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13. ABSTRACT (Maximum 200 Words). This is a molecular epidemiologic case-control study of breast carcinoma <i>in situ</i> (CIS) in Los Angeles County designed to address issues related to the cause and progression of breast CIS by determining epidemiologic risk factors, characterizing selected molecular genetic alterations and prospectively assessing disease progression. The specific aims of the research are 1.) to assess epidemiologic risk factors associated with development of breast CIS, 2.) to determine how frequently specific oncogenes or the p53 tumor suppressor gene are altered in breast CIS, 3.) to investigate potential relationships between various epidemiologic risk factors and somatic genetic alterations and 4.) to assess long-term the association of these factors with disease progression. We have interviewed a total of 1599 women in connection with this investigation. 573 women with breast carcinoma <i>in situ</i> , 480 white women (including Hispanics) and 93 African-American women, have been interviewed. These women were 35-64 years old, diagnosed with breast CIS, residents of Los Angeles County, US-born, and English speaking. As described in our original proposal 1026 control women have been interviewed as part of the Women's CARE Study funded by the NICHD. The DOD has not provided funding for the control interviews and, hence, we have not reported them to the DOD in this progress report although we will use them for comparison purposes when we analyze the breast CIS case data at the conclusion of this study. We have obtained paraffin-embedded tumor tissue from the pathology laboratories for 323 breast CIS patients. We have performed immunohistochemical assays for estrogen receptor, progesterone receptor, HER-2/ <i>neu</i> oncoprotein, p53 tumor suppressor protein and cyclin D1 in these cases. Comparative analysis of the data will be performed at the conclusion of the study when all of the assays have been completed; however, a preliminary analysis was performed with available case data for the purpose of presentation to the DOD to demonstrate our progress.				
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**INTRODUCTION:** This is a molecular epidemiologic case-control study of breast carcinoma *in situ* in Los Angeles County designed to address issues related to the cause and progression of breast CIS by determining epidemiologic risk factors, characterizing selected molecular genetic alterations and prospectively assessing disease progression.

Breast carcinoma *in situ* (CIS) is a significant public health problem in Los Angeles County and throughout the United States. Incidence of this disease has been rising steadily, with little epidemiologic or biologic information about breast CIS available. In Los Angeles county, incidence of breast CIS increased from approximately 4 cases per 100,000 women in 1980 to 15 cases per 100,000 women in 1992.

The specific aims of the research are 1.) to assess epidemiologic risk factors associated with development of breast CIS, 2.) to determine how frequently specific oncogenes or the p53 tumor suppressor gene are altered in breast CIS, 3.) to investigate potential relationships between various epidemiologic risk factors and somatic genetic alterations and 4.) to assess long-term the association of these factors with disease progression.

During the four-year grant period we planned to interview approximately 100 black women and 426 white women (including Hispanics) aged 35-64 years who are diagnosed with breast CIS and who are residents of Los Angeles County, are US-born, and English speaking. The study will utilize 490 black and 490 white control subjects selected by random digit dialing in Los Angeles County who will have been interviewed as part of the Women's CARE Study, a multicentered case-control study of invasive breast cancer being conducted concurrently with this proposed study and funded independently by the NICHD. We proposed obtaining paraffin-embedded tumor tissue from the pathology laboratories where the patients were diagnosed. This case-control study was designed to evaluate reproductive history (early menarche, late menopause, nulliparity or late first birth), lack of participation in physical activity/exercise, positive family history, race (white vs. black), high body mass and exposure to exogenous hormones (oral contraceptives and estrogen or combined estrogen/progestogen replacement therapy) for associations with an increased risk of developing breast CIS. The tumor tissue is being evaluated for HER-2/*neu* and PRAD1 (cyclin D1) gene amplification by fluorescence *in situ* hybridization and for expression by immunohistochemistry. Estrogen receptor and progesterone receptor protein expression is being evaluated in tumor tissue. The p53 tumor suppressor gene, known to be mutated and/or overexpressed in approximately 30% of invasive breast cancers, is being characterized for p53 overexpression by immunohistochemistry. We will determine if any of these molecular characteristics of breast CIS or epidemiologic characteristics are associated with an increased risk of recurrence or progression to invasive disease.

#### **PROGRESS DURING YEAR 04.**

#### **EXPERIMENTAL METHODS AND PROCEDURES.**

##### **1. Epidemiologic Study Methods.**

#### **STATEMENT OF WORK.**

##### **Technical Objectives:**

**Task 1.** Identify and interview 100 African American and 426 white women with breast CIS in Los Angeles County during the first three years of the grant period. A previously tested epidemiologic questionnaire from the Women's CARE Study will be used to interview women entered in this CIS study.

**Task 2.** Identify and interview 490 African American and 490 white control women in Los Angeles County during the first three years of the grant period. These control women will be identified from the controls entered in the Women's CARE Study and interviewed as part of that study.

**Task 3.** Use an epidemiologic interview instrument to determine reproductive history including menarche, menopause, and pregnancy history, participation in physical activity / exercise, family history of breast cancer, race (white vs. black), body mass, and exposure to exogenous hormones (oral contraceptives and estrogen or combined estrogen/progestogen replacement therapy).

**Task 4.** Tissue blocks and slides will be obtained from the hospital laboratory where the diagnosis of breast CIS was made. The histopathology of each case will be reviewed and characterized.

**Task 5.** Gene amplification and expression of HER-2/*neu* and cyclin D1 will be evaluated in CIS, atypical hyperplasia, breast duct proliferative epithelium and normal epithelium using fluorescence *in situ* hybridization and immunohistochemistry.

**Task 6.** Alterations in p53 expression and *p53* gene will be assessed in breast CIS, atypical hyperplasia, breast duct proliferative epithelium and normal epithelium using immunohistochemistry and a combination of SSCP and DNA sequencing.

**Task 7.** The frequency of alterations in the above oncogenes and p53 tumor suppressor gene will be compared with each other and with the epidemiologic data to assess the frequency of various associations. For example, how often is a history of birth control pill use associated with alterations of any of these genes in breast CIS cells?

**Task 8.** Use continued followup through the USC Cancer Surveillance Program and through annual contacts with patients and their physicians to determine how frequently women in the study develop recurrent or invasive breast cancer and assess how often these events are associated with particular genetic alterations in breast CIS.

We have made significant progress with each of the tasks. Our progress with each is summarized as follows:

Task 1. Identify and interview 100 African American and 426 white women with breast CIS in Los Angeles County during the first three years of the grant period. A previously tested epidemiologic questionnaire from the Women's CARE Study will be used to interview women entered in this CIS study. 813 women were diagnosed with breast carcinoma *in situ* in Los Angeles County during the study interval and were eligible for this study. 573 women with breast CIS were interviewed; 480 were white (including Hispanic) and 93 were African-American (Tables 1, 2 and 3). 242 did not participate in the study for the following reasons: 3 were mentally or physically disabled, 95 had their physician refuse permission for participation, 15 moved out of Los Angeles, 91 refused participation and 38 were not available. These women were 35-64 years old, diagnosed with breast CIS, residents of Los Angeles County, US-born, and English speaking. Although we have interviewed seven fewer (93 versus 100) African-American women than proposed, overall we have interviewed the number of breast carcinoma *in situ* patients that we had proposed. Our shortfall for black women is because they are rarely diagnosed with *in situ* tumors. We interviewed only during the time that the Women's CARE Study was fielded because we required controls to have been recruited and interviewed concurrently with our cases. We consider this task to be completed.

Task 2. Identify and interview 490 African American and 490 white control women in Los Angeles County during the first three years of the grant period. These control women will be identified from the controls entered in the Women's CARE Study and interviewed as part of that study. 1026 control women have been interviewed as part of the Women's CARE Study funded by the NICHD as described in our original proposal. The DOD has not provided funding for the control interviews, however, we will use them for comparison purposes when we analyze the breast CIS case data (Tables 1, 2 and 3). This task is completed.

Table 1.

## BREAST CARCINOMAIN SITU DATA: DISTRIBUTION OF AGE AT DIAGNOSIS

## TABLE OF STATUS BY REFERENCE AGE

STATUS (CASE-CONTROL)	REFAGE (REFERENCE AGE-YEARS)							Total	
	Frequency	Percent	Row Pct	Col Pct	35-39	40-44	45-49		50-54
CASE	32	74	109	147	114	97	573		
	2.00	4.63	6.82	9.19	7.13	6.07	35.83		
	5.58	12.91	19.02	25.65	19.90	16.93			
	14.61	26.81	40.52	46.52	41.45	39.75			
CNTRL	187	202	160	169	161	147	1026		
	11.69	12.63	10.01	10.57	10.07	9.19	64.17		
	18.23	19.69	15.59	16.47	15.69	14.33			
	85.39	73.19	59.48	53.48	58.55	60.25			
Total	219	276	269	316	275	244	1599		
	13.70	17.26	16.82	19.76	17.20	15.26	100.00		

Table 2.

BREAST CARCINOMAIN SITU DATA: DISTRIBUTION OF AGE AT DIAGNOSIS BY RACE  
RACIAL ORIGIN = WHITE

## TABLE OF STATUS BY REFAGE FOR WHITE WOMEN

STATUS (CASE-CONTROL)	REFAGE (REFERENCE AGE-YEARS)							Total	
	Frequency	Percent	Row Pct	Col Pct	35-39	40-44	45-49		50-54
CASE	25	62	93	122	99	79	480		
	2.35	5.82	8.72	11.44	9.29	7.41	45.03		
	5.21	12.92	19.38	25.42	20.63	16.46			
	18.38	37.80	51.96	57.01	51.56	43.65			
CNTRL	111	102	86	92	93	102	586		
	10.41	9.57	8.07	8.63	8.72	9.57	54.97		
	18.94	17.41	14.68	15.70	15.87	17.41			
	81.62	62.20	48.04	42.99	48.44	56.35			
Total	136	164	179	214	192	181	1066		
	12.76	15.38	16.79	20.08	18.01	16.98	100.00		

Table 3.  
BREAST CARCINOMAIN SITU DATA: DISTRIBUTION OF AGE AT DIAGNOSIS BY RACE  
RACIAL ORIGIN = BLACK

TABLE OF STATUS BY REFAGE FOR BLACK WOMEN

STATUS (CASE-CONTROL)	REFAGE (REFERENCE AGE-YEARS)							Total
	35-39	40-44	45-49	50-54	55-59	60-64		
Frequency								
Percent								
Row Pct								
Col Pct								
CASE	7	12	16	25	15	18		93
	1.31	2.25	3.00	4.69	2.81	3.38		17.45
	7.53	12.90	17.20	26.88	16.13	19.35		
	8.43	10.71	17.78	24.51	18.07	28.57		
CNTRL	76	100	74	77	68	45		440
	14.26	18.76	13.88	14.45	12.76	8.44		82.55
	17.27	22.73	16.82	17.50	15.45	10.23		
	91.57	89.29	82.22	75.49	81.93	71.43		
Total	83	112	90	102	83	63		533
	15.57	21.01	16.89	19.14	15.57	11.82		100.00

Task 3. Use an epidemiologic interview instrument to determine reproductive history including menarche, menopause, and pregnancy history, participation in physical activity / exercise, family history of breast cancer, race (white vs. black), body mass, and exposure to exogenous hormones (oral contraceptives and estrogen or combined estrogen/progestogen replacement therapy). These epidemiologic interviews have been performed on both cases and controls as summarized in Task 1 and Task 2 above. The information has been entered into a database for future comparison purposes with the results of tissue analyses (Tasks 5 and 6).

Task 4. Tissue blocks and slides will be obtained from the hospital laboratory where the diagnosis of breast CIS was made. The histopathology of each case will be reviewed and characterized. We have obtained paraffin-embedded tumor tissue from the pathology laboratories of 323 breast CIS patients. 266 tissue blocks have been obtained from white women and 57 have been obtained from African-American women. Hematoxylin and eosin tissue sections have been prepared from each tissue block and assessed for histopathology to confirm the presence of breast CIS. Among these breast CIS cases the following intraductal histologic phenotypes were identified: comedocarcinoma (29%), solid (22%), cribriform (20%), micropapillary (19%), and papillary (12%) DCIS. 8% of the breast CIS cases were lobular carcinoma *in situ* and 11% were of mixed histologic types. Tissue blocks from the remaining 250 women with breast CIS have been requested and are currently pending.

Task 5. Gene amplification and expression of HER-2/*neu* and cyclin D1 will be evaluated in CIS, atypical hyperplasia, breast duct proliferative epithelium and normal epithelium using fluorescence *in situ* hybridization and immunohistochemistry. We have performed immunohistochemical assays for HER-2/*neu* oncoprotein and cyclin D1 in 315 of the 323 cases. In addition to analysis of HER-2/*neu* and cyclin D1 proposed in the original application we have also analyzed the estrogen receptor and progesterone receptor content in 309 and 323, respectively,

of these breast CIS cases. The ER and PR data provides an important baseline comparison for these standard markers used in evaluating invasive breast cancers. These results are summarized below in Table 4.

Table 4. Protein Expression in Breast Carcinoma *In Situ* from African-American and White Women.

<u>Protein Product</u>	<u>Black</u>	<u>White</u>	<u>Fisher's Exact Test</u>
ER+ (n = 237)	42 (77.8%)	195 (76.5%)	P = 1.0
ER- (n = 72)	12 (22.2%)	60 (23.5%)	
PR+ (n = 234)	36 (63.2%)	198 (74.4%)	P = 0.10
PR- (n = 89)	21 (36.8%)	68 (25.6%)	
HER-2 High (n = 81)	16 (28.1%)	65 (25.2%)	P = 0.74
HER-2 Low (n = 234)	41 (71.9%)	193 (74.8%)	
P53 Overexp (n = 57)	6 (10.5%)	51 (19.3%)	P = 0.13
P53 Low exp (n = 264)	51 (89.5%)	213 (80.7%)	
Cyclin D1 Hi (n = 81)	12 (30.8%)	52 (30.8%)	P = 1.0
Cyclin D1 Lo (n = 234)	27 (69.3%)	117 (69.2%)	

Expression of estrogen receptor, progesterone receptor, HER-2/*neu* and cyclin D1 proteins showed no statistically significant association with African-American or white racial grouping.

Task 6. Alterations in p53 expression and p53 gene will be assessed in breast CIS, atypical hyperplasia, breast duct proliferative epithelium and normal epithelium using immunohistochemistry and a combination of SSCP and DNA sequencing. We have performed immunohistochemical assays for P53 in 321 cases of the currently available tissue sections as summarized above (Table 4). Expression of p53 protein showed no statistically significant association with African-American or white racial groupings.

Task 7. The frequency of alterations in the oncogenes and p53 tumor suppressor gene will be compared with each other and with the epidemiologic data to assess the frequency of various associations. For example, how often is a history of birth control pill use associated with alterations of any of these genes in breast CIS cells? Analysis of the data will be performed at the conclusion of the study when all of the assays have been completed, however, a preliminary analysis was performed with available case data for the purpose of presentation at the DOD Era of Hope meeting (June 9, 2000) and is summarized here to demonstrate our progress.

Estrogen receptor expression in breast carcinoma *in situ* was associated with increasing age, PR positivity and a history of hormone replacement therapy (Table 5). ER expression in breast CIS was inversely associated with increasing nuclear grade and inversely associated with HER-2/*neu* and P53 expression. No correlation was observed with race, family history of breast cancer, family history of ovarian cancer, age at menarche, history of oral contraceptive use, pregnancy history, menstrual status, smoking status, body mass index or cyclin D1 expression (data not shown).

Table 5. Comparison of Estrogen Receptor Status with Age, Nuclear Grade, Expression of Other Proteins and History of Hormone Replacement Therapy in Breast Carcinoma *In Situ* .

<u>Characteristic</u>	<u>ER-Negative</u>	<u>ER-Positive</u>	<u>Fisher's Exact Test</u>
Age			
35-39	6 (8.3%)	12 (5.1%)	P = 0.035
40-44	12 (16.7%)	28 (11.8%)	
45-49	17 (23.6%)	38 (16.0%)	
50-54	17 (23.6%)	60 (25.3%)	
55-59	12 (16.7%)	49 (20.7%)	
60-64	8 (11.1%)	50 (21.1%)	
Nuclear Grade			
0-1	4 (5.6%)	46 (19.6)	P < 0.001
2	21 (29.6%)	152 (64.7%)	
3	46 (64.8%)	37 (15.7%)	
Unknown (n = 3)			
PR Status			
Negative	58 (80.6%)	30 (12.7%)	P < 0.001
Positive	14 (19.4%)	207 (87.3%)	
HER-2/ <i>neu</i>			
Low	29 (40.9%)	190 (83.0%)	P < 0.001
Overexpression	42 (59.1%)	39 (17.0%)	
P53			
Low	48 (66.7%)	202 (86.3%)	P < 0.001
Overexpression	24 (33.3%)	32 (13.7%)	
Ever used Hormone Replacement Therapy			
No	46 (63.9%)	110 (46.4%)	P = 0.011
Yes	26 (36.1%)	127 (53.6%)	

Progesterone receptor expression in breast carcinoma *in situ* was associated with increasing age, with ER positivity and with a history of estrogen replacement therapy and hormone replacement therapy (Table 6). PR was inversely associated with increasing nuclear grade and inversely associated with HER-2/*neu* and P53 expression. No correlation was observed with race, family history of breast cancer, family history of ovarian cancer, age at menarche, use of oral contraceptives, pregnancy history, menstrual status, smoking status, body mass index or cyclin D1 expression (data not shown).

Table 6. Comparison of Progesterone Receptor Status with Age, Nuclear Grade, Expression of Other Proteins and History of Hormone Replacement Therapy in Breast Carcinoma *In Situ*.

<u>Characteristic</u>	<u>PR-Negative</u>	<u>PR-Positive</u>	<u>Fisher's Exact Test</u>
Age			
35-39	8 (9.0%)	11 (4.7%)	P = 0.001
40-44	12 (13.5%)	28 (12.0%)	
45-49	15 (16.9%)	42 (18.0%)	
50-54	21 (23.6%)	61 (26.1%)	
55-59	21 (23.6%)	43 (18.4%)	
60-64	12 (13.5%)	49 (20.9%)	
Nuclear Grade			
0-1	6 (6.9%)	50 (21.6)	P < 0.001
2	36 (41.4%)	144 (64.7%)	
3	45 (51.7%)	38 (16.4%)	
Unknown (n = 4)			
ER Status			
Negative	58 (65.9%)	14 (6.3%)	P < 0.001
Positive	30 (34.1%)	207 (93.7%)	
Unknown (n = 14)			
HER-2/ <i>neu</i>			
Low	42 (47.2%)	191 (84.9%)	P < 0.001
Overexpression	47 (52.8%)	34 (15.1%)	
Unknown (n = 9)			
P53			
Low	60 (67.4%)	202 (87.8%)	P < 0.001
Overexpression	29 (32.6%)	28 (12.2%)	
Unknown (n = 4)			
Ever used Estrogen Replacement Therapy			
No	76 (85.4%)	163 (70.0%)	P = 0.004
Yes	13 (14.6%)	70 (30.0%)	
Unknown (n = 1)			
Ever used Hormone Replacement Therapy			
No	51 (57.3%)	107 (45.7%)	P = 0.08
Yes	38 (42.7%)	127 (54.3%)	

HER-2/*neu* overexpression in breast carcinoma *in situ* was associated with increasing nuclear grade, with P53 overexpression and with a history of full-term pregnancy (Table 7). HER-2/*neu* overexpression was inversely associated with ER and PR positivity (data not shown). No correlation was observed with race, family history of breast cancer, family history of ovarian cancer,

history of oral contraceptive use, menstrual status, history of hormone replacement therapy, smoking status, body mass index or cyclin D1 expression (data not shown).

Table 7. Comparison of HER-2/*neu* Status with Age, Nuclear Grade, Expression of Other Proteins and Pregnancy History in Breast Carcinoma *In Situ* .

<u>Characteristic</u>	<u>HER-2/<i>neu</i> Low</u>	<u>HER-2/<i>neu</i> Over</u>	<u>Fisher's Exact Test</u>
Age			
35-39	15 (6.4%)	3 (3.7%)	P < 0.001
40-44	27 (11.5%)	12 (14.8%)	
45-49	44 (18.8%)	11 (13.6%)	
50-54	58 (24.8%)	23 (28.4%)	
55-59	45 (19.2%)	18 (22.2%)	
60-64	45 (19.2%)	14 (17.3%)	
Nuclear Grade			
0-1	49 (21.3%)	3 (3.7%)	P < 0.001
2	149 (64.8%)	27 (33.3%)	
3	32 (13.9%)	51 (63.0%)	
Unknown (n = 4)			
ER Status			
Negative	29 (13.2%)	42 (51.9%)	P < 0.001
Positive	190 (86.8%)	39 (48.1%)	
Unknown (n = 15)			
PR Status			
Negative	42 (18.0%)	47 (58.0%)	P < 0.001
Positive	191 (82.0%)	34 (42.0%)	
Unknown (n = 1)			
ER/PR Status			
ER+, PR+	171 (78.1%)	28 (34.6%)	P < 0.001
ER+, PR-	19 (8.7%)	11 (13.6%)	
ER-, PR+	7 (3.2%)	6 (7.4%)	
ER-, PR-	22 (10.1%)	36 (44.4%)	
Unknown (n = 15)			
P53			
Low	195 (84.4%)	59 (72.8%)	P = 0.02
Overexpression	35 (15.2%)	22 (27.2%)	
Unknown (n = 4)			
Ever Full-term Pregnancy			
Never pregnant	38 (16.2%)	6 ( 7.4%)	P = 0.07
Ever pregnant, no FTP	28 (12.0%)	7 ( 8.6%)	
Yes	168 (71.8%)	68 (84.0%)	

P53 overexpression in breast carcinoma *in situ* was associated with increasing nuclear grade and HER-2/*neu* overexpression but was inversely associated with ER and PR positivity (Table 8). No correlation was observed with race, family history of breast cancer, family history of ovarian cancer, history of oral contraceptive use, age at menarche, pregnancy history, menstrual status, history of hormone replacement therapy, body mass index or expression of other proteins (data not shown).

Table 8. Comparison of P53 Status with Age, Nuclear Grade and Expression of Other Proteins in Breast Carcinoma *In Situ*.

Characteristic	P53 Low	P53 Over	Fisher's Exact Test
Age			
35-39	17 (6.4%)	2 (3.5%)	P < 0.001
40-44	31 (11.7%)	9 (15.8%)	
45-49	42 (15.9%)	14 (24.6%)	
50-54	67 (25.4%)	14 (24.6%)	
55-59	56 (21.2%)	9 (15.8%)	
60-64	51 (19.3%)	9 (15.8%)	
Nuclear Grade			
0-1	51 (19.6%)	3 (5.4%)	P = 0.020
2	155 (59.6%)	24 (42.9%)	
3	54 (20.8%)	29 (51.8%)	
Unknown (n = 4)			
ER Status			
Negative	48 (19.2%)	24 (42.9%)	P < 0.001
Positive	202 (80.8%)	32 (57.1%)	
Unknown (n = 15)			
PR Status			
Negative	60 (22.9%)	29 (50.9%)	P < 0.001
Positive	202 (77.1%)	28 (49.1%)	
Unknown (n = 2)			
ER/PR Status			
ER+, PR+	177 (70.8%)	27 (48.2%)	P < 0.001
ER+, PR-	25 (10.0%)	5 (8.9%)	
ER-, PR+	13 (5.2%)	1 (1.8%)	
ER-, PR-	35 (14.0%)	23 (41.1%)	
Unknown (n = 15)			
HER-2/ <i>neu</i>			
Low	195 (76.8%)	35 (61.4%)	P = 0.020
Overexpression	59 (23.2%)	22 (38.6%)	
Unknown (n = 10)			

Cyclin D1 expression in breast carcinoma *in situ* was associated with increasing age and a history of smoking (Table 9). No correlation was observed with race, family history of breast cancer, family history of ovarian cancer, history of oral contraceptive use, age at menarche, history of oral contraceptive use, pregnancy history, menstrual status, history of hormone replacement therapy, body mass index or expression of other proteins.

Table 9. Comparison of Cyclin D1 Status with Age and Smoking Status in Breast Carcinoma *In Situ*.

<u>Characteristic</u>	<u>Cyclin D1 High</u>	<u>Cyclin D1 Low</u>	<u>Fisher's Exact Test</u>
Age			P < 0.001
35-39	5 ( 7.8%)	10 ( 6.9%)	
40-44	12 (18.8%)	14 ( 9.7%)	
45-49	12 (18.8%)	29 (20.1%)	
50-54	12 (18.8%)	37 (25.7%)	
55-59	7 (10.9%)	30 (20.8%)	
60-64	16 (25.0%)	24 (16.7%)	
Smoking Status			P = 0.002
Never Smoked	34 (53.1%)	63 (43.8%)	
Former Smoker	15 (23.4%)	66 (45.8%)	
Current Smoker	15 (23.4%)	15 (10.4%)	

Task 8. Use continued followup through the USC Cancer Surveillance Program and through annual contacts with patients and their physicians to determine how frequently women in the study develop recurrent or invasive breast cancer and assess how often these events are associated with particular genetic alterations in breast CIS. Annual followup of women entered in this study is continuing.

## Conclusions

### Key Accomplishments.

1. Obtained epidemiologic data from 572 women with breast carcinoma *in situ*.
2. Obtained epidemiologic data from 1026 women through our participation in the Women's CARE Study.
3. Obtained tissue blocks from 323 of the 572 women interviewed.
4. Performed immunohistochemical assays for ER, PR, HER-2/*neu*, P53 and cyclin D1 in breast carcinoma *in situ* tissues.
5. Demonstrated an association between ER status and a history of hormone replacement therapy in women with breast carcinoma *in situ*.
6. Demonstrated an association between PR status and either a history of estrogen replacement therapy or a history of hormone replacement therapy.
7. Demonstrated an association between HER-2/*neu* overexpression and a history of full-term pregnancy.
8. Demonstrated an association between cyclin D1 expression and a history of smoking.

Reportable Outcomes.

1. Establishment of a population-based breast carcinoma *in situ* epidemiologic database with associated tissue specimens.

2. Presentation: Press MF, Bernstein L. Molecular epidemiology of breast carcinoma *in situ*. Platform presentation and abstract, Era of Hope, Department of Defense Breast Cancer Research Program meeting, June, 2000.