02-03

Study Title: Determination of GST and XRCC1 genotypes as possible markers of susceptibility to therapy-related subsequent malignancy in CCSS cases Hodgkin's disease.

Working Group and Investigators: This proposal is a joint collaboration between Ann Mertens and Stella Davies. This proposed manuscript will be within the Second Malignancies Working Group. Proposed investigators will include:

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Background and Rationale:

The Childhood Cancer Survivor Study represents a unique population to study late effects of cancer treatment. CCSS received supplemental funding from NCI in 1998 for the collection of buccal cells on 7800 study subjects, and GST genotyping on all Hodgkins disease cases and cases with subsequent malignancies. GST genotyping has been completed on these cases, and this concept proposal describes the analysis of these data. In addition to GST typing, we propose to investigate XRCC1 and its association with radiation induced subsequent malignancies.

Buccal cells are a unique source of genetic material and are currently being successfully utilized in numerous epidemiological studies. Buccal cell collection in CCSS participants (cases and sibling controls) began in May, 1999. To date, viable samples have been collected on 4,412 cases and 666 sibling controls. In addition, exposure to combinations of therapeutic modalities including radiation and chemotherapy has been collected on CCSS cohort members.

The development of subsequent malignancies in survivors of childhood cancer have been ascribed to the mutagenic effects of chemotherapy and radiotherapy, and in some cases to

inherited mutations in tumor suppressor genes. Individual susceptibility to subsequent malignancy is variable; the majority of the patients treated for cancer do not develop this complication. One possible explanation for this is interindividual variability in the ability to detoxify environmental mutagens such as chemotherapy agents and radiotherapy. Two genotypes as possible markers of susceptibility to subsequent malignancy are described below.

Conjugation of electrophilic compounds to glutathione, mediated by glutathione S-transferase enzymes is an important detoxifying pathway for mutagens such as alkylating agents. The glutathione S-transferase M1 (GSTM1) and the GSTT1 genes are polymorphic in humans, and the phenotypic absence of enzyme activity is due to homozygous inherited deletion of the gene. These enzymes are important in the detoxification of environmental carcinogens, and have been implicated in cancers with these exposures.

The human DNA repair gene XRCC1 is involved in the repair of DNA single strand breaks generated in response to either ionizing radiation or alkylating agents. The human XRCC1 gene is polymorphic in humans with single nucleotide polymorphisms occurring in at least 3 sites. Data suggest that this polymorphism is unlikely to be associated with complete loss of protein function, but that reduction in function may importantly influence ability to tolerate potentially carcinogenic DNA damage. In Hodgkin's disease (HD) cases, XRCC1 polymorphism at this locus is believed to influence susceptibility to radiation induced damage. HD cases in this cohort have a higher rate of radiation induced subsequent malignancies, particularly secondary breast cancer which has been associated with radiation exposure.

Specific Aims/Objectives:

For this proposal, there are two outcomes of interest in Hodgkin's disease cases with confirmed subsequent malignancies: a) GST mu, GST theta genotypes and b) XRCC1 genotypes. The specific aims are as follows:

I. Compare GSTT1 and GSTM1 genotypes in five-year survivors of childhood cancer who develop a second malignancy versus survivors who do not develop a second malignancy. Hypothesis: Hodgkin's disease survivors with a GSTM1 and/or GSTT1 null genotype will have an increased frequency of secondary solid tumors after exposure to chemotherapy, independent of radiation exposure.

II. Compare XRCC1 genotypes in five-year survivors of childhood cancer who develop a second malignancy versus survivors who do not develop a second malignancy.
Hypothesis: Possession of one or more glutamine alleles is associated with an increase in radiation-associated subsequent cancers in Hodgkin's disease cases.

Analysis Framework:

Outcomes of interest: GST and XRCC1 genotyping on returned buccal cell specimens from Hodgkin's disease cases.

Subject population: Hodgkin's disease cases that returned a viable buccal cell specimen and had a confirmed diagnosis of subsequent malignancy. The numbers indicated in this proposal are as of the time of this proposal submission. The final number of confirmed subsequent malignancies analyzed in this proposal will be all subsequent malignancies reported in the baseline survey and in the Follow-up survey as of 12/31/01. All buccal cells collected up until the initiation of this analysis will also be included.

Exploratory variables: Demographic variables will be compared between those cases that did and did not return a buccal cell sample to determine the representativeness of the sample. These variables include sex, race, age at diagnosis, metastatic at diagnosis, age at baseline, smoking status, and subsequent malignancy status (see table 1). Genotyping comparisons will be made between the general population and all CCSS Hodgkin's disease cases. Genotyping comparisons will also be made within Hodgkin's disease cases by subsequent malignancy status and type of reported subsequent malignancy. If the sample size allows, interactions will be determined by type of treatment exposure (radiation, alkylating agents, both, neither) and subsequent cancer status.

All subsequent malignancies that have been confirmed at the time of this proposal are divided into two groups: radiation induced, not radiation induced. Those identified in each category are as follows:

Radiation induced: cancer of the breast, thyroid, CNS, mucoepidermoid carcinoma of the parotid, osteosarcoma, AML, melanoma, fibrous histiocytoma.

Not radiation induced: CML, chordoma, leiomyosarcoma, carcinoma in situ of the vulva, uterine cancer, malignant lymphoma.

Suggested tables: (see attached)

Special considerations: This analysis will be conducted by Pauline Mitby and Ann Mertens in conjunction with Gretchen Radloff and Stella Davies in the Molecular Genetics Bank at the University of Minnesota. This laboratory is responsible for the genotyping of specimens on cases eligible for this proposed project.

Since this is the first report to come from the Molecular Genetics Center, this manuscript will describe buccal cell collection in more detail, for reference purposes in future publications.

Table 1. Demographics of Hodgkin's disease cases eligible* for buccal cell collection

	Total eligible cases	Returned buccal cell	No buccal cell returned
Total	1304	643	661
Sex			
Male	702	317	385
Female	602	326	276
Race			
White	1120	585	535
Non-white	184	58	126
Age at diagnosis			
0-5	73	23	50
6-10	215	92	123
11-15	524	277	247
16-20	492	251	241
Metastatic at diagnosis			
Yes	417	216	207
No	622	281	341
Unknown	265	152	113
Age at baseline			
< 21	81	29	52
21-30	547	265	282
31-40	583	301	282
> 40	93	48	45
Smoking status			
Current	198	98	100
Former	189	109	80
Never	732	393	339
Unknown	185	43	142
Subsequent cancer			
Breast cancer	56	35	21
Other radiation-induced cancer	33	21	12
Other cancer	16	9	7
Treatment			
Chemotherapy	61	27	34
Radiation	386	211	175
Both	651	353	298
Neither	156	35	121
Alkylating Agents			
Yes	686	367	319
No	396	220	176
Don't know	6	3	3

* eligible= returned baseline questionnaire, not in tracing, alive at buccal cell send date, and from an institution with IRB approval for buccal cell study.

Table 2. Comparison of genotypes

	Population controls	All CCSS HD cases	HD- no SMN	HD –with SMN	Subsequent breast cancer	Other Radiation induced cancer	Non-radiation induced cancer
GSTM1							
positive							
null							
GSTT1							
positive							
null							
XRCC1							
Arg/Arg							
Arg/Gln							
Gln/Gln							