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<b>13. ABSTRACT (Maximum 200 words)</b>  The UCLA/USC Tissue Bank was established to provide research investigators with a ready source of human tissue samples for basic and clinical research projects directed at a greater understanding of various aspects of human breast cancer. The bank has been successful at accruing some 1020 tissue samples to 5 of the 6 subcomponent banks comprising the main bank. Of these tissues, 371 are breast cancer specimens while the remaining represent specimens from premalignant and non-malignant breast tissue, normal breast tissue, normal non-breast tissue and bone marrow and peripheral stem cells from patients with active breast cancer. Over the course of the past 12 months some 13 investigators have utilized material from this bank. In the coming year we expect expanded utilizing of the bank by a greater number of investigators as well as continued accrual to the bank.  <p style="text-align: right;"><b>DTIC QUALITY INSPECTED 3</b></p>			
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## INTRODUCTION

The nature of the problem addressed by this infrastructure core resource as well as the background of our previous work and the purpose of the present work remains unchanged from the initial grant application. These issues are therefore, restated in this Progress Report submission as originally stated in the initial proposal.

We have had considerable experience in the establishment and utilization of human tumor tissue banks. Prior to 1984, most studies of alterations of human genes had been performed on established cell lines. In 1984, we published the first study using actual human tumor tissue as well as adjacent normal tissue to evaluate differential expression patterns of proto-oncogenes in human malignancies (1). We have had NCI sponsored funding to pursue these types of studies since that initial description and as a result have established and/or utilized human tumor tissue banks to evaluate a number of proto-oncogenes (2-10) and tumor suppressor genes (11-14). These tissue banks have been used by us not only to identify genetic alterations associated with specific human malignancies, but also to provide macromolecules for the cloning of human proto-oncogenes (2,3,5) as well as the initial identification of the proteins encoded by some proto-oncogenes (2,3) and the partial characterization of these and other proto-oncogene proteins (2,3,5,6).

Three of the above mentioned screening studies dealt specifically with human breast cancer (4,5,8) and one of those studies utilized messenger RNA from the tissue bank to clone and characterize a full length c-DNA clone of the HER-2/neu gene (5). This clone has subsequently been used in basic science studies evaluating the role of HER-2/neu amplification and overexpression in the pathogenesis of human breast cancer, underscoring the fact that translational elements can go from clinical material to basic science studies as well as vice-versa. Many investigators have developed banks consisting of hundreds or thousands of samples. Several of these banks, however, may have limitations which restrict the practical utilization of specimens for the development of new reagents and/or new molecular approaches. Frequently tissue banks consist of paraffin-embedded specimens which are appropriate for pathologic and some types of molecular studies but which may have limited use for the analysis of other genetic alterations or protein expression studies. Even though methods now exist for extracting DNA and RNA from paraffin embedded material, these macromolecules are almost always degraded to a greater or lesser extent as a result of the fixation and/or embedding process. PCR technology allows for the assessment of specific sites or regions of genes, however molecular methods requiring high molecular weight DNA or RNA species such as c-DNA cloning or analyses of restriction fragment length polymorphisms (RFLP's) of large DNA segments may be problematic. In

addition, the fixation process can alter the antigenicity of some proteins of interest making immunohistochemical analyses with many antibodies difficult if not impossible. Other banks consist of specimens which are not fixed but stored frozen and while this circumvents some of the previously stated problems, there are frequently other potential problems with such samples. Many of these specimens are stored after other studies have been performed, most notably steroid hormone receptor analyses. As such, some exist as cytosolic preparations or nuclear pellets. Again this presents limitations on the integrity of high molecular weight species of RNA and DNA and such fractionated samples cannot be assessed by immunohistochemistry. Finally, many of these types of specimens have variable clinical and/or follow up data.

Given the nature of the required studies done on breast biopsies and specimens removed for therapeutic purposes, as well as the trend toward earlier diagnosis and thus smaller primary lesions, there are practical limitations to the amount of frozen tissue that can be obtained for a tissue bank. Still, it should be an important objective for any well constructed tissue bank to have significant numbers of specimens which contain sufficient material and appropriate follow-up to circumvent some of the above problems. The ability to perform a comprehensive analysis (ie at the DNA, RNA and protein levels) on a given gene or genetic alteration in an appropriate number of specimens is critical. Our own experience with the human HER-2/neu gene is that this type of analysis can be central to resolving important questions regarding molecular alterations in breast cancer (5) and serves as an example for the necessity of banks which can support comprehensive analyses of a gene or gene product of interest in some specimens. Alteration of the Her-2/neu gene is now known to be of potential biologic and clinical importance in some human breast cancers. However, after publication of our initial paper reporting the correlation of HER-2/neu gene amplification with outcome in breast cancer (4), a number of other reports were published; some confirming and others refuting our data (for a review see discussion in reference 8). The purpose of our subsequent study (5) was not only to examine sufficient numbers of cases to address this issue more completely, but also to critically evaluate the methodologic concerns that might account for discrepancies between studies in the literature. To accomplish this, we performed a comprehensive analysis of 187 tumors that were of sufficient size to obtain DNA, RNA and protein, as well as frozen and fixed material for immunohistochemistry. This part of the study was designed to address several critical issues regarding the HER-2/neu gene in human breast cancer. First, the correlation between a given level of gene amplification and relative expression for both RNA and protein needed to be addressed, since it is known that some genes which are amplified in breast cancer are not expressed (15). These genes may serve as useful markers, but are unlikely to be involved in pathogenesis of the disease. Second, the issue of the specificity of the alteration within a heterogeneous tumor tissue also

needed to be addressed; i.e. were the alterations specific to the tumor cells versus non-tumor cells in the specimen. Solid matrix blotting techniques (Southern, Northern or Western blot analyses) cannot distinguish signals from tumor versus normal cells, and as a result introduce dilutional artifacts which may obscure important findings in the tumor cells. Third, the comprehensive approach was taken to gain some insight into the relative strengths and weaknesses of the various methods used in assessing alterations in the HER-2/neu gene in human tissues. Fourth, since gene expression was found to correlate closely with amplification in this study, it provided an independent assessment of the accuracy of the cases determined by Southern analysis to be amplified.

The relevance of this study for us was not only that it confirmed the observed HER-2/neu amplification rate of 25% and the correlation with clinical outcome, but of equal importance it demonstrated important methodologic considerations which may be operational when measuring any gene or gene product in human breast cancer specimens consisting of heterogeneous cell populations. (5). These considerations are not restricted to HER-2/neu amplification and overexpression. Indeed a comprehensive approach can address several issues such as the resultant change (if any) in messenger RNA or protein size associated with alterations occurring at the DNA level. In addition, it can allow for assessment of changes in expression which may not be accompanied by gross changes at the DNA level. It can also allow for the evaluation of whether specific antibody reagents perform more optimally in frozen or fixed tissue. Finally, and perhaps most importantly, it allows for a determination of the optimal methodologic approach for assessing a change in a gene, gene product or pathway in actual human breast cancer specimens. In addition, comprehensive processing of samples will facilitate isolation of macromolecules which can be used in the generation of reagents for basic science studies, i.e. full length c-DNA and/or genomic clones. Such reagents will have been derived from tumor tissue rather than cell lines, insuring that the resultant clones are representative of genes occurring in actual human tumors rather than cell lines. This circumvents the possibility that any observed alteration may be the result of continuous propagation of cells in vitro. While it is clear that practical considerations will prohibit having sufficient material to perform this type of processing on all specimens stored in a bank, it will be the objective of the UCLA/USC Breast Tissue Bank to have sufficient numbers of specimens in each bank component to allow for a comprehensive analysis of any genes of interest in a large cohort of the samples. We have provided the above narrative not to detail our accomplishments but to convince the reviewers that we have had extensive experience in establishment and utilization of appropriate tissue bank resources for the conduct of relevant translational clinical research projects involving human malignancies and specifically breast cancer.

**BODY**

The UCLA/USC Tissue Bank is actually made up of 6 sub-component banks which include:

- a) Malignant Breast Tissue Component
- b) Pre-malignant/Non-malignant Breast Tissue Component
- c) Normal Breast Tissue Component
- d) Fat Tissue Component
- e) Normal Non-Breast Tissue Component
- f) Bone Marrow/Peripheral Blood Stem Cell Component

**I Accrual**

Accrual to all but one of these component parts of the bank has been excellent. The only subcomponent which has not yet accrued well is the Fat Tissue Component. The accrual to the various other components of the bank over the past 12 months is as follows

- a) Malignant Breast Tissue Component  
A total of 371 new specimens have been accrued to this component of the bank. Of these 52 are DCIS while 319 are invasive carcinomas
- b) Premalignant/non-malignant Breast Tissue Component  
A total of 26 premalignant and non-malignant samples have been collected including breast specimens from biopsies which demonstrate various non-malignant abnormalities including atypical ductal hyperplasias, fibroadenomas etc.
- c) Normal Breast Tissue Component  
A total of 48 normal breast tissue samples from reduction mammoplasties have been accrued to the bank.
- d) Fat Tissue Component  
No tissue has yet been accrued to this portion of the bank due to the fact that we have not yet set up the appropriate storage unit for long term storage of fatty acids. This storage facility will be set up within the next 6 months.
- e) Normal and Non-breast Tissue Component  
A total of 439 tissues have been accrued to the normal and non-breast tissue component of the bank. The break down of these tissues by classification of benign and malignant diagnosis is as follows:

	<u>BENIGN</u>	<u>MALIGNANT</u>
Ovary	39	9
Brain	2	12
Prostate	34	16
Colon	62	58
Rectum		2
Uterus	67	7
Thyroid	13	13
Bladder	3	3
Spleen	4	4 (Metastases)
Lymph Nodes	18	18 (Metastases)
Parotid Gland	3	3
Salivary Gland	1	1
Stomach	8	7
Skin	1	7
Soft Tissue	4	
Testis	6	2
Tongue	1	1

- f) Bone Marrow/Peripheral Blood Stem Cell Component  
There have been a total of 136 specimens accrued to this portion of the bank

## II Utilization

The UCLA/USC Tissue Bank has had a significant utilization over the past 12 months. This productivity is detailed by the list below which includes the investigators who have utilized this resource as well as their funding and the title of the research project they have completed and/or received funding for

Michael E. Press, MD, PhD:

"Pathobiology of Breast Cancer and Hormone Receptors", NCI:2 RO1-CA48780, 1993-1998.

Specimens provided: 215 Breast cancer specimens

Darryl Shibata, MD.

"Genetic Evidence of Early Breast Cancer," funded by the U.S. Army Medical Research & Development Command, DAMD17-94-J-4232, 1994-1996

Specimens Received: 20 paired tumor - normal frozen tissue specimens

Darryl Shibata, MD.

"Biology of Telomere Length Conservation in Breast Cancer." funded by the California Breast Cancer Research Program, 1995-1996

Specimens Received: 40 breast cancer specimens

Peter Daneberg, PhD.,

"Continuous 5-FU infusion and Concomitant Radiation for Locally Advanced Breast Cancer" NCI RO1 CA60899, 1994-1998.

Specimens provided: 22 frozen breast cancer specimens

Thomas Mack, MD.

"Genetic abnormalities in breast cancer tumors: Relationships to Environmental and Genetic Risk Factors Using Twins." U.S. Army Medical Research & Development Command, DAMD17-94-J-4290, 1994-1995

Specimens provided: 77 breast cancer specimens from twins

Franco Muggia, MD.

"Multidrug Resistance in Breast Cancer; Occurrence and Therapeutic Implications". U.S. Army Medical Research & Development Command, DAMD17-94-J-4321, 1994-1988

Specimens Received: 65 frozen breast cancer specimens

Richard Cote, MD. & John Glaspy, MD

National Cancer Institute P50-CA58197. "SPORE in Breast Cancer".

Specimens received: 400 peripheral blood stem cell and bone marrow specimens on 150 patients

Silvia Formenti MD:

"A pilot study of radiosurgery for T1 breast cancers: Clinical, physical and radiobiological correlates", funded from the NCCC Breast Cancer Research Program," NCI P20 CA66222, 1995-1996.

Specimens received: 25 breast cancer specimens

Amy Lee PhD.

"Application of stress protein gene system for drug resistance and gene therapy in breast cancer", funded from the NCCC Breast Cancer Research Program," NCI P20 CA66222, 1995-1996.

Specimens received: 20 frozen breast cancer specimens and 5 normal breast specimens.

Elizabeth Kreckler, MD.

"Breast Cancer in Young Women" (pilot project-not funded)

Specimens received: 89 breast cancer specimens.

Melvin J. Silverstein, MD.

Ductal Carcinoma in Situ of the Breast: A Proposal for a Morphologic Classification based on High Nuclear Grade and Comedo Type Necrosis.

Specimens received: 36 DCIS specimens as well as research personnel assistance in organizing 238 DCIS specimens from his private practice.

Dennis J. Slamon, MD., PhD

"Oncogenes in Physiologic and Pathologic States" NIH RO1 CA36827

Specimens received: 36 DCIS specimens for the collaboration with Dr. Silverstein

Richard J. Pietras, MD., PhD

"Growth Factor Receptor-Directed Therapy in Human Breast Cancer" U.S. Army Medical Research & Development Command, DAMD17-94-J-4370

Specimens received: 105 Breast cancer specimens

## CONCLUSIONS

We are very pleased that in the first 12 months of this funded infrastructure core there has been significant accrual to five of the six component parts of the bank as well as a significant utilization of this resource by 12 funded investigators in the area of breast cancer research. This includes investigations funded by not only the USAMRMC but also by the NIH and NCI. We anticipate that over the ensuing 12 months the accrual will continue at the current pace if not better and we will be able to implement the Fat Tissue component of the bank. We further anticipate increased utilization of the Bank as well as further publications resulting in part or in total from the existence of this critical core resource. This seems certain due to the fact that the resource has only been in existence in its current state for just under 12 months. It is likely that utilization of the Bank will continue to result in the potential development of new diagnostic, prognostic and therapeutic approaches to human breast cancer.

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