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14. ABSTRACT Women with earlier stage breast cancer who receive breast conserving surgery and radiation therapy have a generally good prognosis. However, among 15-20% of these women, breast cancer recurs, and a similar proportion of women also experience severe toxicity with radiation therapy. It is possible that inter-individual differences in capabilities of both tumor and normal cells to protect themselves from radiation-induced damage, and to repair that damage if it does occur, will influence recurrence and toxicity. This variability results from common genetic polymorphisms. This study is conducted in a well-characterized cohort of women who had breast-conserving surgery followed by radiation therapy, and in whom skin reactions were measured and noted. We have extracted DNA from blood to determine genetic polymorphisms in a number of genes that may be important in response to treatment. By conducting follow-up on the women in the study, we will be able to determine how variability in genes that protect cells from damage and in those that repair DNA damage will affect both breast cancer recurrence and toxicity experienced. Follow-up through clinic visits, letters, and home visits has been completed. We evaluated and identified effects of certain genetic polymorphisms on the occurrence of acute toxicity and in the next year, we will correlate genotyping results with late toxicity.

15. SUBJECT TERMS
Radiation sensitivity, DNA repair, oxidative DNA damage, genetic

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INTRODUCTION: Women with earlier stage breast cancer who receive breast conserving surgery and radiation therapy have a generally good prognosis. However, among 15-20% of these women, breast cancer recurs, and a similar proportion of women also experience severe toxicity with radiation therapy. It is possible that inter-individual differences in capabilities of both tumour and normal cells to protect themselves from radiation-induced damage, and to repair that damage if it does occur, will influence recurrence and toxicity. Activity of many of the proteins involved in these processes are determined by common inborn genetic differences, termed genetic polymorphisms. We conducted a pilot study to determine if this were the case, and although the study population was mixed in stage at diagnosis and treatments received, we found that women with variant alleles that would allow more treatment-generated reactive intermediates to reach tumor cells had better survival.

We are conducting the present study in a well-characterized cohort of women who had breast-conserving surgery followed by radiation therapy, and in whom skin reactions were measured and noted. We are extracting DNA from blood to determine genetic polymorphisms in a number of genes that may be important in response to treatment. By conducting follow-up on the women in the study, we will be able to determine how variability in genes that protect cells from damage and in those that repair DNA damage will affect both breast cancer recurrence and toxicity experienced.

BODY: Research accomplishments associated with each Task outlined in the Statement of Work will be addressed within the context of each of the objectives.

Technical Objective 1 Follow-up of breast cancer patients of the parent study regarding therapy outcome and survival and data collection.

The fieldwork has been completed. Of the 478 patients, 5 (1%) patients refused to participate in the follow-up study and 4 (0.8%) could not be traced. Hence, information on the course of disease was available for 469 patients and could be verified with patient records for 463 patients. Fifty-eight (12.3%) women had developed metastases, a secondary carcinoma or a relapse until follow-up. For 467 patients, details on the radiotherapy regimen (total dose, dose per fraction, treatment time, boost dose) were abstracted from irradiation protocols. Of the 469 patients with follow-up information, 27 (5.8%) women had died (12 due to breast cancer, 7 due to other causes and 8 women with unknown cause of death), 45 (9.6%) did not complete the questionnaire and 46 (9.8%) did not agree to an examination of late complications of radiotherapy. Thus, data on late effects of radiotherapy as well as information on demographic and epidemiologic factors were available for 421 (89.8%) women.

Task 1: Months 1-2: Organization of recontact with patients through different sources, development of clinical data forms and questionnaire, and establishment of database.

This task has been completed as reported in 2003.

Task 2: Months 3-24: Recruitment of patients through different sources, perform follow-up examination, obtain informed consent, collect clinical data, complete questionnaire

Follow-up was completed as described above, and as reported in the 2005 annual report.

Task 3: Months 24-36: Data entry with ongoing quality control and plausibility checks
Data entry has been completed.

Task 4: Months 30-36 Perform statistical data analysis; initial descriptive analyses, study of main effects of data derived from questionnaire.

The main effects of questionnaire data in relation to acute toxicities were reported in 2005 and have been published. Vital status was also reported in 2005.

Technical Objective 2 Evaluation of the effect of genetic polymorphisms in certain candidate genes (i.e. alleles that confer reduced protection from ROS damage and variants in DNA repair genes) and outcomes; i.e., breast cancer recurrence and severe skin toxicity.

Task 1: Months 3-6 DNA extraction and shipment of aliquot
Completed as reported in 2003.

Task 2: Months 26-30 Perform DNA analysis for genetic polymorphisms in genes that confer reduced protection from ROS damage, e.g. *MnSOD, GPX1, CAT, GSTT1, GSTM1, GSTA1, GSTP1,* and in DNA repair genes, e.g. *XRCC1, XRCC2, XRCC3, XPD, APE1*
Genotyping has been completed as reported in 2005. In addition to the planned genotypes, SNPs related to cell cycle control were also assessed (*TP53, p21).*

Task 3: Months 31-36 Merge data from laboratory results with questionnaire database. Perform statistical analysis for main effects of polymorphisms on outcomes.
Data analysis to assess the effect of the genetic polymorphisms on occurrence of acute toxicity, was completed and reported in 2005.

Assessment of late toxicities was completed over the last year, and non-genetic predictors have been evaluated. After a median follow-up time of 51 months, 131 (31.4%) patients presented with telangiectasia and 28 (6.7%) patients with fibrosis. We observed a strong association between development of telangiectasia and fibrosis ($p<0.01$). Increasing age of the patient was a risk factor for both telangiectasia and fibrosis (p for trend <0.01 and 0.03, respectively). Patients with acute skin toxicity (OR 1.8, 95% CI 1.0-3.1) were at higher risk to develop telangiectasia. Long-term smoking was associated with a significant increase in risk of telangiectasia compared to non-smokers (OR 2.3, 95% CI 1.2-4.6). Complete data are shown in the tables below.

Table 1. Late side effects among patients who received radiotherapy after breast-conserving surgery (N=421)

	Score		0	1	2	3	4	*missing*
General condition		N	260	94	61	5	1	
Nausea		N	402	15	4			
Lung		N	414	5	1	1		
Heart		N	413	7	1			
Pain		N	279	119	19	4		
Weight change		N	406	7	4	4		
Larynx		N	418	3				
Fibrosis (at operation site)		N	156	233	25	5		*2*
Fibrosis (not at operation site)		N	282	132	5			*2*
Telangiectasia		N	207	79	134	1		
Lymphatic edema (arm)		N	304	97	19			*1*
Lymphatic edema (breast)		N	345	67	7			*2*

Table 2. Association between potential risk factors and development of telangiectasia following radiotherapy for breast cancer

	no telangiectasia		telangiectasia		OR*	95% CI
	N=278	%	N=131	%		
age at end of RT (median)	59.5		62.0		1.05	1.02-1.08
nominal BED skin (median)	61.0		63.0		1.11	1.04-1.19
BMI (median)	25.3		25.5		1.04	0.98-1.10
Boost						
photon	177	63.7	98	74.8	1	Ref
electron	67	24.1	27	20.6	0.71	0.42-1.22
no boost	34	12.2	6	4.6	0.91	0.31-2.69
Chemotherapy	0	0	2	1.5	n.c.	
Hormone therapy						
yes	236	84.9	122	93.1	1	Ref
no	42	15.1	9	6.9	0.53	0.24-1.17
Acute radiosensivity						
no	233	83.8	101	77.1	1	Ref
yes	45	16.2	30	22.9	1.77	1.00-3.13
Allergy	91	32.7	56	42.8	1.64	1.04-2.58
Hypertension	117	42.1	76	58.0	1.60	1.00-2.56
Diabetes	21	7.6	14	10.7	1.30	0.61-2.76
Skin type						
always sunburn/no sun tan	75	27.0	28	21.4	1	Ref
sometimes sunburn/sun tan	150	54.0	80	61.1	1.51	0.88-2.59
never sunburn/sun tan	49	17.6	18	13.7	0.85	0.41-1.79
Breast size						
Cup A, B	154	55.4	71	54.2	1	Ref
Cup C	73	28.4	31	23.7	0.81	0.48-1.36
Cup D, E, F	27	9.7	20	15.3	1.74	0.87-3.49
Marital status						
single/widowed/divorced	66	23.7	49	37.4	1	Ref
married/partner	212	73.3	82	62.6	0.52	0.32-0.85
Alcohol consumption (g/day)						
0	74	26.6	32	24.4	1	Ref
0.1-3.4	71	25.5	30	22.9	1.05	0.57-1.96
3.5-13.2	67	24.1	36	27.5	1.52	0.82-2.83
13.3+	66	23.7	33	25.2	1.41	0.76-2.64
Smoking status						
non-smoker	185	66.6	82	62.6	1	Ref
current smoker	29	10.4	12	9.2	1.45	0.66-3.18
former smoker	54	19.4	30	22.9	1.74	0.99-3.04
Packyears of smoking						
non-smoker	185	66.6	82	62.6	1	Ref
1-9	38	13.7	14	10.7	1.33	0.64-2.74
10-19	15	5.4	12	9.2	2.17	0.92-5.16
20+	22	7.9	15	11.5	2.32	1.07-5.00
Duration of smoking (years)						
non-smoker	185	66.6	82	62.6	1	Ref
1-14	27	9.7	8	6.1	1.06	0.43-2.61
15-29	22	7.9	14	10.7	1.87	0.87-4.02
30+	28	10.1	20	15.3	2.33	1.17-4.64

Percentages for some variables do not add up to 100% due to missing data

*Odds ratios adjusted for age at end of RT, boost, nominal skin BED, follow-up time and BMI; 7 patients with fibrosis only were excluded from the analysis.

We are currently evaluating the effects of genetic polymorphisms on late toxicities among patients, and these results will be published after the funding period.
The numbers of recurrences (12%) was small, and it is unlikely that we will have sufficient power to assess the impact of genetics on breast cancer recurrence. Nonetheless, these analyses are ongoing and will continue beyond this period of grant support.

KEY RESEARCH ACCOMPLISHMENTS:

Follow-up was completed for acute and late toxicities, as well as for recurrence. DNA was extracted and genotyping completed, with data analysis and publication of results. Main research findings are below.

- High body mass index (BMI) was associated with increased risk of acute toxicity.
- *XRCC1-399Gln* and *APE1 148Glu* alleles were associated with reduced risk of acute toxicity among women with normal weight.
- *XRCC3 241Thr, XRCC2 188His, NBS1 185 Gln* alleles were associated with decreased risk of acute toxicity among women of normal weight; with increased protection associated with increasing number of protective alleles.
- GSTP1 low activity alleles were associated with increased risk of acute toxicities HR, 2.28; 95% CI=1.04-4.99). There were no associations between risk of toxicity and variants in *GSTA1*, *GSTT1*, or *GSTM1*.
- Associations between BMI and acute toxicity were greatest among women with genotypes related to higher levels of oxidative stress, particularly *MPO* and *eNOS.*
- Late normal tissue complications (telangiectasia and fibrosis) are more common among women who are older, and who are smokers.

REPORTABLE OUTCOMES:

Posters:

Poster presented at American Association for Cancer Research 96th Annual Meeting:

Chang-Claude J, Popanda O, Tan X-L, Kropp S, Schmezer P, Ambrosone CB. Polymorphisms in DNA repair gene XRCC1, APE1 and XPD and risk fo acute side effects of radiotherapy in breast cancer patients. Proc Amer Assoc Cancer Res 2005;46:113.

Poster presented at DOD Era of Hope meeting, 2005:

Chang-Claude J, Popanda O, Tan X-L, Kropp S, Schmezer P, Tian C, Ahn J, Ambrosone CB. Oxidative stress, DNA repair, and acute side effects of radiotherapy in breast cancer patients.

Poster presented at MEG-AACR Special Conference 2006 :

Chang-Claude J, Popanda O, Lilla C, Tan X, Helmbold I, von Fournier D, Haase W, Sautter-Bihl M. L., Wenz F, Schmezer P, Ambrosone CB. Extrinsic and intrinsic factors associated with the development of telangiectasia following radiotherapy for breast cancer

Poster presented at Society for Epidemiologic Research Meeting 2006:

Lilla C, Ambrosone CB, Kropp S, Helmbold I, Schmezer P, von Fournier D, Haase W, Sautter-Bihl M-L, Wenz F Chang-Claude J. Predictive factors for late normal tissue complications following radiotherapy for breast cancer

Papers published or in press:

Chang-Claude J, Popanda O, Tan XL, Kropp S, Helmbold I, von Fournier D, Haase W, Sautter-Bihl ML, Wenz F, Schmezer P, **AmbrosoneCB^{+}.** Association between polymorphisms in the DNA repair genes, XRCC1, APE1 and XPD, and acute side effects of radiotherapy in breast cancer patients. *Clin Ca Res* 2005;11:4802-9.

Tan X-L, Popanda O, **Ambrosone CB**, Kropp S, Helmbold I, von Fournier D, Haase W, Sautter-Bihl ML, Wnez F, Schmezer P, Chang-Claude J. Association between TP53 and p21 genetic polymorphisms and acute side effects of radiotherapy in breast cancer patients. *Breast Cancer Tr Res* 2006; 97(3):255-62).

Popanda O, Tan X-L, **Ambrosone CB**, Kropp S, Helmbold I, von Fournier D, Haase W, Sautter-Bihl ML, Wenz F, Schmezer P, Chang-Claude J. Genetic polymorphisms in the DNA double-strand break repair genes *XRCC3, XRCC2* and *NBS1* are not associated with acute side effects of radiotherapy in breast cancer patients. *Cancer Epidemiol Biomarkers & Prev* 2006;1048-50.

Ambrosone CB, Tian C, Ahn J, Kropp S, Helmbold I, von Fournier D, Haase W, Sautter-Bihl ML, Wenz F, Chang-Claude J. Acute toxicities related to radiation therapy following lumpectomy for breast cancer: role of glutathione *S*-transferase polymorphisms. *Br Can Res* (in press, 2006).

Ahn, J**, Ambrosone CB^{+}**, Kanetsky PA, Tian C, Lehman TA, Kropp S, Helmbold I, von Fournier D, Haase W, Sautter-Bihl ML, Wenz F, Chang-Claude J. Polymorphisms in genes related to oxidative stress (CAT, MnSOD, MPO and eNOS) and acute toxicities from radiation therapy following lumpectomy for breast cancer. *Clinical Cancer Res* (in press, 2006)

Submitted and in review:

Lilla C, **Ambrosone CB**, Kropp S, Helmbold I, Schmezer P von Fournier D, Haase W, Sautter-Bihl ML, Wenz F, Chang-Claude J. Predictive factors for late normal tissue complications following radiotherapy for breast cancer. *Strahlentherapie und Onkologie*

CONCLUSIONS: The re-contacting and recruitment for participation of the patients has been extremely successful. We achieved 88.1 % full participation and were able to obtain information on clinical course without re-examination from another 9.8% and permission from all these patients to use the blood samples collected in the parent study for genotyping in this project. Analysis is near completion for the relationship between genotypes and acute toxicities, and results infer that low activity *GSTP1* genotypes are associated with increased risk of skin side effects. While genotypes associated with reduced protection from oxidative stress and reduced DNA repair were not significantly associated with toxicities, body mass index modified associations, with associations between genotype and side effects greatest among women with heavier BMI.