

## CHILDHOOD CANCER SURVIVOR STUDY

### Analysis Concept Proposal

**Title** Association of CYP3A5 and second primary breast cancers following Hodgkin Disease: A report from the Childhood Cancer Survivor Study

#### Working Group and Investigators

This publication will be written within the Second Malignancy Working Group, with input from the Epidemiology/Biostatistics Working Group. Proposed investigators include:

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#### Background & Rationale

Childhood cancer survivors have an elevated risk of developing secondary malignancy neoplasm (SMN). Within this group, female survivors of Hodgkin disease (HD) have an unusually high rate of second breast cancer (BC) [1-8] Dose and field of radiotherapy modify SBC risk.[2,4,6,7] The physiology of the breast tissues appears to render it susceptible to the carcinogenic process begun by ionizing radiation.[9] SBC in HD survivors can be considered a model for radiation-induced breast cancer.[9] Genetic risk factors are clearly relevant for SBC in HD survivors as the majority of female HD survivors do not develop SBC despite similar radiotherapy exposures.[2,4-8]

The connection between estrogen exposure and breast cancer risk is well recognized in the general population (i.e., those not exposed to thoracic irradiation). [9-12] As expected, the risk of SBC in female survivors of childhood malignancies is modified by hormone status and family history of breast cancer. [8,13] Local synthesis and catabolism of estrogens may be the most critical factor in mammary carcinogenesis, as the breast is the principal site of heterocyclic amine activation. [14] Interestingly, nipple aspirate fluid (NAF) of estrone (E1) and 17 $\beta$ -estradiol (E2) are substantially increased relative to serum concentrations in premenopausal women at high-risk for breast cancer (e.g., 332x greater than serum concentrations). [15-16] E1 and E2 undergo hydroxylation by cytochrome P450 enzymes (i.e., CYP3A4, CYP3A5, CYP1A1, CYP1B1). [17-22] Most of the variability in the formation rates of 2-OH-E2 and 4-OH-E2, respectively, is explained by a phenotypic marker of CYP3A4/5 activity. [23] CYP3A5 is the predominant extrahepatic CYP3A isoform and recent unpublished data from Dr. McCune's lab suggests that extrahepatic expression is polymorphic and controlled by the *CYP3A5\*1* genotype.[24] Heterologously expressed CYP3A5 metabolizes E1, which is the predominant estrogen in normal breast tissue of premenopausal women, as well as E2. [25-27] This polymorphism may correlate with breast cancer risk, as it favors formation of the more carcinogenic catechol estrogens. [25-26] Carriers of *CYP3A5\*1* may express greater CYP3A5 in breast tissue and could have an altered risk for breast cancers.

### Specific Aims/Objectives/Research Hypotheses

**Hypothesis:** Formation of 4-OH-catechol estrogens is greater with wild-type of *CYP3A5* (*CYP3A5\*1* carriers), conferring a higher risk of breast cancer in survivors of childhood Hodgkin disease.

**Aim 1:** To compare the frequencies of wild-type *CYP3A5* (i.e., *CYP3A5\*1* carriers) in cases of SBC relative to controls (no SBC) in female survivors of childhood HD.

**Aim 2:** To assess the relative role of *CYP3A5* in the 2-, 4-, 16 $\alpha$ -hydroxylation of E1 and E2.

### Analysis Framework

(a) Outcome of interest: Second breast cancer in female survivors of Hodgkin Disease.

(b) Subject Population: All female HD patients in CCSS with available buccal cells

(c) Explanatory variables:

CCSS data: HD initial diagnosis, breast cancer second diagnosis, age at HD dg, age at BC diagnosis, race, number of live births, age at menarche, age at first live birth, pelvic RT, chest RT, alkylating agent score

Laboratory data:

- 1) *CYP3A5* polymorphism genotype in CCSS HD female patients (Use of buccal cell samples)
- 2) Rates of 2- and 4-hydroxylation of E1 and E2 in human liver microsomes previously genotyped for *CYP3A5* and *CYP3A4* promoter (no use of CCSS samples)

### Overall methodology

**Aim 1: Genotyping:** Anonymous DNA samples will be sent to Dr. McCune for genotyping at the Molecular Biomarkers Laboratory of the University of Washington. Human *CYP3A5* polymorphism identification will be conducted using fluorogenic TaqMan™ probes and 5' nuclease-based genotyping (allelic discrimination) assays. The TaqMan™ Detection System uses fluorogenic probes to detect specific nucleic acid sequences and single nucleotide polymorphisms for allelic discrimination by using the 5' nuclease assay. The primer and dye-labeled probe sequences for the established *CYP3A5\*3* have been identified. Quality controls are present, using appropriate positive and negative (no DNA) controls for the fluorescent 5' nuclease-based genotyping analyses. In addition, 10% of the identified alleles will be randomly re-analyzed for the appropriate genetic polymorphisms and compared to previous analyses for quality control.

**Aim 2: Human liver microsomes:** As a surrogate marker of the potential implications of *CYP3A5* upon E1 and E2 catabolism in healthy breast tissue, the rates of hydroxylation of E1 and E2 will be evaluated in human liver microsomes previously genotyped for *CYP3A5* and *CYP3A4* promoter. E1 and E2 hydroxylation will be evaluated in 3 separate groups of human liver microsomes obtained from the University of Washington Tissue Bank of human liver microsomes. The three groups (n=5/group) will consist of: 1) those with high *CYP3A5* protein (*CYP3A5\*1* carriers) and low *CYP3A4* protein; 2) those with low *CYP3A5* protein (*CYP3A5\*3* homozygotes) and high *CYP3A4* protein; 3) those with varying amount of *CYP3A5* protein and average *CYP3A4* protein. This analysis will not require any tissue from CCSS participants, but will provide some additional evidence for functional significance to the *CYP3A5* polymorphism data.

**Specific tables:**

**Comparison of BC risk factors for Hodgkin disease cases with and without buccal cell collection**

	Total Eligible Cases	Returned Buccal Cell	No Buccal Cell Returned	Chi square p-value
Sex				
Race				
Age at HD diagnosis				
Age at BC diagnosis				
Age at menarche				
Number of live births				
Age at first live birth				
Pelvic RT				
Chest RT				
Alkylating Agent Score				
Stem Cell transplant				

**Polymorphism frequencies in CCSS Female HD cases with and without second breast cancer**

	All female HD cases	Female HD cases with SBC	Female HD cases without SBC	P value
CYP3A5 WT				
CYP3A5*1				

**Correlation between CYP3A5/CYP3A4 protein and E1/E2 hydroxylation**

	E1 hydroxylation	E2 hydroxylation	P value
high CYP3A5 protein / low CYP3A4 protein	2- 4- 16-	2- 4- 16-	
low CYP3A5 protein / high CYP3A4 protein	2- 4- 16-	2- 4- 16-	
varied CYP3A5 protein/average CYP3A4 protein	2- 4- 16-	2- 4- 16-	

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