02-12

Childhood Cancer Survivor Study

Analysis Concept Proposal (rev 07 Nov 02)

Submitted: October 1999 / Revised November 2002 [Note: The October 1999 concept submission was not made which was recently brought to our attention. The current concept is a slight modification. A more in depth review can be found in our recent manuscript submitted to Health Physics which could be made available.]

1. **Title:** Genetic and Reproductive Effects of Radiation and Chemotherapy on Survivors of Childhood and Adolescent Cancer

2. **Working Group and Investigators.**

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3. **Background and Rationale:**

Rationale. Studies of the offspring of survivors of childhood cancer offer a unique opportunity to evaluate whether preconception radiation or chemotherapy can result in a detectable increase in genetic effects. The numbers of survivors and their offspring are now sufficient to test genetic hypotheses, and the radiotherapy doses can be determined with precision. The study will determine whether current estimates of genetic effects in humans are reasonable and, at the same time, provide clinical information for patients who are concerned about possible genetic effects from the curative therapies they received.

The proposed concept is in some respects an extension and enhancement of previous concept 98-02 entitled "Pregnancy Outcomes of Survivors of Cancer Diagnosed During Childhood and Adolescence" and several others. The differences include validation of all adverse pregnancy outcomes, quantitative risk assessment for radiotherapy dose to gonads, and evaluation of additional pregnancy outcomes, including those among the spouses of male survivors. While the focus will on the possible genetic effects associated with radiation, chemotherapy and other treatments will also be evaluated to the extent

feasible.

The prognosis for children and adolescents treated for childhood cancer has improved dramatically during the past 20 years. Many survivors have completed their education, entered the workplace, and have made or are planning to make decisions regarding marriage and reproduction. The treatments that have cured the children, however, have the potential to cause germ cell mutations that could be expressed in the offspring. The effect of such mutations could be an increase in the rate of spontaneous abortion, stillbirth, premature birth, low birth weight, neonatal death, congenital malformation, childhood cancer, changes in the sex-ratio (ratio of the number of boys to girls born), sex chromosome abnormalities (such as XXX or XO instead of XX or XY), other chromosomal aberrations, abnormal growth and development, and alterations of the structure and function of nucleic acids or proteins. To date, no such adverse pregnancy outcomes have been consistently linked to prior therapies, but the numbers have been generally limited so that only a relatively high risk could be excluded.

The present cohort is large and includes patients with diverse treatment exposures, and will allow an accurate estimation of the possible effects of treatment on adverse pregnancy outcomes. A cohort of over 14,000 five-year survivors of childhood cancer diagnosed between 1970 and 1986 and over 3,000 sibling controls is derived from 25 participating centers. Cohort members, currently ranging from 13 to 50 years of age (median 27 years), have been extensively characterized by initial cancer diagnosis, therapy received, and baseline health status (Robison et al. 2002).

Contact and recruitment of eligible participants began in August of 1994. Survivors 18 years of age or older were contacted directly, while parents were contacted for survivors under 18 years of age. Subjects, or their parents, were asked to complete a 24-page questionnaire, provide consent for release of medical records and for future contact to update the health history and to consider participation in other research projects, such as the current proposed study of adverse pregnancy outcomes.

Among the 14,000 participants, 54% are male, the median age is 27 years, 38% are married, 90% have a high school education, 33% were diagnosed with leukemia, 13% CNS, 13% Hodgkin's disease, 7% non-Hodgkin lymphoma, 7 % neuroblastoma, 9% Wilms tumor, 9% soft tissue sarcoma, 9% bone cancer. Of the several thousand radiotherapy records preliminarily evaluated, 8% indicated that the gonads were near the radiation field (< 3 cm), 4% were in the radiation field but shielded, 11% were in the radiation field unshielded, and 77% were beyond 3 cm of the radiation field. Crude estimates of gonadal doses from this sample imply that 79% of survivors received between 1-1,000 cGy, 13% 1,000-2,499 cGy, 4% 2,500-3,499 cGy, and 3% greater than 3,500 cGy. Abdominal radiotherapy was received by 27% of survivors, pelvic radiotherapy by 24% and total body irradiation by 3%. These numbers indicate a broad ranges of gonadal doses that will be available for correlation with reported and confirmed adverse pregnancy outcomes (Boice et al. 2000).

Among the 12,459 sexually active survivors of childhood cancer, 2,978 reported 6,017 pregnancies. The questionnaire included items regarding sexual activity, attempts to begin a pregnancy, occurrence of pregnancy, and the outcome of pregnancy (live birth, stillbirth, miscarriage, abortion, neonatal death). Medical records of all members of the cohort are available to obtain chemotherapeutic agents administered, and the dates and details of administration of all radiation therapy.

The proposed study will provide valuable clinical information that can be used in counseling survivors of childhood cancer as to their potential risk for adverse pregnancy outcomes. In addition, it will provide important scientific information as to the level of genetic risk possibly due to radiation or chemical exposures. The different outcomes to be measured and validated will enhance our ability to detect adverse effects should they exist. A more comprehensive review has been submitted for publication (Boice et al. 2002).

Selected Bibliography

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4. Specific Aims/Objectives/Research Hypotheses: The specific aim of this study is to evaluate

reproductive outcomes of survivors of childhood and adolescent cancer (diagnosis before 21 years of age) and to learn whether there is an excess of birth defects, stillbirths, neonatal deaths, and miscarriage and whether the occurrence can be related to radiation dose to the gonads of survivors. Additional measure of heritable genetic damage would include altered sex ratios, the incidence of childhood leukemia and cancer, the occurrence of spontaneous abortions and low birth weight. In the analysis, we will both evaluate and, where possible, adjust for any effect from concurrent chemotherapy as well as uterine dose among female survivors. To the extent that other variables might be important, such a paternal or maternal age, social class, and calendar year, they too will be accounted for in the analysis. Information on other potentially important factors, such a extent of cigarette smoking has been obtained.

From a practical starting point, though, the first objective will be validation. The 14,000 childhood cancer survivors reported having 6,000 pregnancies and over 4000 live births. A major effort of the proposed study will be in validating the self-reported cases of adverse pregnancy outcomes.

Specific tasks include:

1. *Stillbirths and neonatal deaths*. Cases of stillbirths and deaths within the first week will be identified from questionnaire responses and verified by medical record acquisition and review. Over 80 stillbirths have been reported to date. The medical records of mothers and children will be requested for all stillbirths and neonatal deaths.

2. *Congenital malformations*. Congenital malformations would be determined from responses of the parents and validated from medical records by a medical geneticist and/or pediatrician. Medical records of stillbirths and neonatal deaths would also be evaluated for congenital anomalies.

3. *Cancer incidence*. Cancer incidence among the offspring will be determined from the responses of the parents and validated from medical records.

4. *All deaths*. All deaths occurring among the offspring of cancer survivors will be determined and death certificates obtained and cause of death coded.

5. *Birth weight*. Birth weight has been collected based on interview response which has a high level of accuracy (Olsen JE, et al. Am J Epidemiol 145:58-67, 1997). Analyses will be performed relating low birth weight with prior therapies given the cancer survivor. A separate concept has been developed in this regard.

6. *Sex ratio*. The gender of the offspring can be determined and the sex ratio evaluated. For irradiated mothers, it would be anticipated that there would be a deficit of male births and thus the male to female ratio would be decreased. For irradiated fathers, it would be anticipated

that there would be a deficit of female births and thus the male to female ratio would be increased.

7. Radiotherapy and chemotherapy. Radiotherapy and chemotherapy records will be obtained and evaluated on parents who had a child/fetus with one of the reproductive outcomes considered. Radiotherapy will be evaluated at the MD Anderson Hospital in Houston, Texas, USA and doses to the gonads (testes/ovaries) determined. Dose to uterus will also be determined. Chemotherapy will be characterized as to whether alkylating or non-alkylating agents.

8. Confirmation. All information will be interpreted for alternate explanations, such as family history, teratogenic exposure, possible syndrome diagnosis, and genetic etiology. Adverse pregnancy outcomes will be validated from medical record review by a pediatric oncologist. A medical geneticist would evaluate the extent that family history might play in the adverse outcomes as well as consult with regard to congenital malformation evaluations. Medical records for both the child and the mother will be requested for validation as needed.

9. Other. To the extent possible, other adverse pregnancy outcomes will be considered, such as chromosomal abnormalities, chromosome aberrations, miscarriages, growth and development, and blood protein alterations. Miscarriages that required a hospitalization or those occurring in the 2nd trimester might be considered. Similarly voluntary abortions occurring in the 2nd trimester might be related, in part, to prenatal diagnoses of congenital anomalies. Sampling approaches might be considered.

Hypotheses to be tested. Does radiation dose to the gonads increase the occurrence of untoward pregnancy outcomes (stillbirths, congenital malformations and neonatal deaths)? Do the offspring have a higher than expected risk of childhood leukemia and cancer? Similar hypotheses can be restated substituting chemotherapy for radiation dose, however, only a characterization by alkylating versus non-alkylating agents will be sought initially. An additional hypothesis, if numbers permit, would be an evaluation of the combined effect of radiation and chemotherapy on adverse pregnancy outcomes.

5. Analysis Framework

The observed numbers of stillbirths, congenital malformations, and neonatal deaths will be compared with those expected based on prevalence rates and mortality in the general population and among the sibling control group. Furthermore, it will be evaluated whether the occurrence of adverse pregnancy outcomes can be related to radiation dose to gonads (or to chemotherapy) in a case-control design within the cohort. For the comparisons with the sibling comparison group as well as for the internal dose response analyses, a variety of analytical methods will be used, including Poisson regression and logistic regression.

A. Comparisons

Comparisons with the offspring of siblings of the cancer survivors will be made. Cancer incidence will be contrasted with general population rates (from the National Cancer Institute's Surveillance, Epidemiology and End Results registries) and O/E ratios computed. Comparisons over levels of gonadal dose by gender will be made, and these internal evaluations will be the primary way to assess any association between preconception irradiation and adverse pregnancy outcomes. Birth defects and genetic disease incidences will be compared with published population-based rates in similar fashion; and dose response analyses conducted.

B. Risk assessment in a nested case-control design

1. Selection of cases and controls

It is envisioned that at least 350 offspring with one of the selected reproductive outcomes will be identified.

An equal number of comparison subjects for detailed dosimetry evaluation will be selected within the cohort of offspring of the survivor of childhood cancer. The details for the selection criteria will be similar to those taken in a parallel study being conducted in Denmark. A case-cohort approach was decided upon after extensive discussions with statisticians around the world. The family unit will be the sampling frame. A 25% sample of all families with 1-3 liveborn and stillborn children will be selected, and a 100% sample for families with >3 pregnancies. Multiple-birth pregnancies will be excluded.

Offspring of the sibling controls will also be evaluated as comparison subjects, accounting to some extent for genetic predisposition. Because of the sampling scheme in the CCSS study, approximately 40% of the cases (cancer survivor with adverse pregnancy outcome) will have a matched sibling who has had a child eligible for comparison. This comparison, with data collected in similar fashion as on the case, will be in addition to the population rate comparison. Some siblings were also treated for cancer and it will be determined whether such treatment occurred prior to pregnancy.

2. Radiation dose assessment

For all cases and controls, review of medical records of survivor parents will be performed in order to evaluate doses of radiotherapy and chemotherapy. Gonadal doses will be evaluated in USA. The dosimetry will take into account treatment, age, and type of therapy unit. For example, the testes doses for a 5-yr old boy treated for Wilms tumor with either orthovoltage, cobalt-60, and 6 MV x-rays might range from 20-200 cGy. For a 5-yr old boy treated with cranial irradiation, the range of testes dose might be 2-15 cGy. The range depends on energy, with orthovoltage resulting in the highest dose and 6 MV the lowest dose. In the 1940s and 50s orthovoltage was most common. In the 1960's-70's, most radiation treatments were by cobalt-60; later years were by cobalt-60 and 6 MV. These testes doses are indicative of the range for all tumors since these (Wilms and brain) are about as far away and as close to the gonads as occurs for pediatric cancer. Also, of course, younger children, for the same

type of treatments, would have higher doses and older children, lower doses. Dosimetry will involve both anthropomorphic phantom measurements, water phantom measurements, and three-dimensional mathematical models. The child's age, size, gender, calendar year of treatment and type of therapy unit (orthovoltage, cobalt-60, 6 MV) will be accounted for in the dosimetry reconstructions.

The approach will include measurements and calculations necessary to estimate absorbed radiation dose to gonads of the cancer survivor, including measuring dose with anthropomorphic phantoms, using thermoluminescent dosimeters (TLD), under conditions that simulate patient irradiation; measuring dose outside radiation beams in water phantoms and applying these data to a computerized mathematical phantom; measuring components of dose outside radiation beams, such as internal scatter, head leakage, and collimator scatter, in order to determine differences among therapy machines of the same nominal radiation energy. Individual data will be obtain for each case and control from the 25 participating medical centers.

3. Analysis

Gonadal radiation doses will be estimated for those with pregnancies resulting in stillbirths, neonatal deaths or congenital malformations and for twice as many controls. Relative risks of adverse pregnancy outcomes will then be calculated as a function of preconception radiation dose. Additional measure of heritable genetic damage would include altered sex ratios, the incidence of childhood leukemia and cancer, the occurrence of spontaneous abortions and low birth weight. In the analysis, we will both evaluate and adjust, where possible, for any effect from concurrent chemotherapy as well as uterine dose among female survivors. To the extent that other variables might be important, such a paternal or maternal age, social class, and calendar year, they too will be accounted for in the analysis. Information on other potentially important factors, such a extent of cigarette smoking has been obtained.

Attached are rough outlines of tables to be prepared. The key analysis will be dose response, contrasting gonadal doses received by survivors with adverse pregnancy outcomes with those of the comparison cohort. Crude estimates will be made first, similar to what has been done for Denmark and then more in depth analyses as described above.

Table 1.

Potential Genetic Disease in Offspring of Survivors and Sibling Controls

Type of Potential Genetic Disease	Survivor offspring	Sibling offspring	Prevalence Ratio 95% CI
Cytogenetic abnormality			
Single gene (Mendelian) disorder			
Simple malformation			
Cancer			
Stillbirth			
Neonatal deaths			
All deaths			
Total			

Table 2.

Observed (OBS) and Expected (E) Malformations Among Offspring of Survivors and Sibling Controls[a]

	Cancer Survivor Offspring			Sibling Offspring		
Malformation	**Obs**	**O/E**	**95% CI**	**Obs**	**O/E**	**95% CI**
All congenital malformations						
Anencephaly						
Spina bifida						
Hydrocephalus						
Other malformations of nervous system						
Eye						
Ear, face and neck						
Heart						
Blood vessels						
Respiratory organs						
Lip and palate						
Upper digestive system						
Lower digestive system						
Genitalia						

Table 2.
Observed (OBS) and Expected (E) Malformations Among Offspring of Survivors and Sibling Controls[a]

	Cancer Survivor Offspring			Sibling Offspring		
Malformation	**Obs**	**O/E**	**95% CI**	**Obs**	**O/E**	**95% CI**
Urinary organs						
Foot						
Other malformations of extremities						
Other malformation in musculoskeletal system						
Skin, hair, nails						
Malformations not further specified						
Multiple malformations						

[a] Population statistics may be able to provide expected numbers, similar to Denmark.
If not, direct comparisons might be possible.

Table 3.

Congenital Malformations by Radiation Exposure Potential[a]

Exposure Potential	OBS	EXP	O/E
None			
Low			
Low-Medium			
Medium			
Medium-High			
High			

[a] Tables can be prepared by untoward pregnancy outcomes which include the combination of malformations, stillbirths and neonatal deaths. Expected numbers from population statistics similar to what was done in Denmark will be considered. Direct comparisons for dose response.

Table 4.

Cancer Risk among Offspring of Survivors and Sibling Controls

	Survivor Offspring				Sibling Offspring			
Primary cancer of offspring	**Obs**	**Exp**	**O/E**	**95% CI**	**Obs**	**Exp**	**O/E**	**95% CI**
All malignant neoplasms								
Retinoblastoma (Denmark only)								
Brain and nervous system								
Connective tissue								
Non-Hodgkin's lymphoma								
Melanoma of skin								
Kidney								
Ovary								
Leukemia								
Testis								
All other sites								

Expected value can be obtained from population statistics. Direct comparisons can be made if numbers are sufficient. It is recognized, however, that the number of cancers will unlikely be too small for such detail.

Table 5

Genetic Disease by Radiation Exposure Potential[a]

Exposure Potential Gonadal Dose	Cases	Comparison Cohort	RR 95% CI
None			
Low			
Low-Medium			
Medium			
Medium-High			
High			

[a] Tables can be prepared by untoward pregnancy outcomes which include the combination of malformations, stillbirths and neonatal deaths. Format to follow for all adverse pregnancy outcomes, separately or combined. Also, the categories will be replaced with actual gonadal dose and analyses conducted separately for male and female survivors.

6. Special Considerations (and possible future initiatives)

The funding for this project comes from a grant from the Westlakes Research Institute in England (URL: http://www.wri.co.uk/index.htm). The funding to the University of Minnesota will be through the International Epidemiology Institute in Maryland which is also conducting a collaborative parallel study of adverse pregnancy outcomes among survivors of childhood cancer in Denmark (see below) and is receiving funds for both studies from the Westlakes Research Insitute. It is envisioned that both studies will be combined sometime in the future. Dosimetry and the nested case-control approach will be the same in both investigations. Westlakes Research Institute is a non-profit charity for the encouragement of higher education and economic regeneration in Cumbria, UK. There are over 70 technical staff and we would be working closely with the Genetics group, led by Dr Jan Tawn. The Department conducts research to help understand the nature and scale of inherited and somatic genetic variation and the influence of environmental and occupational exposures on individuals and populations. The work involves collaboration with universities and research institutes nationally and internationally in studies of genetic

changes at cellular, chromosomal and molecular levels. Westlakes Research Institute is an independent scientific research organization that also receives partial funding from BNFL, Ltd (British Nuclear Fuels), the organization that oversees and runs the nuclear energy program of the United Kingdom (and also has been given responsibilities in the United States for remediation of radiation plants and contaminated lands). The British government is the sole owner of BNFL.

POOLED ANALYSIS WITH DANISH STUDY

Analysis of CCSS data will be carried out independently of a similar Danish study being conducted in parallel. However, it is anticipated that a pooled analysis will be possible. The Danish study will involve over 12,000 survivors of childhood cancer, among whom 4,000 are estimated to be sexually active and among whom 2000 life births have occurred. Dose to gonads will be reconstructed from radiotherapy records by the same medical physicists at the MD Anderson Hospital as in the CCSS study using similar methodologies. Outcomes include congenital malformations (estimated to be at least 100 major ones), stillbirths, neonatal deaths, total deaths, leukemia and childhood cancer incidence, and altered sex ratio. Malformations would be determined from stillbirths, live births, neonatal deaths, elective abortions (after prenatal diagnoses), and autopsy findings after spontaneous abortions. The pooled analysis will include analysis of original data from both the Danish and the CCSS study for those outcomes that are the same: untoward pregnancy outcomes (stillbirths, neonatal deaths, congenital malformations), altered sex ratios, leukemia and childhood cancer in the offspring. The other outcomes will be similarly evaluated to assess the potential impact of preconception irradiation, i.e., total deaths, low birth weight, and spontaneous abortions. The pooled analysis will address consistencies across the studies as well as provide stronger numbers on which to base conclusions. Further, it may be possible to incorporate other Nordic studies in a study of adverse pregnancy outcomes among survivors of childhood cancer who were treated with radiotherapy. If Finland and Sweden, and possibly Norway, agree to participate, the Nordic component will equal that of the CCSS and the inferences with regard to genetic effects of radiation would be based on large numbers. Funding for these initiatives will be through the International Epidemiology Institute.

SCIENTIFIC ADVISORY COMMITTEE

A Scientific Advisory Committee is envisioned to provide overall advice, review the study protocol, monitor study progress and evaluate the study findings. Experts in radiation genetics, epidemiology, radiation biology, reproductive biology, statistics and epidemiology currently serve on the advisory committee, including Dr William Jack Schull at the University of Texas at Houston, Dr Allen Wilcox (NIEHS), Dr Roger Cox (NRPB, UK, radiation biologist, animal experimentalist), Dr Gerald Draper (Oxford University, epidemiologist, Chairman), Dr Godfrey Oakley (CDC, malformation registry), Dr. Ranajit Chakaborty (University of Cincinnati, medical geneticist).

OTHER STUDY POSSIBILITIES

The CCSS study also has a mechanism to obtain biological specimens from survivors and possibly their offspring. In the future, it might be informative to obtain blood samples from both parents and the offspring and evaluate whether changes in blood characteristics could be detected that might be related to paternal irradiation. The G2 radiation sensitivity assay may be able to determine whether radiation sensitivity is inherited. With 6 DNA-gene repair gene pathways, and six critical pathways of cell survival following radiation exposure, there would be about 80 to 100 SNP sites that could be assayed. Radiation damage inherited a changes in minisatellites might be evaluated also, as in Denmark. The study of atomic bomb survivors evaluated changes in blood protein charge and function using electrophoresis and other assays. Similar or new and more sophisticated biomedical approaches to evaluate nucleic acid changes might be incorporated in future studies. If a follow-up questionnaire is sent to the CCSS cohort in the near future, additional cases of adverse pregnancy outcomes could be identified and potentially included. Finally, similar studies are being conducted in Scandinavian countries other than Denmark and an even larger sample size may be possible in the foreseeable future if all comprehensive studies of the offspring of survivors of childhood cancer were incorporated into a pooled analysis.