: $264.00

Depositors: CM McGrath

Shipped: frozen

Growth Properties: adherent

Morphology: epithelial

PHOTO

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

Cellular Products: insulin-like growth factor binding proteins (IGFBP) BP-2; BP-4; BP-5

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 (Nucleofection technology from Lonza
Roche FuGENE® Transfection Reagents)

Receptors: estrogen receptor, expressed

Antigen Expression: Blood Type O; Rh+

DNA Profile (STR):
Amelogenin: X
CSF1PO: 10
D13S317: 11
D16S539: 11,12
D5S818: 11,12
D7S820: 8,9
THO1: 6
TPOX: 9,12
vWA: 14,15

Cytogenetic Analysis: modal number = 82; range = 66 to 87.
The stemline chromosome numbers ranged from hypertriploidy to hypotetraploidy, with the 2S component occurring at 1%. There were 29 to 34 marker chromosomes per S metaphase; 24 to 28 markers occurred in at least 30% of cells, and generally one large submetacentric (M1) and 3 large subtelocentric (M2, M3, and M4) markers were recognizable in over 80% of metaphases. No DM were detected. Chromosome 20 was nullisomic and X was disomic.

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

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Cell Biology

ATCC® Number:	**HTB-132™** Order this Item	**Price:**	**$264.00**
Designations:	MDA-MB-468	**Depositors:**	R Cailleau
Biosafety Level:	1	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	adherent
Organism:	*Homo sapiens* (human)	**Morphology:**	epithelial

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 (Nucleofection technology from Lonza
Roche FuGENE® Transfection Reagents)

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

Tumorigenic: Yes

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