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Award Number: DAMD17-03-1-0571

TITLE: Development of a Combination Cell and Gene Therapy  
Approach for Early-Stage Breast Cancer

PRINCIPAL INVESTIGATOR: Michael T. Lewis, Ph.D.

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REPORT DATE: February 2005

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

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20051227 206

# REPORT DOCUMENTATION PAGE

Form Approved  
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

|                                     |                                 |   |
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| 1. AGENCY USE ONLY<br>(Leave blank) | 2. REPORT DATE<br>February 2005 | 3. REPORT TYPE AND DATES COVERED<br>Final (15 Jul 2003 - 14 Jan 2005) |
|-------------------------------------|---------------------------------|---|

|  |  |
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| 4. TITLE AND SUBTITLE<br>Development of a Combination Cell and Gene Therapy Approach for Early-Stage Breast Cancer | 5. FUNDING NUMBERS<br>DAMD17-03-1-0571 |
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|---|--|
| 6. AUTHOR(S)<br><br>Michael T. Lewis, Ph.D. |  |
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| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)<br>Baylor College of Medicine<br>Houston, TX 77030<br><br>E-Mail: mtlewis@breastcenter.tmc.edu | 8. PERFORMING ORGANIZATION REPORT NUMBER |
|---|--|

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|---|--|
| 9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)<br>U.S. Army Medical Research and Materiel Command<br>Fort Detrick, Maryland 21702-5012 | 10. SPONSORING / MONITORING AGENCY REPORT NUMBER |
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|-------------------------|
| 11. SUPPLEMENTARY NOTES |
|-------------------------|

|   |                        |
|---|------------------------|
| 12a. DISTRIBUTION / AVAILABILITY STATEMENT<br>Approved for Public Release; Distribution Unlimited | 12b. DISTRIBUTION CODE |
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| 13. ABSTRACT (Maximum 200 Words)<br><br><p>The unique biology of the breast presents the opportunity to fuse cell and gene therapy techniques in a way that circumvents many of these technical limitations for the treatment of early stage breast cancer. In principle, it should be possible to obtain a patient's breast cells, genetically modify them, and reintroduce those cells into the affected regions of the breast via intraductal methods. Reintroduced cells would become incorporated into existing ductal structures and survive long-term to attack cancers.</p> <p><b>Objective:</b> <i>We propose to conduct a "proof-of-principle" combined cell and gene therapy study in a preclinical mouse model which, if successful, could be optimized and adapted for use in breast cancer patients.</i></p> <p><b>Task 1:</b> To isolate and culture primary mammary epithelial cells from the genetically tagged Enhanced Green Fluorescent Protein (EGFP) mouse strain.</p> <p><b>Task 2:</b> To exploit polyethylenimine (PEI)-mediated transfection to transfect primary cultures of mammary epithelial cells with a Red Fluorescent Protein reporter gene.</p> <p><b>Task 3:</b> To use intraductal injection to reintroduce transfected mammary epithelial cells into the ducts of genetically tagged Enhanced Cyan Fluorescent Protein into host mice</p> <p><b>Task 4:</b> To assay reconstituted ducts for efficiency with which re-introduced, transfected epithelial cells are incorporated into duct walls</p> |
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| 14. SUBJECT TERMS<br><br>Cell therapy; gene therapy; breast cancer | 15. NUMBER OF PAGES<br>13 |
|  | 16. PRICE CODE            |

|   |  |   |   |
|---|--|---|---|
| 17. SECURITY CLASSIFICATION OF REPORT<br>Unclassified | 18. SECURITY CLASSIFICATION OF THIS PAGE<br>Unclassified | 19. SECURITY CLASSIFICATION OF ABSTRACT<br>Unclassified | 20. LIMITATION OF ABSTRACT<br>Unlimited |
|---|--|---|---|

## Table of Contents

|                                   |   |
|-----------------------------------|---|
| Cover.....                        | 1 |
| SF 298.....                       | 2 |
| Introduction.....                 | 4 |
| Body.....                         | 4 |
| Key Research Accomplishments..... | 7 |
| Reportable Outcomes.....          | 7 |
| Conclusions.....                  | 8 |
| Appendices.....                   | 9 |

## INTRODUCTION

The unique biology of the normal breast presents the rare opportunity to fuse cell and gene therapy techniques in a way that circumvents many of these technical limitations for the treatment of early stage breast cancer. In principle, it should be possible to obtain a patient's own breast cells, genetically modify them to perform a therapeutic function, and reintroduce those cells into the affected regions of the breast via intraductal methods. Reintroduced cells would then become incorporated into existing ductal and alveolar structures and survive long-term to attack early stage cancers. Such an approach would therefore combine cell and gene therapy techniques, be minimally invasive, enhance efficiency of gene delivery, and reduce the risk of adverse treatment responses.

**Objective:** *We propose to conduct a "proof-of-principle" combined cell and gene therapy study in a preclinical mouse model which, if successful, could be optimized and adapted for use in breast cancer patients.*

## RESULTS

**Task 1:** To isolate and culture primary mammary epithelial cells from the genetically tagged Enhanced Green Fluorescent Protein (EGFP) mouse strain. (Months 1-12)

- We have optimized our protocol for isolation of single cells and small cell clusters using extensive, but gentle, mechanical disruption coupled with filtration through a 40 $\mu$ m filter.

**Task 2:** To exploit our polyethylenimine (PEI)-mediated transfection technique to transfect primary cultures of EGFP mammary epithelial cells with a Red Fluorescent Protein (RFP) reporter gene. Newly-developed PEI derivatives (1) will also be tested in parallel to obtain the maximum transfection efficiency possible using this technique. (Months 1-6)

- We find that the 25kDa form of polyethylenimine performs well at 7-9 nitrogen equivalents/DNA phosphate and consistently yields approximately 10-30% transfection of primary mammary epithelial cells. An added benefit is that fibroblasts are not transfected as efficiently at this concentration (fibroblasts transfect maximally at 5 equivalents) and, in fact, do not survive the procedure very well. Thus, transfected primary cultures are enriched in epithelial cells by the end of the procedure.

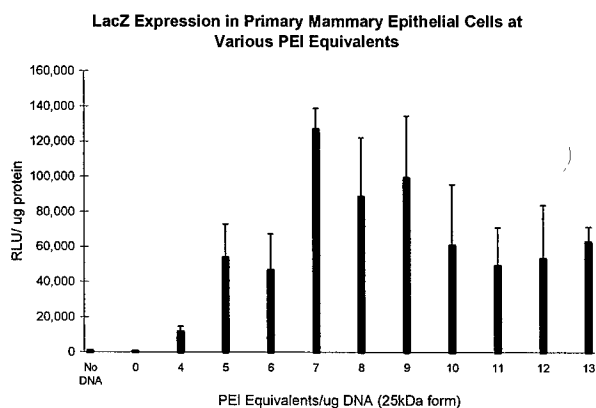


Figure 1. LacZ reporter expression in primary mammary epithelial cells upon transfection of 2 $\mu$ g pCMV- $\beta$  DNA using a range of PEI nitrogen equivalents. Seven PEI nitrogen equivalents per DNA phosphate was adopted for the final protocol.

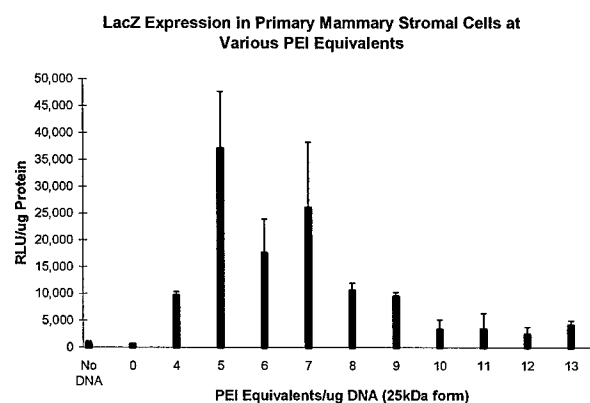


Figure 2. LacZ reporter expression in primary mammary stromal cells upon transfection of 2 $\mu$ g pCMV- $\beta$  DNA using a range of PEI nitrogen equivalents. Five PEI nitrogen equivalents per DNA phosphate was adopted for the final protocol.

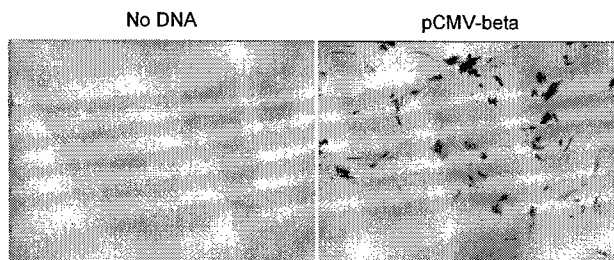


Figure 3. Transfection efficiency of epithelial cells at 7 PEI equivalents. Approximately 20% of the epithelial cells express LacZ as evidenced by blue staining.

- We have also developed protocols for use of electroporation in transfection of primary mammary epithelial cells using an Amaxa electroporation system. This method is superior to PEI, with approximately 50% viability after transfection and about 30-40% of viable epithelial cells being transfected. We have since adopted this method as our method of choice.

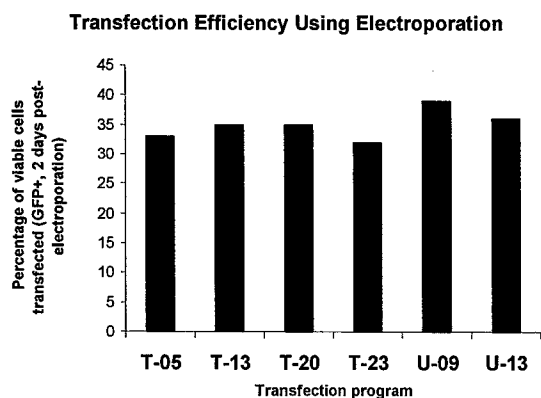


Figure 4. Transfection efficiency of primary mammary epithelial cells by electroporation using different electroporation conditions (e.g. T-05, T-13 etc.) shown along the X axis.

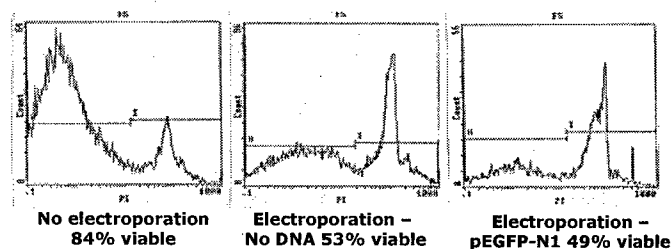


Figure 5. Viability of primary mammary epithelium upon electroporation using the propidium iodide staining method. Propidium iodide does not permeate viable cells. Thus, dead cells show a peak of fluorescence toward the right side of each flow cytometry plot.

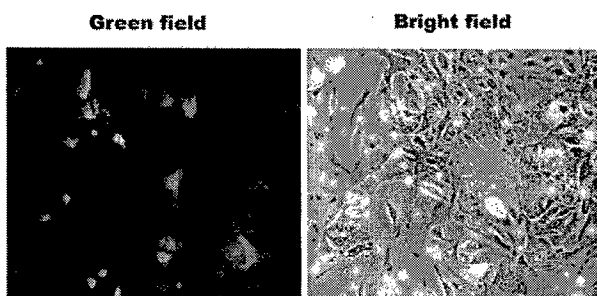


Figure 6. GFP expression in primary mammary epithelial cells after transfection via electroporation using the U-09 program.

**Task 3:** To use intraductal injection to reintroduce transfected mammary epithelial cells into the ducts of genetically tagged Enhanced Cyan Fluorescent Protein (ECFP) host mice whose endogenous epithelium has been treated, either with a function-blocking antibody against P-cadherin (2) or with 2mM EGTA [or EDTA], to disrupt adherens and tight junctions. (Months 6-10).

- The technician assigned to complete this portion of the project was found to be incapable of doing so and has since been terminated from employment. This project has been reassigned as of 2/1/2005 with the hiring of an experienced Postdoctoral Research Associate who is eager to complete the project.
- The P-cadherin antibody is available only in limited quantities and so has not been used routinely. We have data demonstrating that 2mM EGTA or EDTA delivered in a volume of 10-20ul is sufficient for focal disruption of tissue architecture. We have since begun to use 2 mM EDTA; 0.25% trypsin to enhance overall disruption of tissue architecture within the gland. Unfortunately, not all ducts are affected equally, with ducts closest to the nipple showing enhanced disruption. This effect was reversed within 24-48 hours.

Not surprisingly, it was found that disruption of tissue architecture prior to injection of transfected cells did not allow dissociated cells to be delivered into the ductal system. Instead, injected cells were localized to the nipple region. The reason for this is obvious. When tissue architecture is disrupted, epithelial cells are stripped from the duct walls and are dispersed in the lumen. Cell movement is therefore blocked by the pre-existing dispersed cells that are already filling the duct space. Thus, disruption of tissue architecture must be performed during the injection of the transfected cells, or after the transfected cells are introduced. We have chosen to adopt the simultaneous disruption/injection procedure.

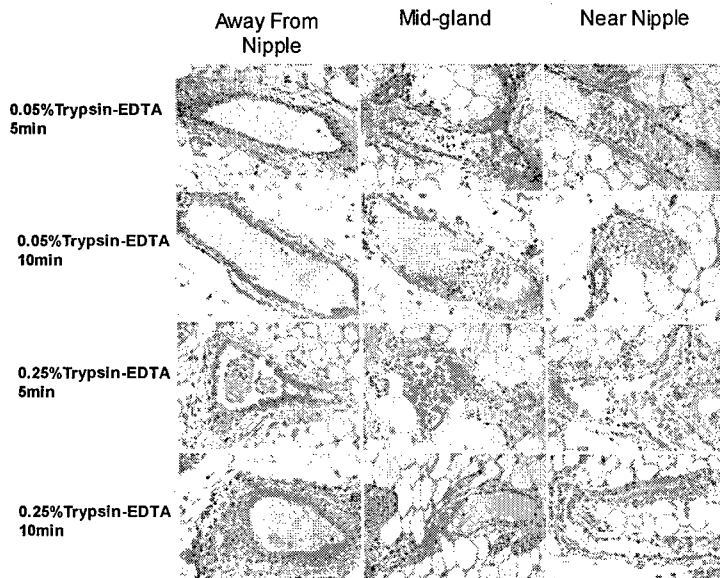


Figure 7. Transient disruption of mammary ductal histoarchitecture using Trypsin:EDTA.

Using the simultaneous disruption/injection procedure, we have successfully introduced untransfected mammary epithelial cells expressing CFP into wild type (non-fluorescent) hosts and have examined ducts 2 and 7 days post-injection. Introduced CFP+ cells persist and appear to have become incorporated into ductal structures at least through 7 days.

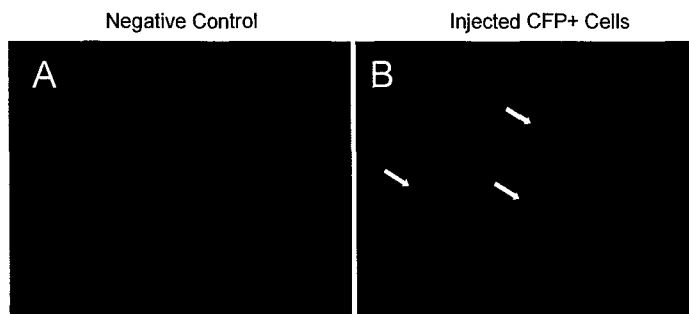


Figure 8. Persistence of CFP+ cells injected into the nipple using our simultaneous disruption/injection procedure. A. Negative control gland from a wild type mouse. B. CFP expressing regions (arrows) in injected gland.

**Task 4:** To assay reconstituted ducts for efficiency with which re-introduced, transfected epithelial cells are incorporated into duct walls and to measure duration of reporter gene expression after reintroduction. (Months 6-10)

Histological analysis for the ability of re-introduced cells to assimilate into normal structures is underway using the glands generated from task 3. We anticipate that the histoarchitecture of the re-formed ducts will be entirely normal. All that remains to be accomplished is to inject transfected cells and examine their behavior over time.

#### **KEY RESEARCH ACCOMPLISHMENTS**

- We have optimized two different transfection techniques that will be generally useful to the research community.
- We have confirmed published reports that tissue architecture can be reversibly disrupted in vivo and have developed a new method using EDTA and trypsin.
- We have demonstrated that genetically-tagged cells can be reintroduced to the mammary ductal tree by intraductal injection and can persist for at least 7 days.

#### **REPORTABLE OUTCOMES**

##### **Presentations**

- Baylor College of Medicine (2004)
- Texas A&M University (2004)

#### **EMPLOYMENT RECEIVED AND RESEARCH OPPORTUNITIES**

##### **Research funding**

Title: Novel Gene Networks in Breast Development and Cancer, Project 4: The Ptc1 Hedgehog Receptor in Mammary Ductal Development and Progression to Neoplasia

Agency: NIH P01 CA30195

Role: PI (Osborne/Lewis)

Period: 4/1/04-3/31/09

The major goals of this large Program Project are to identify and characterize the role of novel genetic pathways, which are found to be important in the normal breast, in the pathogenesis and progression of human breast cancer. The goal of Project 4 is to determine the role of Ptc1 in these processes.

Title: Novel Gene Networks in Breast Development and Cancer, Animal Handling and Imaging Core

Agency: NIH P01 CA30195 (Osborne/Lewis)

Role: PI

Period: 4/1/04-3/31/09

The major goals of this large Program Project are to identify and characterize the role of novel genetic pathways, which are found to be important in the normal breast, in the pathogenesis and progression of human breast cancer. The goal of Core C is to provide for purchase and housing of animals, and to provide specialized surgical and imaging support for all 5 projects included in this Program Project.

Title: Unmasking Stem/Progenitor Cell Properties Using Short-Term Transplantation

Agency: Department of Defense

Role: PI

Period: 6/30/05-6/29/06

The goal of this project is to unmask stem cell behaviors in differentiated cells using transplantations.

PENDING

R01 CA30195 (Lewis)

10/1/05-9/30/10

NIH

Hedgehog Network Regulation of Mammary Ductal Development and Neoplasia

ACS (Lewis)

10/1/05-9/30/09

American Cancer Society

Hedgehog Network Regulation of Mammary Ductal Development and Neoplasia

#### **CONCLUSIONS**

- Transfected primary mammary epithelial cells can be reintroduced to the mammary gland by intraductal injection. It remains to be demonstrated that they can survive long-term and continue to express a therapeutic gene of interest.

#### **APPENDICES:**

**Curriculum vitae for Michael T. Lewis**

Principal Investigator/Program Director (Last, First, Middle):

## BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.  
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| NAME<br><b>Michael T. Lewis</b>   | POSITION TITLE<br><b>Assistant Professor</b> |           |                           |
|---|--|-----------|---------------------------|
| eRA COMMONS USER NAME<br><b>mtlewis</b>   |  |           |                           |
| EDUCATION/TRAINING ( <i>Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.</i> ) |  |           |                           |
| INSTITUTION AND LOCATION  | DEGREE<br>(if applicable)                    | YEAR(s)   | FIELD OF STUDY            |
| College of William and Mary, Williamsburg, Virginia USA   | B.S.   | 1982-1986 | Biology                   |
| University of California, Santa Cruz, California USA  | Ph.D.  | 1989-1995 | Biology                   |
| University of California, Santa Cruz, California USA  | Post-doc.                                    | 1995-1998 | Biology                   |
| University of Colorado, Denver, Colorado USA  | Post-doc.                                    | 1999      | Physiology and Biophysics |

### A. Positions

- 10/86-7/88 **Biologist** – National Biomedical Research Foundation  
7/88-8/89 **Research Scientist** – National Biomedical Research Foundation – Protein Information Resource (NBRF-PIR). 3900 Reservoir Rd., N.W., Washington, D.C., 20007.  
9/89-6/95 **Graduate Researcher** – University of California, Santa Cruz. Department of Biology. Laboratory of Dr. Jerry Feldman.  
9/89-6/95 **Teaching Assistant** – University of California, Santa Cruz. Department of Biology.  
7/95-12/98 **Post Graduate Researcher** – University of California, Santa Cruz, CA 95064. Department of Biology. Laboratory of Dr. Charles Daniel.  
1/99-6/99 **Postdoctoral Research Associate** – University of Colorado School of Medicine, Denver, CO 80262. Department of Physiology and Biophysics. Laboratory of Dr. Peggy Neville.  
6/99-6/01 **Instructor** – University of Colorado School of Medicine, Denver, CO 80262. Department of Physiology and Biophysics.  
7/01-present **Assistant Professor** – Baylor College of Medicine Breast Center and the Department of Molecular and Cellular Biology, Houston, TX 77030.

### B. Honors and Awards

- 1989 University of California Regents Fellowship  
1992-Present Charter member - Sigma Xi (Santa Cruz chapter)  
1996 University of California Breast Cancer Research Program Postdoctoral Research Award  
2000 Department of Defense IDEA Award  
2001 Organizer – Gordon Research Conference in Mammary Gland Biology Workshops  
2001-2002 Member - Pathogenesis Study Section – Univ. California Breast Cancer Research Program  
2001-Present Member - San Antonio Breast Cancer Symposium Organization Committee  
2003 Organizer – NIH Workshop on Homeobox genes in mammary gland development

2003-Present Member - Tumor Progression Study Section – Univ. Cal. Breast Cancer Research Program  
 2003-Present Member – Molecular Biology Genetics 2 Study Section - Dept. Defense Breast Cancer Research Program  
 2003 Susan Love MD Breast Cancer Research Foundation Grant Award  
 2003 Department of Defense CONCEPT Award  
 2004 Department of Defense CONCEPT Award

### C. Publications

1. **Lewis, M.T.**, Hunt, L.T., and Barker, W.C. (1989) Striking sequence similarity among sialic acid-binding lectin, pancreatic ribonucleases, and angiogenin: possible structural and functional relationships. *Protein Seq. Data Anal.* 2: 101-105.
2. Ron, D., Zannini, M., **Lewis, M.T.**, Wickner, R.B., Hunt, L.T., Graziani, G., Tronick, S.R., Aaronson, S.A., and Eva, A. (1991) A region of proto-*dbl* essential for its transforming activity shows sequence similarity to a yeast cell cycle gene, *CDC24*, and the human breakpoint cluster gene, *bcr*. *The New Biologist* 3: 372-379
3. **Lewis, M.T.**, and Feldman, J.F. (1993) The putative *frequency (frq)* clock protein of *Neurospora crassa* contains sequence elements that suggest a nuclear transcriptional regulatory role. *Protein Seq. Data Anal.* 5: 315-323
4. **Lewis, M.T.** and Feldman, J.F. (1996) Evolution of the *frequency (frq)* clock locus in fungi. *Mol. Biol. Evol.* 13:1233-1241
5. **Lewis, M.T.**, Morgan, L.W., and Feldman, J.F. (1997) Analysis of *frequency (frq)* clock gene homologs: evidence for a helix-turn-helix transcription factor. *Mol. Gen. Genet.* 253:401-414
6. **Lewis, M.T.** and Feldman, J.F. (1998) Genetic mapping of the *band (bd)* locus of *Neurospora crassa*. *Fungal Genet. Newsl.* 45:21.
7. **Lewis, M.T.**, Ross, S., Strickland, P.A., Snyder, C.J. and Daniel, C.W. (1999) Regulated expression patterns of *IRX-2*, an Iroquois-class homeobox gene, in the human breast. *Cell Tissue Res.* 296:549-554
8. **Lewis, M.T.**, Ross, S., Strickland, P.A., Sugnet, C., Jimenez, E. Scott, M.P. and Daniel, C.W. (1999) Defects in mouse mammary gland development caused by conditional haploinsufficiency of *Patched-1 (Ptc1)*. *Development* 126:5181-5193
9. Nguyen, D., Beeman, N., **Lewis, M.T.**, Schaack, J. and Neville, M.C. (2000) Intraductal injection into the mouse mammary gland. *Methods in Mammary Gland Biology and Breast Cancer Research.* M.M. Ip and B.B. Asch (eds.) Kluwer Academic/Plenum Publishers, New York. 259-270
10. **Lewis, M.T.** (2000) Homeobox genes in mammary gland development and neoplasia. *Breast Cancer Research* 2: 158-169
11. **Lewis, M.T.**, Ross, S., Strickland, P.A., Sugnet, C., Jimenez, E., Hui, C-c. and Daniel, C.W. (2001) The *Gli2* transcription factor is required for normal mouse mammary gland development. *Dev. Biol.* 238:133-144
12. **Lewis, M.T.** (2001) Hedgehog signaling in mammary gland development. *J. Mammary Gland Biol. Neoplasia* 6:53-66
13. Salomon, D.S. and **Lewis, M.T.** (2004) Embryogenesis and Oncogenesis: Dr. Jekyll and Mr. Hyde. *J Mammary Gland Biol Neoplasia.* 9:105-7.

14. **Lewis, M.T.**, and Veltmart, J.M. (2004) Next stop, the twilight zone: hedgehog network regulation of mammary gland development. *J. Mammary Gland Biol. Neoplasia* 9:165-181.
15. Chang J.C., Wooten E.C., Tsimelzon A., Hilsenbeck S.G., Gutierrez M.C., Tham Y.L., Kalidas M., Elledge R., Mohsin S., Osborne C.K., Chamness G.C., Allred D.C., **Lewis M.T.**, Wong H., O'Connell P. (2005) Patterns of resistance and incomplete response to docetaxel (Taxotere) by gene expression profiling in breast cancer patients. *Journal of Clinical Oncology* 23(6):1169-77.

**DATA COLLECTIONS:**

Protein Sequence Database. Barker, W.C., Hunt, L.T., George, D.G., Yeh, L.S. Chen, H.R., Blomquist M.C., Seibel-Ross, E.I., Elzanowski, A., Bair, J.K., **Lewis, M.T.**, Marzec, C.R., Davalos, D.P. and Ledley, R.S. National Biomedical Research Foundation, Washington, D.C. (Updated quarterly) (1986-1989).

**D. Research Support**

Title: Novel Gene Networks in Breast Development and Cancer, Project 4: The Ptc1 Hedgehog Receptor in Mammary Ductal Development and Progression to Neoplasia

Agency: NIH P01 CA30195

Role: PI (Osborne/Lewis)

Period: 4/1/04-3/31/09

The major goals of this large Program Project are to identify and characterize the role of novel genetic pathways, which are found to be important in the normal breast, in the pathogenesis and progression of human breast cancer. The goal of Project 4 is to determine the role of Ptc1 in these processes.

Title: Novel Gene Networks in Breast Development and Cancer, Animal Handling and Imaging Core

Agency: NIH P01 CA30195 (Osborne/Lewis)

Role: PI

Period: 4/1/04-3/31/09

The major goals of this large Program Project are to identify and characterize the role of novel genetic pathways, which are found to be important in the normal breast, in the pathogenesis and progression of human breast cancer. The goal of Core C is to provide for purchase and housing of animals, and to provide specialized surgical and imaging support for all 5 projects included in this Program Project.

Title: Hedgehog Signal Transduction Inhibitors in Breast Cancer Treatment and Prevention

Agency: Department of Defense (IDEA) 17 00 1 0477

Role: PI

Period: 7/1/00-6/30/05

The major goals of this project are to determine whether constitutive activation of hedgehog signaling can lead to mammary lesions in transgenic mice using an activated form of *Smo* that signals independently of hedgehogs and is unresponsive to *Ptc-1* inhibition, to test the *in vivo* effect of specific hedgehog protein inhibitors on hedgehog network-induced lesions and the normal mammary

gland, to test the *in vivo* effect of specific hedgehog protein inhibitors on hedgehog-independent lesions, and to test the effect of hedgehog inhibitors on the growth and morphology of human breast cancer cell lines *in vitro*.

Title: SPORE in Breast Cancer-Project 5: Genetic Expression Profile of Taxotere Versus AC Sensitivity

Agency: NIH (P50 CA58183)

Role: Co-Investigator

Period: 12/1/02-11/30/07

The main goal of this project is to identify, confirm and validate prospectively and retrospectively, two genetic pathways involved in the sensitivity and resistance of the two main treatment regimens in breast cancer, Taxotere (T) and Adriamycin plus cyclophosphamide (AC).

Title: Combining cell and gene therapy for treatment of early stage breast cancer

Agency: Department of Defense (DAMD17-03-1-0571)

Role: PI

Period: 7/1/03-8/31/05

The goal of this project is to define conditions under which genetically modified cells will persist when reintroduced to the mammary gland.

Title: Unmasking Stem/Progenitor Cell Properties Using Short-Term Transplantation

Agency: Department of Defense

Role: PI

Period: 6/30/05-6/29/06

The goal of this project is to unmask stem cell behaviors in differentiated cells using transplantations.

Title: Development of an Intraductal Cell and Gene Therapy Approach for Treatment of Early Stage Breast Cancer

Agency: Susan Love MD Breast Cancer Research Foundation

Role: PI

Period: 7/1/03-6/30-04

The goal of this pilot project is to perform a "proof of principle" experiment to determine whether a patient's own breast cells can be removed, genetically modified to perform a therapeutic function, and reintroduced intraductally to survive long-term to combat cancer.

Title: Induction of mammary cancer by signaling molecules

Agency: NCI (R01 CA85736 Anderson, PI)

Role: Co-Investigator

Period: 4/1/00- 3/31/05

The major goals of this project are to determine whether constitutive activation of either the prolactin receptor or one of its downstream effectors (Akt) will contribute to neoplastic progression or developmental defects in the mouse mammary gland.

Title: Functional Development of the Mammary Gland

Agency: NIH (PO1 HD38129 Neville, PI)

Role: Co-Project Leader/Animal Core Director

Period: 7/1/00-6/30/01 (6/30/05)

\*Before relocating to Baylor College of Medicine, Dr. Lewis devoted 20% time as Co-Principal Investigator with Dr. Dean Edwards (UCHSC Department of Pathology) on a project to define the mechanisms of inhibition of milk secretion by progesterone during pregnancy and 20% time as the Animal Core Director for the program project group. He continues to collaborate with the group from the University of Colorado.