Second cancers following treatment for retinoblastoma

Ruth Anne Kleinerman, MPH

Submission for
Doctor of Philosophy in Health Services Research (via prior publication)

School of Health Sciences

October 2016

Table of contents

List of figures

List of tables

City, University of London
Northampton Square
London
EC1V 0HB
United Kingdom

T +44 (0)20 7040 5060

THE FOLLOWING PARTS OF THIS THESIS HAVE BEEN REDACTED FOR COPYRIGHT REASONS:

p. 51-3, Kleinerman RA, Tarone RE, Abramson DH, Seddon JM, Li FP, Tucker MA. Hereditary retinoblastoma and risk of lung cancer. J Natl Cancer Inst 92;2037-39, 2000

p. 68-75, Kleinerman RA, Tucker MA, Tarone RE, Abramson DH, Seddon JM, Stovall M, Li FP, Fraumeni, JF, Jr. Risk of new cancers after therapy in long-term survivors of retinoblastoma: An extended follow-up. J Clin Oncol 2005;23:2272-9

p. 84-91, Kleinerman RA, Tucker MA, Abramson DH, Seddon JM, Tarone RE, Fraumeni JF, Jr. Risk of soft tissue sarcomas by individual subtype in survivors of hereditary retinoblastoma. J Natl Cancer Inst, 2007;99:24-31.

p. 106-116, Yu CL, Tucker MA, Abramson DH, Furukawa K, Seddon JM, Stovall M, Fraumeni JF Jr, Kleinerman RA. Cause-specific mortality in long-term survivors of retinoblastoma. J Natl Cancer Inst, 2009:101:581-91.

p. 129-136, Kleinerman RA, Yu CL, Little MP, Li Y, Abramson DH, Seddon JM, Tucker MA. Variation of second cancer risk by family history among long-term survivors of retinoblastoma. J Clin Oncol, 2012;30:950-957.

p. 143-151, Wong JR, Morton LM, Tucker MA, Abramson DH, Seddon JM, Sampson JN, Kleinerman RA. Risk of subsequent malignant neoplasms in long-term retinoblastoma survivors following chemotherapy and radiotherapy. J Clin Oncol, 2014;32:3284-3290.

p. 204-206, An Update from the Retinoblastoma Followup Study, National Cancer Institute, 2001

p. 207-211, An Update from the Retinoblastoma Followup Study, National Cancer Institute, 2005

List of abbreviations and specialist terms

CI	Confidence interval
EAR	Excess absolute risk
Gy	Gray (unit measure of radiation)
HR	Hazards ratio
ICDO	International classification of the diseases of oncology
LMS	Leiomyosarcoma
NCI	National Cancer Institute
NDI	National Death Index
RB	Retinoblastoma
RB1	Retinoblastoma gene
RR	Relative risk
SEER	Surveillance, epidemiology and end results program
SIR	Standardised incidence ratio
SMR	Standardised mortality ratio
SSA	Social Security Administration
SSN	Social Security number
STS	Soft tissue sarcoma
TEM	Triethylenemelamine

Acknowledgements

I am grateful to my advisors Professor John Lawrenson and Dr Caroline McGraw for their thoughtful comments and advice throughout the entire process of producing this dissertation. Special thanks go to my Branch Chief Dr Amy Berrington for her unwavering support and encouragement during this process. My colleagues in the Division of Cancer Epidemiology and Genetics at the National Cancer Institute enthusiastically supported my work towards this degree. Annelie Landgren helped immensely with technical issues. Thank you also goes to Drs John Boice and the late Fred Li who jointly started the original retinoblastoma study on which my research is based. I am also grateful to Dr Peggy Tucker for her support on all of these studies.

Special thanks go to my husband Steve Kleinrock and my daughter Elizabeth whose encouragement and support carried me all the way from beginning this journey to the end. I would also like to thank my friends for supporting me and cheering me on throughout this process.

Declaration

I declare that I grant powers of discretion to the University Librarian to allow the thesis to be copied in whole or part without further reference to the author. The permission covers only single copies made for study purposes, subject to normal conditions and acknowledgements and copyright relating to the individual papers contained in the thesis.

Abstract

Improvements in treatment over the past century have greatly increased survival for retinoblastoma, a rare childhood tumour of the eye, caused by mutations of the *RB1* tumour suppressor gene. However, as survival for retinoblastoma has improved, those with the hereditary form of the disease (*RB1* germline mutation) have elevated risks of developing additional cancers, mostly bone and soft tissue sarcomas and melanoma. Despite advances in understanding of second cancer risks following treatment for retinoblastoma, key research questions remained including 1) risks of common adult-onset cancers, some of which have somatic mutations in the *RB1* pathway and are associated with ionizing radiation; 2) persistence of increased risks of bone and soft tissue sarcomas into adulthood; 3) clarification of risks of second cancers following chemotherapy to treat retinoblastoma and 4) role of genetic susceptibility to second cancers following retinoblastoma.

In response to these questions, a large cohort of 1852 long-term survivors of retinoblastoma was assembled to evaluate systematically the risk of second cancers. The work described in this thesis, which comprises six major studies, that have used this cohort to identify a higher risk than previously assumed of lung cancer; confirmed the increased risk of second cancers in survivors with a *RB1* germline mutation and past radiotherapy; documented for the first time that risk of soft tissue sarcomas varied by subtype; demonstrated that mortality from second cancers exceeded that from retinoblastoma; provided new information on variation in second cancer risk by family history of retinoblastoma; and clarified that chemotherapy in addition to radiotherapy for retinoblastoma confers a higher risk for second cancers compared with radiotherapy alone. These studies collectively have provided risk data that can be used to inform survivors and their health care providers to facilitate screening or surveillance and early identification of second cancers.

Preface

The original National Cancer Institute study of second cancers in retinoblastoma survivors was started in 1984 with the aim of estimating the risk of second cancers in relation to radiotherapy. A study of retinoblastoma patients treated in New York had estimated a 90% risk of second cancers in patients who had survived 30 years after their RB diagnosis Abramson et al. (1984). My colleagues at the National Cancer Institute thought that this was a higher than expected risk that indicated a possible reporting bias, i.e. those with a second cancer were more likely to respond to the study than those without a second cancer. Another unusual finding was that the investigators did not find a relationship between increasing radiation dose and second cancers. Therefore, a retrospective cohort of RB survivors was assembled by Drs John Boice and the late Fred Li in collaboration with investigators from two well-known treatment centres for RB - one in New York City (included in the previous study) and one in Boston, Massachusetts with the aim of improving the completeness of follow up of survivors. Prior to my undertaking the current set of analyses, two major analyses in this cohort had been conducted by my colleagues. The first analysis conducted in this cohort was a mortality study of 1601 RB patients (Eng et al., 1993) that reported that patients with bilateral RB (*RB1* germline mutation) had a 26% risk of dying of a second cancer by age 40 years, whereas those with non-hereditary RB (*RB1* somatic mutations) had only a 1.5% chance in the same time period. Following on that study, I collaborated in an incidence analysis (Wong et al., 1997) of the cohort that extended the follow up and found a 51% risk of

subsequent incident cancer by age 50 years in hereditary survivors (*RB1* germline mutation) and a 5% risk in non-hereditary survivors (*RB1* somatic mutations). In addition, we conducted a case-control study within the cohort of soft tissue sarcomas and bone cancers and estimated the dose to the specific cancer site. A significant positive radiation dose-response for an increasing risk of soft tissue sarcomas and bone cancers with increasing dose of radiotherapy for RB was observed in these data. Many of the bone and soft tissue sarcomas had been diagnosed in the head and they were clearly related to past radiotherapy for RB (Wong et al., 1997).

In 1996, I became the lead investigator at the National Cancer Institute for the study of second cancers in RB survivors. The two studies described above by Eng et al (Eng et al., 1993) and Wong et al (Wong et al., 1997) formed the foundation for the subsequent studies that I initiated and conducted in this cohort. As the lead investigator, I continued to monitor the population for risk of second cancers by following up the population using a variety of sources including commercial databases and survivor interviews, ascertaining vital status and cause of death, and initiating a telephone survey to capture new information on second cancers, basic cancer risk factors and current medical procedures and conditions. In addition, I expanded the original cohort (cohort 1) to include more recently treated patients with the goal of evaluating the effect of newer treatments such as chemotherapy on the risk of second cancers in this population. For the new cohort (cohort 2), I sought out the necessary approvals and designed and directed the data collection and follow-up including a

telephone survey to ascertain up to date cancer information and risk factors. In addition, I developed newsletters and a study website (see Appendix 1) to inform the cohort of study findings, make recommendations for cancer screening and answer general questions about the study (https://rbstudy.cancer.gov).

Objective

Quantify the risk of second cancers in a large cohort of long-term retinoblastoma survivors in the United States.

Aims

1) Systematically evaluate the risk of second cancers in a large number of survivors over a long period of time and quantify the risks of second cancers focusing on genetic susceptibility and treatment.

2) Identify the risk of death from second cancers in relation to treatment.

Chapter 1. Retinoblastoma – a rare paediatric ocular tumour

1.1 Introduction

In this chapter, I am providing information on the aetiology and treatment of retinoblastoma and explain what makes it a unique paediatric tumour. I explain about the features that distinguish the two forms of this cancer, and then go on to describe incidence and survival of this malignant ocular tumour. Typical treatments for RB are presented. This section is followed by a discussion on second cancers in general and specifically in relation to treatment for a first cancer. Lastly, I discuss some key previous literature on the risk of second cancers in long-term survivors of RB.

1.2 Aetiology of Retinoblastoma

Retinoblastoma is a rare paediatric cancer of the eye with an autosomal dominant inheritance pattern. It is caused by mutations in the *RB1* tumour suppressor gene, located on chromosome *13q14* (Weinberg, 2007). The *RB1* gene is one of the most commonly mutated genes in childhood cancer (Zhang et al., 2015). Approximately 80%-90% of *RB1* mutation gene carriers develop ocular tumours (Harbour, 2001). The *RB1* gene encodes the cell cycle regulatory RB gene protein (p*Rb*), which controls cellular differentiation during both embryogenesis and in adult tissues, regulates apoptotic cell death, maintains cell cycle arrest and preserves chromosome stability (Burkhart and Sage, 2008). When the *RB1* gene is mutated, it no longer functions to suppress tumours and causes tumours of the retina to form.

1.3 Pathogenesis

Retinoblastoma is a unique type of tumour because it can occur in one of two forms: hereditary (30-40%) and non-hereditary (60-70%). Hereditary (or familial) RB is caused by a germline mutation in one allele of the *RB1* gene and an acquired somatic mutation in the other allele, whereas the non-hereditary form (or sporadic) is caused by somatic mutations in both alleles of the gene, referred to as bilallelic inactivation (Weinberg, 2007) (see Figure 1.1).

The *RB1* germline mutation is either inherited from one parent or the mutation occurs *de novo* during formation of a sperm or an egg. Children born to a parent with RB have a 50% risk of inheriting the *RB1* mutation. The majority (80-94%) of the *de novo RB1* germline mutations originate from the father due to multiple cycles of cell divisions when sperm are formed compared with the relatively smaller number cell cycle divisions preceding formation of eggs in the mother, whereas the parental origin of somatic *RB1* mutations does not show a paternal preference (Weinberg, 2007, Dryja et al., 1989, Zhu et al., 1989).

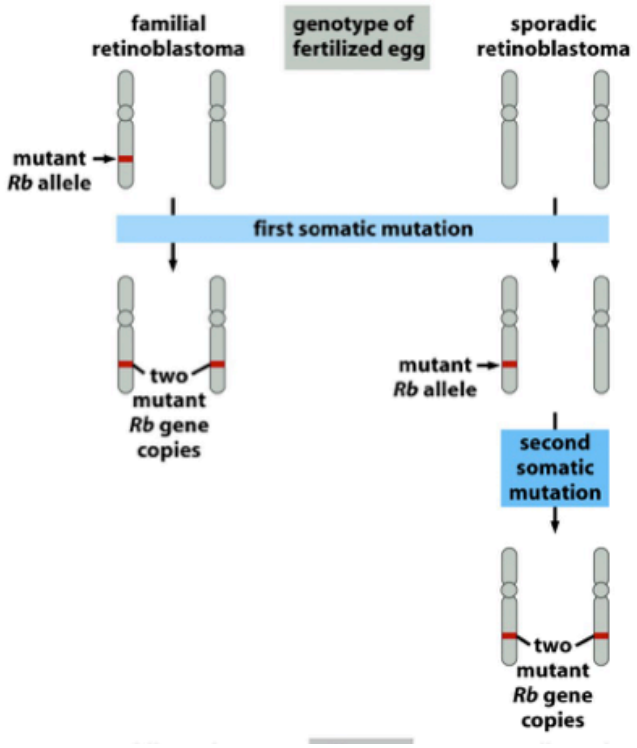


Figure 1.1. Mutations in bilateral and unilateral retinoblastoma (Weinberg R, Cancer 2006). In the familial form, a germline mutation in the RB1 gene is inherited and a somatic mutation occurring at random is required for the disease to appear in the retina in both eyes, whereas in the sporadic form of retinoblastoma, two somatic mutations occur at random in the gene and cause the cancer in one eye.

In contrast to the functional role that the *RB1* gene plays in RB, the *RB1* gene is somatically mutated in many adult cancers such as lung, bladder and colon (Maris and Knudson, 2015). Somatic mutations in the *RB1* gene have been reported in other tumours such as osteosarcomas and soft tissue sarcoma (Friend et al., 1987, Kansara et al., 2014). The loss of *RB1* function is known to be associated with both initiation and

progression of more common adult cancers via several hypothesised mechanisms (Burkhart and Sage, 2008). The frequency of *RB1* inactivation varies by cancer type. Somatic *RB1* mutations have been identified in the pathway of small cell lung cancer (Harbour et al., 1988) and the *RB1* gene is inactivated in 90% of these cancers. *RB1* inactivation is also considered an initiating event in some familial cases of melanoma (Shennan et al., 2000). Progression of prostate, breast, bladder, brain, oesophageal, ovarian and liver cancer as well as chronic myeloid leukaemia have also been attributed to inactivation of the *RB1* gene (Burkhart and Sage, 2008, Sharma et al., 2010, Song et al., 2006).

1.4 Clinical Features

The affected eye or eyes with RB most commonly presents with leukocoria ("white pupil") (about 54%) and less commonly with strabismus (cross-eyes) (19%)(Abramson et al., 2003). RB is sometimes diagnosed as a result of an incidental finding of an abnormal red reflex following flash photography in children (Damasco and Dire, 2011).

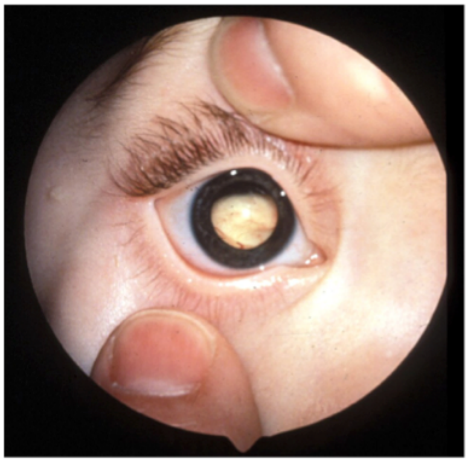


Figure 1.2 Eye with retinoblastoma presenting with leukocoria (Weinberg, 2007).

The hereditary form of RB is characterized clinically by disease in both eyes (bilateral RB) and is typically diagnosed before 12 months of age, whereas, the non-hereditary form affects one eye (unilateral RB) and is typically diagnosed between 2-5 years of age.

These differences in age at diagnosis of bilateral and unilateral RB led Knudson to develop the two-hit theory (Knudson, 1971), in which only one additional somatic mutation or hit is needed for hereditary RB, hence the younger age at diagnosis. However, two somatic mutations (hits) are required for non-hereditary RB, hence the older age at diagnosis, because it takes longer to acquire the two mutations.

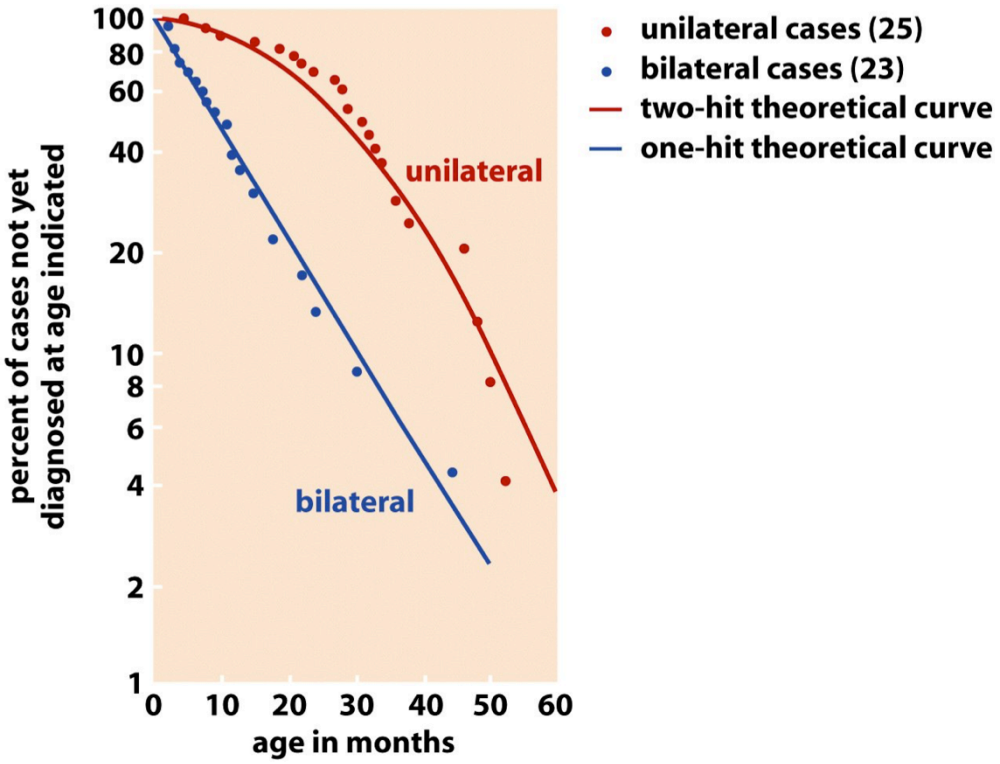


Figure 1.3. Graph shows earlier age at diagnosis of bilateral retinoblastoma cases compared with unilateral cases (Weinberg, 2007).

About 10-15% of patients with unilateral RB carry a germline *RB1* mutation and have a family history of RB. These *RB1* germline mutations are considered to be a less penetrant form of RB because only one eye rather than both have tumours. This type of mutation is sometimes referred to as mosaicism, because only some but not all of the germline genes are mutated. The proportion of unilateral patients with mutations that were mosaic was estimated to be 3.8% (Rushlow et al., 2009) but recent studies have indicated that up to 10% of unilateral patients have a *RB1* germline mutation that was mosaic (Amitrano et al., 2015, Dommering et al., 2014).

Table 1.1 Features distinguishing hereditary and non-hereditary retinoblastoma

Feature	**Hereditary (familial)**	**Non-hereditary (sporadic)**
Type of *RB1* mutation	Germline + somatic	Somatic only
Inherited mutation	Inherited (15%) or *de novo* (85%)	No
Typical age at diagnosis	<1 year	2-5 years
Number of eyes with disease	Both eyes or one eye with family history of RB	One eye only *
Family history of RB	Yes	No
Proportion in general population	40%	60%
Mosaicism	8.8%	1.2 -10%

1.5 Incidence and Survival

The age-adjusted annual incidence rate of retinoblastoma from 2008-2012 in the US for both sexes and all races for ages 0-19 years is 3.2 per 10^7, with a small but significant decline of 0.4 per cent in incidence over the past 37 years (Howlader, 2016). Retinoblastoma is the most common childhood cancer under age 4 for both boys and girls. Among children under one year of age, the incidence was 29.2 per 10^7 children and among ages 1-4, the incidence is 8.7 per 10^7 children. The 5-year relative survival for

the most recent time period (2005-2011) is 97.5% in the U.S. and did not differ by sex (Howlader, 2016). RB is diagnosed in one in 20,000 births, and approximately 300 new cases are diagnosed each year in the United States (Wong et al., 2014). In lower income countries, RB tends to be diagnosed at later stages that negatively affect survival, and is more likely to be non-hereditary that Stiller and Parkin suggest could be related to poor living conditions and a possible infectious aetiology (Stiller and Parkin, 1996).

1.6 Treatment of Retinoblastoma

In high-income countries, patients with RB are usually treated at specialist centres by ocular oncologists or ophthalmologists. Treatment for RB historically has consisted primarily of radiotherapy (both external beam and/or radioactive plaques), enucleation (removal of the eye), chemotherapy, focal therapies such as laser or cryotherapy, or a combination of these modalities depending upon the extent of the cancer, laterality and the ability to preserve vision (Rodriguez-Galindo et al., 2015). For children with unilateral RB, the eye is often removed (enucleation) and no further treatment is needed. These children are fitted with a prosthetic eye that is periodically replaced as the child grows older. However, for children with bilateral RB, one eye is often removed and the other eye is treated with radiation (either fractionated external beam radiation or radioactive plaque depending upon the location and size of the tumour) to preserve sight in the less diseased eye. The location of the tumour within the eye determines the likelihood that external beam radiation will be successful. In addition, depending upon the stage of the RB, both eyes may be treated with radiation or both eyes may be removed. Although external beam radiation may preserve sight in the eye, there is often cosmetic facial

deformity of the orbital bones by the radiation because orbital growth that is in progress during early childhood is disrupted (Rodriguez-Galindo et al., 2015).

Systemic chemotherapy has been used since 1950 to treat RB mainly in combination with radiotherapy; treatment patterns shifted in the 1990s with less use of radiotherapy to greater use of chemotherapy to treat RB. Recent trends in the US indicate a significant decline in use of radiotherapy to treat RB from 30.5% in the 1980s to 2.6% after 1999 pointing to the increased use of chemotherapy (Shinohara et al., 2014).

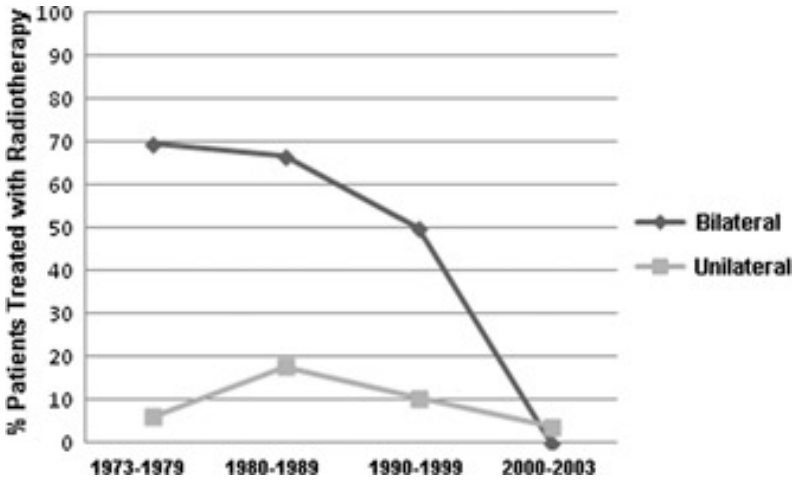


Figure 1.4. Radiotherapy trends by calendar year by laterality (Shinohara et al., 2014)

In the last decade, there has been a significant shift to the use of ophthalmic artery chemosurgery (local administration of chemotherapy to the ophthalmic artery) and intravitreous chemotherapy (Gobin et al., 2011) (Abramson et al., 2015b), in order to spare children exposure to systemic chemotherapy, which has been linked to the development of acute myeloid leukaemia in RB survivors (Gombos et al., 2007). This new

method continues to be refined and used to treat advanced unilateral and bilateral RB patients (Abramson et al., 2015a, Abramson et al., 2015b).

Table 1.2 Retinoblastoma treatments by laterality and decade in the US cohort

Years of Treatment	Bilateral – typical treatments	Unilateral – typical treatments
1914-1949	Enucleation in one eye and radiation (fractionated external beam or brachytherapy) in other Radiation in both eyes Enucleation in both eyes	Enucleation Radiation
1950s-1960s	Enucleation in one eye, radiation in other eye and systemic chemotherapy (Triethylenemelamine)	Enucleation Radiation
1970s-1990	Enucleation in one eye, radiation in one eye and systemic chemotherapy Focal therapies: e.g. laser, cryotherapy	Enucleation Chemotherapy
1990s-	Focal therapies: laser photocoagulation, cryotherapy and brachytherapy Enucleation and systemic chemotherapy External beam radiation when other treatments have failed.	Enucleation Chemotherapy
2000-2006	Chemotherapy (intra-arterial, intravitreal or systemic)	Chemotherapy (systemic or intra-arterial or intravitreal) and focal therapies

1.7 Second cancers

Second cancers have become an important concern for childhood cancer survivors due to major improvements in treatment and increased survival of the first cancer (Reulen et al., 2011, Morton et al., 2014b). Second cancers represent 17-19% of all new cancers in children and adults (Morton et al., 2014b). In order to study second

cancers in paediatric cancer populations, large numbers of patients are needed, because childhood cancers are rare and many years of follow up are required to study increased risks with sufficient statistical power (Morton et al., 2014b).

Second cancers are histologically different malignant tumours that develop in patients with a first cancer. Second cancers can be diagnosed at the same time as the first cancer (synchronous cancer) or at later time (metachronous tumours). Treatment, genetic predisposition, host, medical, lifestyle and environmental factors such as tobacco can all contribute to the risk of second cancers. However, the most important factors that account for the risk of second cancer in children and young adults are treatment for the first cancer and genetic susceptibility (Morton et al., 2014b).

Both radiotherapy and chemotherapy have been linked to increased risks of second cancers in children and adults (Morton et al., 2014a). Multiple studies of paediatric and adult cancer survivors have reported that second solid cancers typically develop 10-15 years after radiotherapy (Friedman et al., 2010, Ng and Travis, 2008). Patients are exposed to multiple sources of scatter radiation during treatment from the external beam itself, the collimator head as well as scatter throughout the body.

Chemotherapy for treatment of a first cancer, in particular alkylating agents or epidophyllotoxins are known to cause DNA damage that can lead to diagnosis of an acute myeloid leukaemia as early as 18 months after the first cancer (Morton et al., 2014a). Recently, studies of childhood cancer survivors have reported increased risks of selected second solid tumours related to chemotherapy for a first cancer (Henderson et al., 2015, Swerdlow et al., 2011).

Genetic susceptibility or inherited cancer syndromes, such as retinoblastoma, Li Fraumeni, neurofibromatosis 1 (NF-1) and Nevoid Basal Cell Cancer (NBCCS) syndromes increase risk of specific cancers due to the presence of germline mutations, and exposure to radiotherapy may increase the risk of additional cancers (Kleinerman, 2009, Zhang et al., 2015). For example, children and adults with Li-Fraumeni syndrome are at increased risks of sarcoma, breast cancer and adrenocortical cancers due to a germline *p53* mutation. Radiotherapy to treat these cancers has been reported to be related to the risk of another cancer (Hisada et al., 1998).

Environmental factors such as sun exposure (ultraviolet radiation) or lifestyle factors such as smoking tobacco can increase the risk of second cancers either independently or through an interaction with other factors, although these factors are more of an issue for adult than paediatric patients (Morton et al., 2014b).

1.8 Second cancers after retinoblastoma: Literature review

Second cancers have been recognized as a risk among hereditary retinoblastoma survivors in numerous case reports, several clinical series and in two population-based cohorts in the UK (MacCarthy et al., 2013) and the Netherlands (Marees et al., 2008b). These two population-based cohorts are the most relevant to the US cohort in size, length of follow-up and risk estimates for second cancers (Table 1.3).

Table 1.3. Risk of second cancers in cohort studies of long-term survivors of retinoblastoma

		Hereditary			Non-hereditary		
Cohort	Diagnosis years	No. of patients	No. 2nd cancers	SIR[1]	No. of patients	No. 2nd cancers	SIR[1]
USA (n=1601)[2], [3]	1914-1984	963	260	19 (16-21)	638	17	1.2 (0.7-2.0)
UK (N=1927)[4]	1951-2004	806	112	14 (11-16)	1121	20	1.5 (0.9-2.3)
Netherlands (n=608)[5]	1945-2005	298	62	20 (16-26)	370	12	1.9 (1.0-3.2)

[1]SIR, Standardised incidence ratio and 95% confidence interval. This is the number of observed cancers divided by the number of cancers expected from the general population.
[2] Number in parentheses is the total number of subjects in the cohort.
[3] (Kleinerman et al., 2005) 1-year survivors, hospital-based
[4] (MacCarthy et al., 2013) 3-year survivors, population-based
[5] (Marees et al., 2008a) all survivors, register-based

Initially the UK cohort was comprised of 884 children diagnosed with RB from 1962-77. Draper et al (Draper et al., 1986) reported a cumulative risk of 8.4% for all second cancers after 18 years and 6.0% for osteosarcoma among the 384 hereditary RB survivors. Within the field of radiation, the cumulative risk was 6.6% for all second cancers. This study also suggested relationships between cyclophosphamide and second cancers and melanoma in hereditary RB survivors. Subsequent study by Hawkins et al (Hawkins et al., 1987) in 363 three-year hereditary survivors reported cumulative risks of second cancers at 15 years after treatment were 2.7% for surgery only, 6.8% for radiotherapy alone and 13% for radiotherapy and chemotherapy. Further modelling of

the risk of osteosarcomas in relation to chemotherapy and radiotherapy in this population indicated a positive dose-response for increasing risk with increasing dose of both types of treatment (Hawkins et al., 1996). MacCarthy et al have recently reported increased risks of second cancers similar to those noted in our cohort (MacCarthy et al., 2013). Although that study did not report treatment data, it did report risks within the head and neck region and outside the region, which served as a surrogate variable for radiation to the head.

DerKinderen studied 141 children diagnosed with hereditary RB from 1945-1970 in the Dutch Retinoblastoma Registry and reported a cumulative risk of second cancers of 19% at 35 years (Derkinderen et al., 1987). Moll et al (Moll et al., 1996) updated the Register to 1994 and reported a cumulative incidence of 17.7% for second cancers in 639 hereditary survivors. This cohort has been expanded to include more recently treated patients and continues to be followed for risk of second cancers in relationship to treatment (Marees et al., 2008b, Marees et al., 2010). Their findings are very similar to the US cohort in terms of relative risk estimates for all second cancers in relation to radiotherapy. The UK and Dutch cohorts however excluded pineoblastomas as independent second cancers in their studies, whereas we have included them in the US studies, because they all occurred at least one year after RB diagnosis.

The earlier studies of second cancers after RB from the Netherlands (Derkinderen et al., 1987) (Moll et al., 1996) and the UK (Draper et al., 1986) (Hawkins et al., 1996) preceded development of the US cohort of RB patients. However, several of the follow-up studies of these same cohorts were published either after or contemporaneously with

the publication of the initial US studies (Fletcher et al., 2004, Marees et al., 2008b, MacCarthy et al., 2013). The next chapter presents the development and details of the US cohort of RB patients.

Chapter 2. Description of the National Cancer Institute retinoblastoma cohort

2.1 Introduction

This chapter describes the source of study population that comprises the US cohort, methods of data collection, ascertainment of second cancer incidence and mortality as well as treatment. The statistical methods used in the analyses that are presented in the subsequent chapters are described in this chapter.

2.2 Overview of the cohort

The National Cancer Institute (NCI) retinoblastoma study is a multi-institutional, hospital-based retrospective cohort design with long-term follow up of subsequent cancer incidence and mortality.

RB patients were originally identified from medical records at two medical centres: in New York and in Boston. These two medical centres were selected because they are major treatment centres for RB and the clinical investigators at these sites wanted to collaborate in the research. Study subjects were identified solely through medical records at these two treatment centres and the subjects were treated between 1914-1984 (cohort 1) and 1985-1996 (cohort 2).

The Special Studies Institutional Review Board (SSIRB) of the NCI as well as the Institutional Review Boards (IRBs) of the participating medical centres approved the study. Continuing annual IRB review of the study and approval is obtained from the NCI.

2.3 Study population

Eligible study subjects had to have been diagnosed with either unilateral (one eye affected) or bilateral (both eyes affected) RB as determined by diagnoses recorded in medical records. All bilateral patients were classified as hereditary as well as unilateral patients with a documented history in the medical record of RB in a first or second degree relative, excluding having a child with RB. All other unilateral patients were classified as non-hereditary. There were no age restrictions on eligibility. All patients had to be US residents so they could be followed with available US databases (e.g., the US National Death Index, NDI) and they had to survive at least one year after their RB diagnosis. This restriction was included in order to assure that the subsequent cancer was diagnosed at least one year after the RB and was not a synchronous tumour nor likely to be associated with intense medical surveillance during the first year after RB diagnosis.

Cohort 1: The original cohort (Cohort 1) was the larger of the two cohorts and included RB patients treated in New York or Boston from 1914-1984. Medical records were found for a total of 1,729 RB patients who were treated during this time period. We excluded 114 (6.4%) patients who died within 12 months of diagnosis of RB, 11 (0.6%) who died outside the US, 2 (0.1%) patients with an unknown birth year, 1 (0.1%) patient who was determined not to have RB, which left 1,601 (92.7%) one-year survivors of RB eligible for study.

Cohort 2: I expanded the original cohort to develop cohort 2 in order to capture information on patients who were treated more recently and therefore were more likely

to have been treated with newer treatments such as chemotherapy. Medical records were identified for 262 RB patients, however we excluded 7 patients (2 died within 1 year, 2 were seen for consultation only and 2 were lost to follow-up (no usable tracing information). After these exclusions, cohort 2 is comprised of 253 RB patients treated from 1985-1996 at one medical centre in New York. I selected the New York centre due to the large volume of RB patients treated annually at that institution.

The proportion of hereditary (60%) and non-hereditary (40%) RB patients in the study (cohorts 1 and 2) differs from the proportion in the US general population in which only 40% have hereditary disease and 60% have non-hereditary disease. This is likely due to the source of the RB patients from major medical centres specialising in the treatment of RB. Because RB is a rare disease as are second cancers, the advantage of having more hereditary patients in the cohort allowed a larger pool of patients who were more prone to developing second cancers, and thus we were able to conduct more detailed analysis of second cancers.

2.3 Data collection

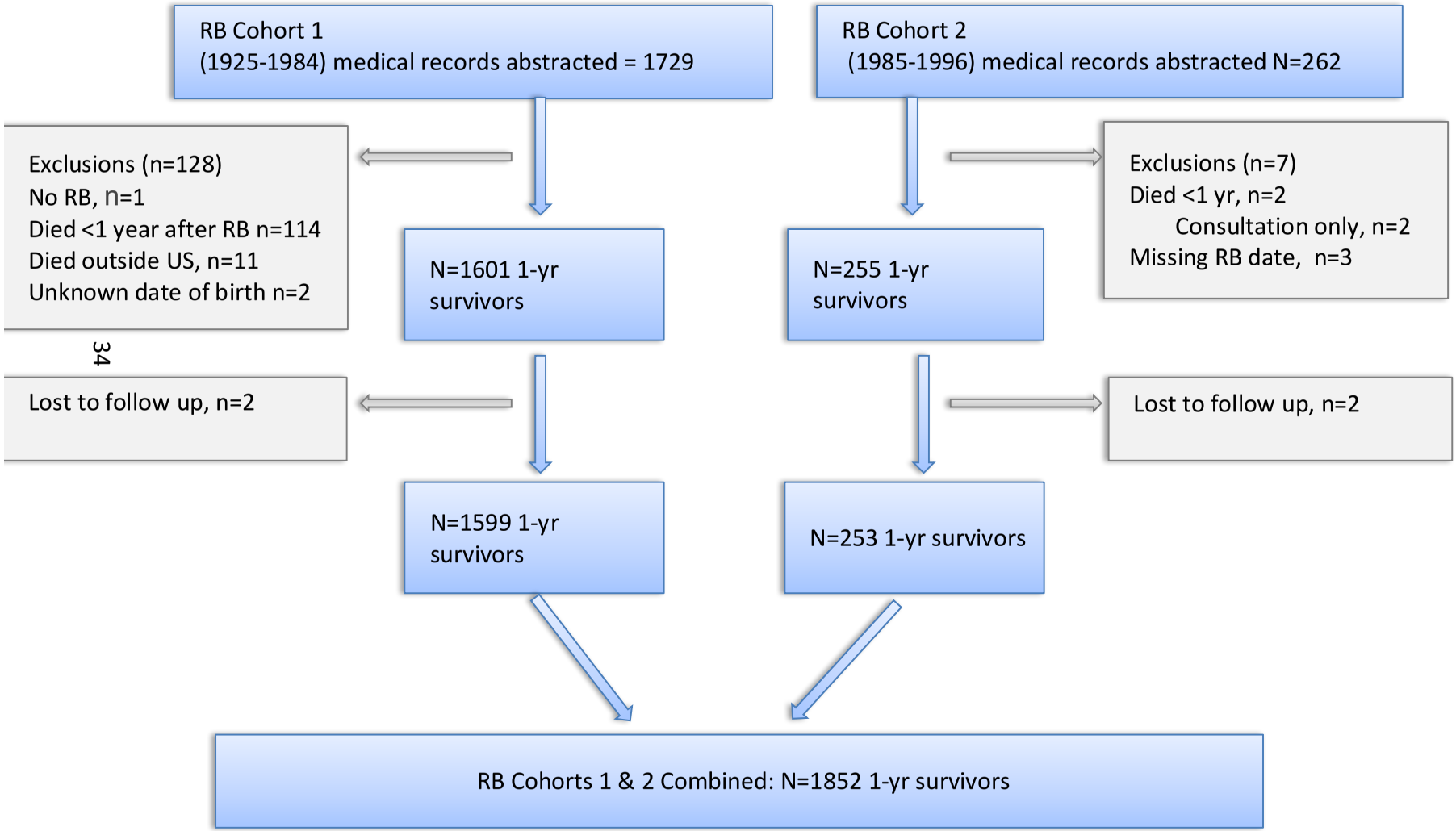
Data collection efforts consisted of two phases and were similar in both cohorts -- 1) medical record abstraction to establish baseline data on diagnosis and treatment, and 2) Follow up via interview and electronic data linkages to determine vital status and identify second cancers.

Using a standardised medical record abstract form (Appendix A1), trained medical record abstractors recorded baseline diagnosis and treatment information from hospital records including dates of diagnosis and treatment, laterality, mention of family history

of RB, type of treatment (radiotherapy – external beam, radioactive plaque or both), chemotherapy (name of drug), surgical procedures – type specified including removal of eye or enucleation) as well as any mention of a subsequent cancer or death.

For cohort 2, we initially reviewed the roster of RB patients manually to identify patients. Because patients made multiple visits to the hospital ocular oncology clinic, we needed to be sure that we were only counting each patient one time to develop the cohort. Next we abstracted data from medical records for these patients using the same medical record abstract form as cohort 1 and recorded contact information so that we could contact either the survivors or their parents, if under age 18, to gain additional information about subsequent cancers. We contacted this same group by telephone in 1998 and 2009 to update their subsequent cancer information. (Figure 2.1)

Figure 2.1 Description of Cohort



2.4 Vital status and death ascertainment and follow up procedures

The lack of available personal identification numbers and a national cancer registry in the US makes locating study subjects, updating vital status and obtaining second cancer information very challenging. As a result, we used a variety of sources to ascertain vital status and current address that included post office correction updates, commercial credit bureau linkages, social security administration linkage, individual tracing through publicly available sources and linkage with the NDI.

The Social Security Administration (SSA) is responsible for paying survivor death benefits to US citizens. They maintain mortality files as well as a 'presumed living' file for people with a social security number who have not collected death benefits. We linked our study subjects with a social security number (about 90%) with the SSA to ascertain current vital status. Ascertainment of death from the Social Security Death Master File relied on submitting a correct having a social security National Death Index depended upon having good matching information for linkage.

The NDI provides information on deaths for 87%-98% of the US population (Cowper et al., 2002). Individual states have to report date and cause of death to the NDI. Cause of death has been available since 1979 and coded cause of death since 1984. There is a two-year lag in reporting deaths by the NDI. The NDI uses a matching algorithm to determine matches, and the best matches are those that are an exact match on date of birth and social security number. If a social security number is missing or the subject has a common name, then the match would be less certain. When the NDI reports death matches, we review the matches and in some cases we may reject the

match if we think it is not the same person because of a difference in place of birth, for example. Over the lifetime of the cohort, we have been linking the cohort with the NDI usually every 2-3 years rather than every year due to the cost involved in the linkage. We estimate that we may be missing up to 5% of the deaths in this study, based on reports from relatives for deaths that we missed because the subject either died out of country or was missed by the NDI.

For deaths prior to the establishment of the NDI, we had requested death certificates from the individual state where a patient died, unless the death certificate was available in the medical record. In addition, using multiple vital status tracing methods has been reported to enhance mortality follow-up in large cohort studies (Schall et al., 2001).

In order to collect cancer incidence data, we conducted multiple telephone interviews in 1987, 1993, 1996, 2000 and 2009 to ascertain current health, subsequent tumours (benign and malignant) and basic cancer risk factor data (2000 telephone survey only).

2.5 Ascertainment of second cancers

All subsequent invasive cancers were ascertained from medical records, physician notes, autopsy reports, interviews with subjects or their parents, and the NDI. There is no national cancer registry in the US. There are individual state cancer registries but they vary in quality and years of coverage. Given the lack of a national cancer registry in the United States, we were primarily dependent on contact with survivors to obtain incident cancers. If we were able to contact a survivor then we had current information. But if we

were unable to contact a survivor whom we knew was alive and/or they refused to participate, then we had no information on their second cancers, unless there was already documentation of a second cancer in the medical record from when we abstracted medical records at the beginning of the study in 1984. The majority of the cohort had been treated for RB in the 1960s, and we did identify 80 confirmed second cancers at the time of the medical record abstraction. If subject had died prior to 1984 or since that time and we were never able to contact them to ask about second cancers, then we relied on what we found in their medical record at the time of start of the study in 1984 and/or at their cause of death if it was a cancer.

Because many of the subsequent tumour reports were self-reports, we attempted to confirm these by obtaining pathology reports from the hospital where the cancer was diagnosed. Persons trained to code cancers coded all pathology reports according to the International Classification of Diseases for Oncology (ICDO version 2 or 3 according to the year of diagnosis) and coded all death certificate reports according to the ICD version corresponding to the year of death. All analyses were conducted using only confirmed cancer reports. If the same second cancer was reported on both a pathology report and on a death certificate as well, we used the incident cancer confirmed by the pathology report as the preferred source for purpose of analysis, unless we were specifically analysing mortality patterns. The end of the follow-up varied depending upon the type of analysis. For mortality analyses, the end of follow-up was the date of the most recent deaths reported by the NDI. The end of follow-up for the

incident cancers was based on the date of the most recent survey available at the time of the analysis.

2.6 Treatment

2.6.1 Radiotherapy

Most hereditary patients (85%) in this cohort were treated with radiotherapy whereas non-hereditary patients were not (18%). Patients were treated typically with external beam radiotherapy (90%), radioactive plaques (1%) or both (9%). Before 1960, most patients received external orthovoltage x-irradiation. After 1960, the beam energies increased, and patients were treated with 22 to 23 MV Betatron megavoltage photons or cobalt-60 gamma rays. The higher energy beams deposited more radiation at the eye with somewhat less scatter radiation to surrounding normal tissues.

We collaborated with the Medical Physics department at M.D. Anderson Medical Center (Houston, Texas) to estimate the sources of scatter radiation from a typical radiation treatment to organs in the body of a 1-year old and an infant (6 months) with RB. Based on the available radiation records, medical physicists used a water phantom to measure the scatter radiation to the patients from three potential sources (radiation leakage from the collimator of the external beam machine, leakage from the head of the machine, and scatter from within the body from the radiotherapy) for the various external beam energies. These measurements were then applied to mathematical phantoms that could be scaled to the size of the child being treated taking into account the field size (Stovall et al., 2006). Typical radiation scatter doses to organ sites are

presented in Table 2.1 for a one-year old treated for RB with Orthovoltage and Betatron radiotherapy. These dose data permitted us to categorise patients according to whether their second cancer was heavily, moderately or lightly irradiated as well as diagnosed in or out of the radiation field.

Table 2.1 Typical Radiation Doses (Gray)* to organ sites in a 1-year old

Organ site	Orthovoltage (<1960)	Betatron ≥1960
Brain	3.6	1.6
Eye treated	60	45
Eye untreated	18	34
Nasal region	34	3.2
Salivary	4.3	1.6
Head (soft tissue)	22	11
Facial bones	28	8
Thyroid	2.0	0.9
Lung	0.5	0.4
Breast	0.4	0.4
Kidney	0.1	0.3
Stomach	0.2	0.4
Colon	0.1	0.2
Bladder	0.1	0.2
Uterus	0.1	0.2
Bone marrow	1.2	1.0

* Left eye treated, 4 cm x 4cm, 50 Gy given dose to lateral & nasal fields
Source: personal communication from Marilyn Stovall, MD Anderson Cancer Center

2.6.2 Chemotherapy

Twenty-eight percent of patients in cohorts 1 and 2 were treated with chemotherapy and 88% of these patients were also treated with radiotherapy. Beginning in the 1950's through 1970, RB patients in this cohort received an alkylating agent called

TEM (Triethylenemelamine). After 1970, survivors were treated with a range of other alkylating agents, including nitrogen mustard, cyclophosphamide and thiotepa. Very few patients (2%) who received chemotherapy were treated with non-alkylating agent chemotherapy in this cohort. Because the main focus of the study of second cancers in RB survivors was risk in relation to radiation, little emphasis was placed on collecting details of the chemotherapy other than name of agents and dates. In total, there were 36 different combinations of chemotherapeutic agents that we grouped into six categories based on their toxicity. Dose data were generally not available. If we decide in the future to conduct another case-control study of sarcomas, where we have noted an increased risk related to chemotherapy in addition to radiation, then we would attempt to retrieve information on chemotherapy dose from hospital records. We would have to conduct a small feasibility study first to find out whether the records are still available and the level of detail provided.

2.7 Statistical Methodology

The purpose of the analyses was to quantify the effects of treatment on the risk of a second cancer in the RB cohort. The majority of the analyses evaluated the risk of incident second cancers in the cohort. We used rates from two US registry sources (Connecticut Tumor Registry [1935-72] and the SEER 9 registries [1973-present] to calculate expected number of cancers. The Connecticut Registry was used because it was the only US cancer registry with data as far back as 1935. In addition, the population of Connecticut was similar racially, ethnically and economically to that of New York and Boston, where the cohort was treated. We also used the SEER nine registries to derive

expected numbers of cancers because it provided a larger population base than Connecticut. Unless otherwise specified in a particular analysis, all person-year accrual began one year after diagnosis of RB and ended at date of second cancer diagnosis, date of death, date of last contact, or end of study date, whichever occurred earliest. We excluded the person time and events during the first year after RB diagnosis because we wanted to exclude any simultaneously diagnosed cancers as well as deaths due to RB.

For analyses that investigated the specific causes of death among RB patients, we compared the causes of death in the cohort with the US mortality rates. As with the incidence analyses, follow-up began one year after RB diagnosis but ended on the date the patient was last known to be alive, date of death or end of follow-up (date of the NDI search), whichever occurred earliest. As with the incident analyses, we excluded the person time and events during the first year after RB diagnosis because we wanted to exclude any simultaneously diagnosed cancers as well as deaths due to RB.

The specific methods used in both types of analyses are described below and within each chapter.

2.7.1 Standardised incidence ratio (SIR)

We estimated SIRs and 95% confidence intervals (CI) for the risk of second cancers compared with the incidence of these cancers in the general population. We divided the observed number of second cancers by the expected number of cancers based on age, sex and 5-year calendar year-specific incidence rates from two sources – the Connecticut Tumour Registry rates for 1935-1972 and SEER rates (Surveillance,

Epidemiology and End Results program) for 1973 onwards. Rates were not adjusted for race or ethnicity. Confidence intervals were calculated using the Wald method (Breslow and Day, 1987). We compared the incidence of observed second cancers relative to those expected from the general population, because the general population rates provided the most stable rates for comparison of risk for many different types of second cancers, including histologic types of soft tissue sarcomas. Comparison of SIRs for specific characteristics of RB survivors (e.g. SIR for patients treated <1 year of age RB diagnosis versus SIR for ≥1 year of age) was based on the chi-square test of homogeneity (Breslow and Day, 1987).

2.7.2 Cumulative incidence

We used the Gooley method (Gooley et al., 1999) to calculate the cumulative incidence per cent and 95% CI of second cancers after RB diagnosis up to 50 years later. For the cumulative incidence of all second cancers combined, that is the probability of developing a second cancer by the number of years after treatment for RB, deaths from RB and all other non-second cancer causes were considered as a competing risk. For the cumulative incidence of a specific second cancer, deaths from other second cancers were treated as a competing risk, because deaths from other causes would have precluded the occurrence of the specific cancer of interest. Practically, this means that patients were no longer at risk from the time of competing event. *P*-values were calculated according to the method of Gray (Gray, 1988) and conducted using the *cmprsk* in R statistical software (http://www.r-project.org).

2.7.3 Standardised mortality ratio and cumulative mortality

We estimated standardised mortality ratios and exact Poisson 95% CIs (Monson, 1974) by dividing the observed number of deaths by the expected number from the general population by applying US mortality rates (by 5-year age, 5-calendar year and sex-specific categories) to the appropriate person-time accrued by the RB survivors. US mortality rates were available from 1925 onwards. For the few survivors diagnosed prior to 1925, we reset their start date to January 1, 1925.

Cause-specific cumulative mortality up to 50 years after RB diagnosis was calculated using the *cmprsk* program in *R* statistical software *(*http://www.r-project.org*).* Causes of death from other than the specific second cancers of interest were treated as competing risks.

2.7.4 Excess absolute risk (incidence and mortality)

We calculated the excess absolute risk (EAR) as the observed number of second cancers minus the expected number of second cancers divided by the person years at risk times 10,000. This estimate provided the burden of incident second cancers or burden of mortality from specific second cancers.

2.7.5 Univariate and multivariate analyses

In order to evaluate the effect of modifying variables such as age at exposure, year of exposure, sex and type of treatment on the risk of second cancers, we fitted a Poisson regression model that evaluated the association of each variable individually

(unadjusted or univariate relative risk) and simultaneously adjusted for all other variables in the model (adjusted or multivariate risk) using Epicure software (Preston DL, 1993). The statistical significance of each variable was assessed by a likelihood ratio test comparing the model with the variable of interest to the model without the variable. Two-sided *P* values <0.05 were considered statistically significant. The variables were stratified according to hypotheses associated with particular attributes. For example, calendar year of RB diagnosis was stratified into three categories, i.e., before 1960, 1960-69 and 1970+ to reflect radiotherapy treatment trends. Prior to 1960, orthovoltage external beam was used and after 1960, cobalt-60 and betatron external beam was use. Although chemotherapy began to be used more commonly after 1990, there were too few survivors treated exclusively with chemotherapy during this time period to be able to evaluate the risk of second cancers with modern chemotherapy. The majority of survivors in the cohort were treated in the 1960s. Age at RB diagnosis was classified as <12 months, 12-23 months and ≥24 months. An early analysis of the New York cohort of survivors (Abramson and Frank, 1998) indicated that children with RB treated at <12 months of age are more susceptible to the harmful effects of radiotherapy to induce second cancers compared with older children.

For the detailed chemotherapy analyses of risk of second cancers (see chapter 8), we used a Cox proportional hazards regression model with age as the time scale to investigate the risk of second cancers in different treatment subgroups (e.g. with and without alkylating agents, TEM) using SAS, version 9.3. These models were stratified by

calendar year of diagnosis to account for the temporal changes in treatment practices for RB. Two-sided *P* values <0.05 were considered statistically significant.

Chapter 3. Risk of lung cancer and smoking

Kleinerman RA, Tarone RE, Abramson DH, Seddon JM, Li FP, Tucker MA. Hereditary retinoblastoma and risk of lung cancer. J Natl Cancer Inst 92;2037-39, 2000

3.1 Introduction and rationale

Somatic mutations in the *RB1* gene contribute to the risk of lung cancer and these mutations have been identified in small cell lung cancer (Harbour et al., 1988). However, at the time of this study, it had been hypothesised but was unknown whether RB survivors were at increased risk of lung cancer, because none had been reported in the previous mortality analysis, likely due to the median years of follow up the cohort of only 17 years (Eng et al., 1993). Because lung cancer is a fatal cancer, we investigated risk of dying of lung cancer in our cohort of RB survivors. This chapter describes this investigation.

3.2 Methods

We conducted a search of the National Death Index (NDI) to identify new causes of death through the end of 1997. This added 7 more years of follow up since the previous NDI search in 1990 that was used as the basis for the publication of the original study of this cohort (Eng et al., 1993). The entire cohort 1 was submitted to the NDI to identify new deaths in the cohort. At the time of the analysis, cohort 2 was not yet available for analysis. If the subject was not matched to a record in the NDI, we assumed

that they were still alive. For subjects who matched, we used the underlying cause of death for analysis.

We evaluated the risk of dying from lung cancer in 1604 RB patients (964 hereditary and 640 non-hereditary). Overall, 16.5% (n=264) of the survivors were 40 years and older (median age=24 years) and 24% had died (n=381). Vital status differed by hereditary status with a larger proportion of deaths in the hereditary patients compared with non-hereditary patients (32% vs. 11%) (Table 3.1).

Table 3.1 Characteristics of 1604 one-year survivors of retinoblastoma

Characteristic	Hereditary n=964	Non-hereditary n=640
Sex	**No. %**	**No. %**
Male	513 (53.2)	334 (52.2)
Female	451 (46.8)	306 (47.8)
Age at last follow up		
<40 years	825 (85.6)	515 (80.5)
40+ years	139 (14.4)	125 (19.5)
Vital status		
Alive	604 (62.7)	519 (81.1)
Lost to follow up	51 (5.3)	49 (7.7)
Death	309 (32.1)	72 (11.3)

We conducted a Standardised Mortality Ratio (SMR) analysis by dividing the observed deaths in the RB cohort by the expected number of deaths from the general population based on the person-years at risk and adjusted for age, sex and calendar year of the death. Analyses were stratified by hereditary status.

3.3 Main findings

Overall, we found an increased risk of death from all cancers in hereditary patients (observed=129, SMR=47, 95%CI 39-56) compared with non-hereditary patients

(observed =10, SMR=3.8, 95%CI 1.8-7.0). Over the 7 years of additional deaths from NDI since the previous study by Eng et al (Eng et al., 1993), 50 new deaths had occurred, mainly cancers of the bone (n=12), connective tissue (n=13) and lung (n=5). We noted a statistically significantly elevated risk of dying from lung cancer (observed=5, expected=0.33, SMR=15.2, 95%CI 4.9-35). All of the lung cancer deaths occurred in hereditary patients and only 2 of the 5 were diagnosed in patients who had received radiotherapy (Table 3.2). Additionally, all five deaths were in females, and 75% of those with histology were small cell carcinomas. We were able to document smoking history and found 4/5 cases had smoked. Most notably, 3/5 deaths were in women 40 years of age or younger.

Table 3.2 Characteristics of the lung cancer cases

Obs.	Sex	Radio-therapy	Lung cancer histology	Age at death, years	Smoking history
1	Female	Yes	Unknown	39	60 pack-yrs
2	Female	No	Small cell/large cell mixed	40	19 pack-yrs
3	Female	Yes	Small cell	40	20 pack-yrs
4	Female	No	Adenocarcinoma	52	Non-smoker
5	Female	No	Small-cell	64	Modest smoker for many years

Although there have been case reports of lung cancer in *RB1* mutation carriers and mostly in males (Smith and Bedford, 1976), a UK study of 4101 relatives of RB patients including 117 *RB1* carriers reported 10 lung cancers in *RB1* mutation carriers with a similar SMR to our study (SMR=15.4, 95%CI 6.6-30) (Sanders et al., 1989). Overall, our results together with the earlier reports establish that germline *RB1* mutations

confer an increased risk of dying of lung cancer. Previous reports of lung cancer in males likely points to a chance occurrence of lung cancer in females in our study and could be due to higher smoking rates in men than women in earlier time periods.

The number of lung cancer deaths was small, however, they were only diagnosed in hereditary patients. Four of the five lung cancer deaths were confirmed by pathology reports as well. Several factors support an increased susceptibility of carriers of a *RB1* germline mutation to smoking-related lung cancer. These include the young age at onset of lung cancer, histology (small cell lung cancer), and positive smoking history. Although tobacco use in the entire cohort was incomplete and this is a major factor for lung cancer, we were able to obtain some preliminary cigarette smoking data from a telephone survey of the cohort conducted in 2000 that indicated that hereditary patients were no more likely to smoke than non-hereditary patients, yet no lung cancers deaths were diagnosed in the non-hereditary patients (Foster et al., 2006). Smoking rates of 17% in both the hereditary and non-hereditary survivors were similar to the US population.

3.4 Significance

This study provided epidemiological evidence for increased risk of lung cancer mortality in RB survivors, which had been hypothesised based on previous biologic evidence of *RB1* gene somatic mutations identified in the pathway of small cell lung cancer. The results from this study (Kleinerman et al., 2000) were cited in an editorial in 2004 as evidence of a clinical relationship between a RB germline mutation and small cell lung cancer that was originally identified through a molecular genetic study: *"For many years, the intuitive notion has been that a subset of tobacco users*

exhibits a genetic susceptibility to developing lung cancer, but there was never evidence to directly link a specific genetic locus to this elevated risk. The RB1 gene may now serve as yet another paradigm for the concept of a genetic susceptibility locus for lung and bladder cancer among tobacco users" (Kaye and Harbour, 2004).

3.5 Public Health Message

Survivors with hereditary RB should be targeted for smoking cessation because they are at greater risk of dying from lung cancer. On the RB study website, we have provided recommendations for survivors to quit smoking as well as sources to contact for support.

3.6 Role in the study

Role: I initiated the study, analysed the data in consultation with Dr Tarone and drafted the manuscript. Study team: Dr Tarone was the statistician, Drs Abramson and Seddon were the clinical collaborators and provided the patients; Drs Li and Tucker were the senior study investigators. My contribution: 75%

3.7 Publication

Chapter 4. Risk of second cancers due to radiotherapy

Kleinerman RA, Tucker MA, Tarone RE, Abramson DH, Seddon JM, Stovall M, Li FP, Fraumeni, JF, Jr. Risk of new cancers in long-term survivors of retinoblastoma: An extended follow-up. J Clin Oncol 2005;23:2272-9

4.1 Introduction and rationale

Based on previous studies in this cohort indicating statistically significant increases in second cancers related to past radiotherapy for RB (Eng et al., 1993) (Wong et al., 1997), and the hypothesis that the risk of second epithelial cancers would be expected to increase above background rates as the cohort ages (Kaye and Harbour, 2004), we surveyed the cohort in 2000-2001 to update the information on second cancers. In this analysis, we provided new information on cancer risk based on seven additional years of follow-up. This chapter provides a summary of those findings.

4.2 Methods

This study was conducted in cohort 1 only (n=1601 survivors), because cohort 2 was not available for analysis. We updated the vital status and the contact information for survivors in 2000. Next, I designed a survey to be conducted among living members of the cohort (computer aided telephone interview) (Appendix A.1) to ascertain basic cancer risk factor information and obtain diagnoses of new cancers. There were 1100 living individuals and we were able to successfully contact 875 (75%) (Foster et al., 2006).

At the time of the survey conducted in 2000 (Foster et al, 2006) for cohort 1, we found through various tracing efforts that 1169 (73%) of subjects were alive, 385 (24%) were deceased and 47 (2.9%) were lost to follow up. Lost to follow-up was defined as any study subjects for whom we had no information that they were alive or dead based on linkages with the National Death Index and Social Security Administration. Of the 1169 eligible subjects, 875 (75%) responded to the computer-aided telephone questionnaire and 294 (25%) did not respond. Non-responders did not differ significantly from responders by year of birth, age at survey, sex, hereditary status, age at RB diagnosis or treatment. However, there were slightly more respondents who reported a family history of RB (17.4% vs. 11.9%) indicating that perhaps those with a second cancer were more likely to respond to the survey. However, the SIR for all second cancers combined for hereditary and non-hereditary survivors as well the SIR for bone cancer (Kleinerman et al 2005) were very similar to those reported by the Dutch RB cohort (Marees et al., 2008), which was similar to our study in terms of treatment of RB patients, making it less likely that there was a bias due to over-reporting of second cancers. It remains a concern today that survivors with a second cancer are more likely to respond than those without a second cancer. They are more likely to be motivated to respond to a survey as well as likely being under surveillance for other tumors, such as non-melanoma skin cancers.

We confirmed reports of new primary cancers by pathology reports (60.7%), hospital or physician records (20.6%), death certificates (15.5%) and autopsy reports (3.2%). All of the cancers were coded to ICDO version-2 or -3 for topography only. Although we had pathology reports with histology coded, we did not use histology in this

analysis, because the expected rates from the Connecticut Tumour Registry and SEER were based on topography only.

I worked with our colleagues at MD Anderson Cancer to estimate typical radiation scatter doses to all organs in the body for an infant and for a one-year old patient receiving radiotherapy for RB (see Chapter 2, Table 2.1). The dose data allowed us to stratify the subsequent cancer sites into three major exposure groups (heavily irradiated sites, ≥ 1 Gy; moderately irradiated sites, 0.4-1.0 Gy; and lightly irradiated sites, <0.4 Gy), in order to evaluate how risk varied by dose category.

Accrual of person-years began 1 year after RB diagnosis and ended at date of death, second cancer, date last known alive or December 30, 2000, which ever occurred earliest. As mentioned earlier, we used a very broad definition of lost to follow up. These subjects were mainly those for whom we could not contact due to lack of a good address or telephone number, nor could we get a positive match with either the social security administration presumed living search or death master file, nor link with the National Death Index. However, even if we were unable to contact a subject, if they were matched with the social security presumed living search, we considered them alive and not lost to follow up.

We calculated SIRs as the ratio of observed cancers to expected cancers using 5-year age-specific, sex-specific and 5-year calendar year-specific rates from the Connecticut Tumour Registry and SEER. Excess absolute rates were calculated as (number of observed cancers minus expected cancers divided by person-years at risk) times 10,000. Cumulative incidence rates were estimated with adjustment for competing

risk of death for 1) hereditary status (hereditary, non-hereditary), 2) radiotherapy among hereditary survivors (yes/no), and 3) type of radiotherapy (Orthovoltage and Cobalt-60 or Betatron) among hereditary survivors.

4.3 Main findings

At the end of follow-up, more non-hereditary than hereditary survivors were alive (84.8% vs. 64.6%), and only 28 hereditary (2.9%) and 25 (3.9%) non-hereditary survivors were lost to follow-up. The average years of follow up were 25.2 years for hereditary and 29.5 years for non-hereditary. At the time of this follow-up, 22% of hereditary and 35% of non-hereditary were over age 40, the primary age group of interest for epithelial tumours. Selected characteristics of the study population are presented in Table 4.1.

Table 4.1 Selected characteristics of 1-year survivors of retinoblastoma

Characteristic	Hereditary (%)	Non-Hereditary (%)
No. of subjects	963 (100)	638 (100)
Laterality		
Unilateral	47 (4.9)	638 (100)
Bilateral	916 (95.1)	0 (0.0)
Sex		
Male	512 (53.2)	334 (52.3)
Female	451 (46.8)	304 (47.7)
Age at Rb diagnosis		
< 1 yr	545 (56.6)	140 (21.9)
1 yr	267 (27.7)	197 (30.9)
2 yr	110 (11.4)	159 (24.9)
3—7 yrs	41 (4.3)	142 (22.3)
Yr. of Rb diagnosis		
1914-49	106 (11.0)	75 (11.8)
1950-59	200 (20.8)	100 (15.7)
1960-69	312 (32.4)	198 (31.0)
1970-79	253 (26.3)	192 (30.1)
1980-84	92 (9.5)	73 (11.4)
Family history of Rb		
Yes	283 (29.3)	0 (0.0)
No	497 (51.6)	499 (78.1)
Uncertain	183 (19.1)	139 (21.9)
Treatment		
Surgery	95 (9.9)	480 (75.2)

Chemotherapy	16 (1.6)	38 (6.0)
Radiation	466 (48.4)	67 (10.5)
Radiat/Chemo	383 (39.8)	47 (7.4)
Unknown	4 (0.3)	6 (0.9)
Any Radiotherapy		
Yes	849 (88.2)	114 (17.5)
No	114 (11.8)	524 (82.5)

In the additional 7 years of follow-up since 1993-2000, 78 new second cancers were confirmed, and one-half of these were either bone or soft tissue sarcomas. Overall, there was a significantly elevated risk for second cancers in the hereditary survivors (SIR=19, 95% CI 16-21; Observed=260) compared with a non-significant risk in the non-hereditary group (SIR=1.7, 95% CI 0.7-2.0; Observed=17) (Table 4.2). In the hereditary survivors, significant SIRs over 100 were noted for cancers of the bone, connective tissue, eye and orbit and nasal cavities. Risks were also elevated (SIR >10) for pineoblastoma, melanoma, and cancers of the brain, buccal cavity and corpus uteri. Lower but significantly increased risks (SIR<10) were also noted for cancers of the lung, breast and colon.

Table 4.2 Risk of new cancers in 1-year survivors of retinoblastoma by hereditary status.

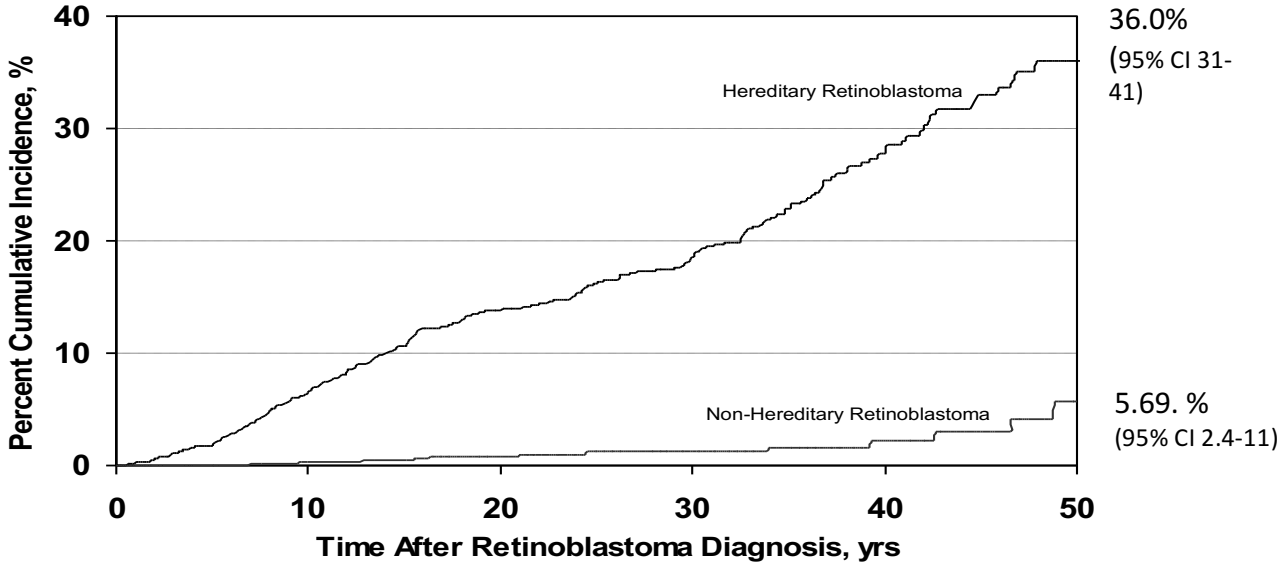
	Standardised Incidence Ratios*					
No. of persons	963			638		
Person years at risk	25,309			18,972		
	Hereditary			Non-hereditary		
Cancer Site (ICD-0 Classification)	O	E	SIR (95% CI)	O	E	SIR (95% CI)
All sites[†]	260	13.9	19 (16-21)	17	13.9	1.2 (0.7-2.0)
Bone (170)	75	0.21	360 (283-451)	0	0.16	0.0 (0.0-22.6)
Connective and soft tissue (171, 192.4, 192.5)	34	0.28	122 (84-170)	0	0.22	0.0 (0.0-16.8)
Nasal Cavities (160)	32	0.03	1111 (760-1569)	0	0.03	0.0 (0.0-135)
Cutaneous melanoma (173 and M872-878)	29	1.05	28 (18-40)	0	1.00	0.0 (0.0-3.7)
Eye and orbit (190)	17	0.06	266 (155-426)	0	0.05	0.0 (0.0-81)
Brain, CNS (191-192.0-.3,192.9)	10	0.74	13.6 (6.5-25)	2	0.58	3.43 (0.4-12)
Female Breast (174)	10	2.52	3.96 (1.9-7.3)	7	2.46	2.84 (1.1-5.9)
Corpus uteri (182)	7	0.35	20 (8.0-41)	0	0.35	0.0 (0.0-10)
Buccal cavity (140-149)‡	7	0.34	20 (8.2-42)	0	0.37	0.0 (0.0-9.9)
Lung (162)	5	0.84	5.94 (1.9-14)	0	1.11	0.0 (0.0-3.3)
Pineoblastoma (194.4)	5	0.06	90.8 (29-212)	0	0.04	0.0 (0.0-93)
Colon (153)	3	0.48	6.28 (1.3-18)	0	0.58	0.0 (0.0-6.3)
Hodgkin lymphoma (M9650-67)	3	0.88	3.4 (0.7-10)	1	0.70	1.4 (0.04-8.0)
Bladder (188, 189.9)	2	0.32	6.15 (0.7-22)	0	0.41	0.0 (0.0-8.8)
Leukemia (204-207)	2	0.89	2.25 (0.3-8.1)	1	0.66	1.47 (0.04-8.2)
Excess absolute risk per 10,000 person-years§			97.2			1.63

Comparison of hereditary patients who did and did not receive radiotherapy revealed that hereditary patients treated without radiation still had a 7-fold increased risk of second cancers compared with the general population (SIR=6.9, 95% CI 4.1-11). Radiotherapy further increased the risk by 3-fold in hereditary patients (SIR 22 vs. SIR=6.9). When we stratified the organ sites by heavily, moderately and lightly irradiated, the risk patterns were consistent with the highest risks noted for the heavily irradiated sites with the exception of thyroid cancer that was not significantly elevated. Interestingly, cutaneous melanoma was significantly elevated in hereditary patients both with and without radiation exposure. The risk for corpus uteri was also significantly increased in both treatment groups as well. When we investigated further, we found that 5/7 of these uterine tumours were leiomyosarcomas, a rare smooth muscle tumour.

When we compared risk among hereditary patients with radiotherapy treated with and without chemotherapy, we noted a modest difference in risk for all cancers combined (SIR=25, 95%CI 21-30 with chemotherapy vs. SIR=19, 95%CI 16-23 without chemotherapy).

The cumulative incidence of second cancers varied by hereditary status, radiotherapy status and type of radiotherapy machine. At 50 years after RB diagnosis, there was a 36% cumulative risk of a second cancer in hereditary compared with a 5.6% cumulative risk in non-hereditary survivors (Figure 4.1). Among the hereditary survivors the cumulative incidence reached 38% (95% CI 33%-44%) and 21% (95% CI 9.4%-36%) respectively for those with and without radiotherapy, based on a small number of 50-year survivors (Figure 4.2).

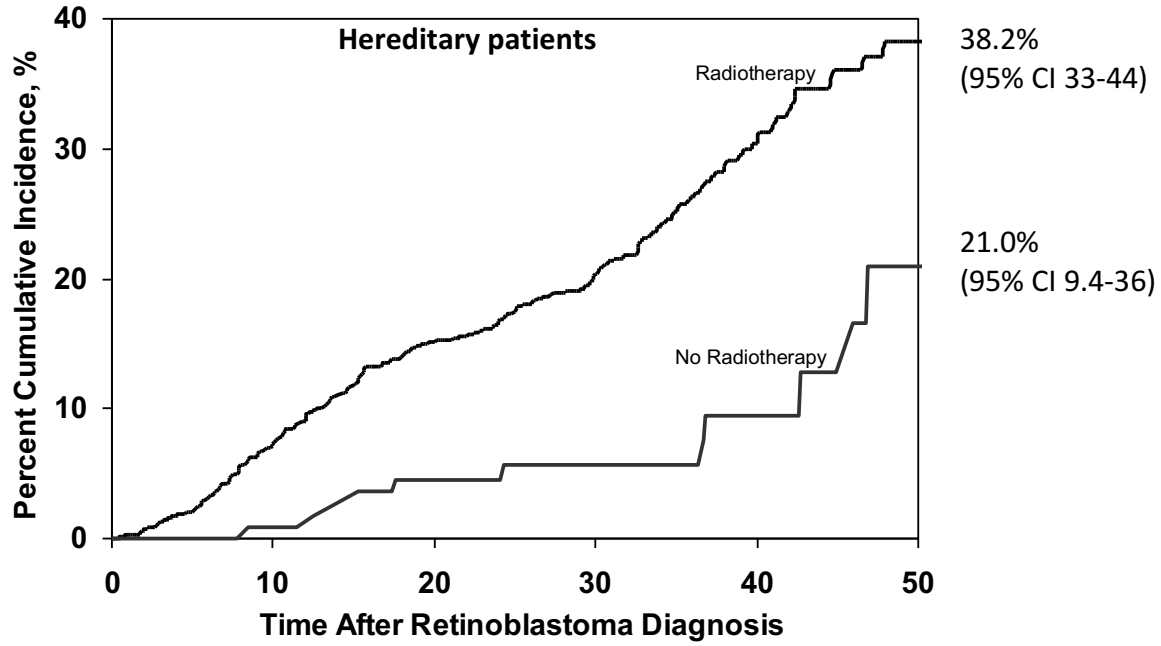
Figure 4.1 Cumulative incidence of 2nd cancers by hereditary status



Hereditary						
963	760	615	401	147	30	
Non-Hereditary						
638	570	500	317	13	46	

Number of Patients at Risk

Figure 4.2 Cumulative incidence of 2nd cancers by radiation



Radiotherapy					
849	658	529	339	112	19
No Radiotherapy					
114	102	86	62	35	11

Number of Patients at Risk

4.4 Significance

This analysis provided new information on risks of epithelial cancers in relation to radiation treatment and confirmed previous increases in second cancers, mainly bone and soft tissue sarcomas, in hereditary survivors (Kleinerman et al., 2005). When treated with radiotherapy, hereditary survivors were three times more likely to develop a subsequent cancer compared with hereditary survivors who were treated without radiotherapy. We observed the highest risks for cancer of organ sites that received the highest doses. At 50 years after RB diagnosis, one out of three hereditary survivors and one of out of 20 non-hereditary survivors had developed a subsequent non-ocular cancer.

One of the main reasons for undertaking this analysis was to identify the risk of epithelial tumours in this cohort, especially those cancers with a somatic mutation in the *RB1* pathway. Although based on small numbers, we identified new risks in this population for cancer of the salivary gland, tongue and nasopharynx. All of these three sites received high doses of radiation due to their proximity to the eye, which was the target of radiation. Salivary gland cancer has been linked previously to radiotherapy in children treated for enlarged tonsils (mean dose=4.6 Gy) and tinea capitis (ringworm of the scalp) (0.4 Gy) (Ron, 2003), which was consistent with the radiation dose received by those sites in RB patients (mean dose 1.6-4.2 Gy) in this study. Cancer of the tongue has been associated with tobacco smoking, but neither of the two survivors smoked. One of two nasopharyngeal cancers was a sarcoma. All of these three types of cancer occurred in irradiated hereditary survivors, supporting a link with radiation.

We also identified new increased risks for uterine cancer, the majority of which were leiomyosarcomas (LMS), a rare smooth muscle tumour, as well as a risk for colon cancer. Doses were low to both of these organs, yet the SIRs were significantly elevated. Increased risks for bladder cancer have been reported in other RB cohorts (Fletcher et al., 2004, Marees et al., 2008a) but only two bladder cancers were diagnosed in this cohort.

Previously, breast cancer was significantly elevated only in the non-hereditary survivors, which was surprising since somatic *RB1* mutations have been identified in sporadic breast cancer (Bosco and Knudsen, 2007). In the current analysis we found breast cancer to be significantly elevated in both hereditary and non-hereditary survivors, but among the hereditary survivors, it was only significantly elevated only in those who were irradiated. The radiation dose to the breast (mean dose= 0.4 Gy) in RB survivors is consistent with the doses received by other childhood populations that have reported increases in breast cancer in adults irradiated for suspected enlarged thymus glands or haemangioma in childhood (Preston et al., 2002).

One of the biggest differences with this analysis compared with the previous analyses by Wong et al (Wong et al., 1997) was a decrease in the cumulative incidence of second cancers at 50 years from 50% to 36% in hereditary survivors in the current analysis. We did not detect a change in the cumulative incidence for non-hereditary survivors. The previous analysis used the Kaplan Meier method, whereas the Gooley method that we used in the current analysis likely accounted for some of the difference, because it takes competing risk of death into account. We also had accumulated

additional person years in this analysis compared with the earlier analysis that would have led to more stable cumulative risk estimates for the more recent time period.

We found a lower cumulative incidence for hereditary patients treated without radiation compared with those treated with radiation (21%, 95%CI 9%-36% vs. 38%, 95%CI 33%-44%). Although patients were not randomised to treatment groups, most patients were diagnosed with RB at approximately the same age, so age should not have confounded the cumulative incidence estimates. If the two treatment groups were markedly different ages at diagnosis then the cumulative incidences could not be compared because they would not have the same opportunity to develop second cancers at similar ages.

Interestingly, we noted a lower cumulative incidence for survivors treated with non-orthovoltage radiotherapy compared with orthovoltage radiotherapy (26% vs. 33%), although the confidence intervals did overlap. There is less scatter radiation associated with non-orthovoltage radiation compared with orthovoltage radiation. However patients were treated with orthovoltage in earlier time periods and therefore had more time to develop a second cancer, so this could account for some of the difference in the cumulative incidence.

The source of the population was hospital-based rather than population-based and included only two institutions that may limit the generalisability of the findings from the study to the general population. The cohort was not representative of the true proportion of hereditary patients in the general population, because it included a much larger proportion of hereditary compared with non-hereditary survivors typically found in

the general population. Hereditary patients are more likely to develop an additional cancer due to their germline mutation and this could have biased the risk estimates upwards. However, the loss to follow-up was similar in both groups and each group was independently compared with the general population. In addition, RB is a rare cancer and second cancers are rare, so the inclusion of more hereditary survivors increases the value of the population to identify risks. Treatment differed greatly by hereditary status with almost all hereditary patients receiving radiotherapy (85%) compared with a very small proportion of non-hereditary patients treated with radiotherapy (15%). This could have confounded the results, but when we stratified the risks by radiation dose in three categories (high, moderate and low), we observed the highest risks for organ sites in the head region that received the highest doses of scatter radiation. Although these data suggest a dose-response relationship in hereditary survivors, given the number of organ sites evaluated, the few cases for some of the sites, and the number of variables that could have affected the risk, e.g., age at exposure, current age, chemotherapy in addition to radiation and smoking, these data should be interpreted cautiously. Further, it was difficult to evaluate the heterogeneity of risk in this cohort.

Because we did not conduct mutation testing on all of the survivors, we did not know whether unilateral patients had a germline mutation that was less penetrant. Therefore we relied on laterality of RB and mention of family history of RB in the medical record. It is likely that some unilateral patients survivors may have been misclassified as non-hereditary, due to unknown family history of RB or a less penetrant form of a germline RB1 mutation (mosaicism). This would have biased the risk in the non-

hereditary upward, and biased the ratio of risks of hereditary: non-hereditary towards the null. But given the very large increase in risk in the hereditary patients, the misclassification would likely have had a very small effect. Interestingly, when we segregated the unilateral patients with a family history whom we had classified as hereditary, their second cancer risk resembled that of the bilateral patients.

Although we collected reports of subsequent cancer from several sources (medical record abstracts, self-reports and death certificates), it is possible that we may have missed some cancers that are non-fatal. However, given the large ratios of observed to expected number of cancers compared with the general population, it is possible that we are actually underestimating the risks of these cancers (e.g. thyroid, breast) in this cohort. A comparison of the incident non-fatal cancers such as breast and thyroid with deaths for these two cancers in hereditary survivors indicated that we are ascertaining incident cancers but missing non-fatal cancer deaths. There were 10 incident breast cancers whereas there were only 2 deaths from breast cancer, and there were 2 incident thyroid cancers but no deaths attributed to this cause.

This analysis establishes that one in three hereditary survivors of RB is at risk of developing a subsequent cancer by age 50 years likely related to their past radiotherapy for RB. Sarcomas and melanomas are the predominant second cancers. However, new increased risks were noted for epithelial cancers in this cohort, notably those of the lung, buccal cavity and breast as well as leiomyosarcomas of the uterus.

4.5 Public health message

The persistently elevated risk of subsequent cancers in hereditary but not non-hereditary survivors points to the need for life-long surveillance of subsequent cancers in RB patients who have a *RB1* germline mutation and for those treated with radiotherapy.

4.6 Role in the study

Role: I initiated the study, conducted the analysis and drafted the manuscript. Study team: Dr Tarone was the consulting statistician; Drs Abramson and Seddon were the clinical collaborators and provided the patients; Dr Stovall provided the dose data; Drs Li, Fraumeni and Tucker were the senior study investigators who contributed to the interpretation of the data. My contribution: 75%

4.7 Publication

Chapter 5. Risk of Soft tissue sarcomas by histology

Kleinerman RA, Tucker MA, Abramson DH, Seddon JM, Tarone RE, Fraumeni JF, Jr. Risk of soft tissue sarcomas by individual subtype in survivors of hereditary retinoblastoma. J Natl Cancer Inst, 2007;99:24-31.

5.1 Introduction and rationale

Hereditary retinoblastoma survivors have a high risk of soft tissue sarcomas (STS) compared with the general population (Wong et al., 1997), but the risk for individual subtypes of STS had never been previously estimated. An earlier analysis of STS risk in relation to radiation in this cohort found a clear dose-response for increasing risk of STS with increasing dose up to 11-fold for doses of 60 Gy and higher (Wong et al., 1997). However, radiation dose was only available on a subset of STS patients (31 out of 44) so we did not calculate the risks separately by individual subtype due to a small number of affected individuals. In addition, STS are a diverse group of tumour types, and we wanted to quantify the risks by subtype to investigate whether any specific subtype was preferentially elevated after RB and radiotherapy. Identification of risk of specific STS subtype(s) and age at occurrence would aid in the surveillance of survivors at risk for second cancers.

5.2 Methods

STS are often coded according to the site or organ in which they occur (e.g. shoulder, bladder, colon, uterus). In previous analyses in this cohort, we only used the

category connective tissue (ICDO2 code = 171) to define STS. For example, a sarcoma could be diagnosed in a uterus, and unless the histology code is also used, it would be coded as a uterine cancer rather than a soft tissue sarcoma. Therefore, for this analysis, STS were coded according to both the location of the tumour or topography and its subtype or histology. Using this approach increased the number of soft tissue sarcomas available for analysis (n=69). This was far larger and included the 44 STS reported as connective tissue cancers. The 69 STS were confirmed by pathology report (70%), autopsy (4%), other hospital records (19%) or death certificate only (7%) and were coded according to ICDO-2 and ICDO-3.

This analysis was restricted to the 963 hereditary survivors in cohort 1, because no STS were diagnosed in any non-hereditary survivors. Radiotherapy was treated as a yes/no variable, but chemotherapy was scored according to the type and number of individual alkylating agents (0, none; 1, low; 2, medium; 3 or more, high). This was a modification of the method developed by Tucker et al (Tucker et al., 1987) and each type of chemotherapy was assigned a score based on the type of alkylating agent and level of toxicity. The individual scores were then summed over all of the chemotherapy courses to calculate an alkylator score for each subject. Almost all of the patients who received chemotherapy also received radiotherapy. The small number of survivors who received chemotherapy alone precluded any meaningful analysis of this group.

The expected number of STS by subtype was based on rates from SEER. Rates were calculated for the following histologic subtypes: fibrosarcoma; malignant fibrous

histiocytoma; liposarcoma; leiomyosarcoma; and rhabdomyosarcoma; STS other and STS, not otherwise specified.

The statistical analysis included calculation of standardised incidence ratios for the six main subtypes of STS, stratified by type of treatment (radiotherapy: any, none; chemotherapy yes, no; and alkylating agent score) and time since RB diagnosis. In addition, we calculated cumulative incidence for a period up to 50 years after RB diagnosis with adjustment for competing risk of death due to other causes. Accrual of person-years at risk began one year after RB diagnosis and ended at date last known alive, date of death or December 31, 2000, whichever occurred earliest.

5.3 Main Findings

Most of the survivors were treated with radiotherapy (n=849) and 45.1% (n=383) of these survivors also received chemotherapy. We identified 69 STS in 68 survivors. Leiomyosarcoma (LMS) was the most common subtype (33%), followed by fibrosarcoma (19%), malignant fibrous histiocytoma (17%), soft tissue tumours and sarcomas NOS (15%), rhabdomyosarcomas (12%) and liposarcoma (4.3%). As a group, the STS were more often diagnosed in the head and face (71%). The majority of LMS, however, were diagnosed more often outside the radiation field in contrast to the other STS subtypes.

5.3.1 Treatment

SIRs were significantly elevated for all of the STS subtypes with the highest SIRs noted for leiomyosarcomas (SIR=390) and fibrosarcomas (SIR=398). Most of the STS were diagnosed in irradiated survivors; 3 STS developed in survivors who had not been

irradiated. SIRs were also elevated similarly in irradiated patients who did and did not receive chemotherapy. The excess absolute risks were similar for those who also received chemotherapy (EAR=33.5) compared with those who did not receive chemotherapy (EAR=27.7), with the exception of LMS that had higher EARs associated with chemotherapy and rhabdomyosarcoma that only occurred in those without chemotherapy.

We did not observe a trend of increasing risk for STS (all types combined) with increasing alkylator score. The most common chemotherapy agent used in this population was Triethylenemelamine (TEM).

5.3.2 Latency

The SIRs were highest in the first 10 years after RB diagnosis and continued to be diagnosed up to 30 years and later for all subtypes except LMS and liposarcomas. LMS were not diagnosed until 20 years after RB diagnosis and most occurred 30 years and later (Table 5.1).

5.3.3 Gender

Risks for STS did not differ by gender, however, the location of the LMS did differ by sex with the majority of the LMS diagnosed in the head in males (64%) and in the uterus in females (58%).

Table 5.1 Risk of soft tissue sarcoma in 1-year survivors of hereditary retinoblastoma by time since diagnosis of retinoblastoma

	Number of years (yrs) since retinoblastoma diagnosis							
	1 - 9 yrs		10 - 19 yrs		20 - 29 yrs		30+ yrs	
No. of subjects starting each interval	963		791		658		465	
No. of person-years	7649		7381		5691		4573	
Histology and ICD-O Classification	O *	SIR, 95%CI†	O	SIR, 95%CI	O	SIR, 95%CI	O	SIR, 95%CI
Soft tissue tumors and sarcomas, NOS	4	229 (62-585)	2	85 (9.5-307)	1	34 (0.4-192)	3	88 (18-258)
Fibrosarcoma	4	598 (161-1531)	4	387 (104-904)	2	250 (28-904)	3	393 (79-1147)
Malignant Fibrous Histiocytoma	2	488 (55-1760)	4	209 (56-534)	1	24 (0.3-136)	5	90 (29-210)
Liposarcoma	0	0 (0-5038)	2	836 (94-3019)	0	0 (0-467)	1	51 (0.7-282)
Leiomyosarcoma	0	0 (0-3468)	1	213 (2.8-1187)	4	336 (90-861)	1 8	435 (258-687)
Rhabdomyosarcoma	4	340 (92-871)	3	286 (57-835)	0	0 (0.0-908)	1	428 (5.6-2380)
Total	14	335 (183-562)	16	227 (129-368)	8	79 (34-155)	3 1	193 (131-274)

*observed

†Standardized incidence ratio and 95% confidence interval

5.4 Significance

This was the first study of RB survivors with sufficient long-term follow up to identify increased risks of STS by subtype (Kleinerman et al., 2007). The most common type and highest risks were noted for LMS. Although LMS were diagnosed in irradiated survivors, the majority of LMS was diagnosed outside the radiation field. Higher risks for LMS were noted for those who also received chemotherapy, and LMS was not diagnosed until age 20 years and the majority occurred after age 30. The location of the LMS also differed by gender with males having LMS diagnosed in the head and females diagnosed with LMS of the uterus.

The novelty of this study was the analysis of the STS by histologic sub-type. We created rates from the general population based on the main STS subtypes. This permitted us to report risks by individual subtypes for this population. Interestingly, we found variation by subtype in time to appearance after RB diagnosis as well variation in the location of the STS in relation to the radiation field. This has important implications for screening survivors in relation to when they should be screened for specific STS.

This analysis also moved beyond the previous analyses by attempting to take into account the influence of alkylating agents on the risk of STS. Most of the previous analyses of these data (Eng et al., 1993, Wong et al., 1997) focused on the effect of radiation on second cancers and did not take into account the influence of chemotherapy that was given to almost half of those patients who were irradiated in the cohort. Although this analysis did not indicate an increasing risk with increasing alkylating agent

score for all STS combined, we did find a heightened risk for LMS with chemotherapy combined with radiation.

This was also the first analysis to note the different locations of the LMS by gender. Based on these findings, female survivors 30 years and older need to be aware of the possibility of LMS of the uterus. This finding prompted another analysis in these data focused on the risk of uterine LMS (Francis et al., 2012) that suggested that female survivors may want to consider undergo hysterectomies at the end of childbearing in order to avoid this possible second cancer.

In an accompanying editorial to this analysis (Meadows, 2007) Meadows noted "*The report by Kleinerman et al in this issue of the Journal contains new information that is important for clinicians who follow retinoblastoma survivors."..."Improved treatment can be expected to reduce the incidence of soft tissue (and bone) sarcomas associated with radiation. The importance of this report lies in its emphasis on leiomyosarcoma, a tumour that occurs in RB1 gene carriers whether or not they were treated with radiation and that can be expected to continue to occur in older survivors, who will require more careful follow-up."*

5.5 Public Health Message

Survivors with hereditary RB have a genetic predisposition to soft tissue sarcomas. Long-term surveillance for sarcomas is warranted for these survivors, because leiomyosarcomas, which are the most common soft tissue sarcomas in survivors, mainly occur 30 years after RB diagnosis.

5.6 Role in study

Role: I initiated the idea for the study, conducted the analysis and drafted the manuscript. Study team: Dr Tarone was the consulting statistician, Drs Abramson and Seddon were the clinical collaborators and provided the patients; Drs Tucker and Fraumeni were the senior study investigators. My contribution: 80%

5.7 Publication

Chapter 6. Mortality of second cancers

Yu CL, Tucker MA, Abramson DH, Furukawa K, Seddon JM, Stovall M, Fraumeni JF Jr, Kleinerman RA. Cause-specific mortality in long-term survivors of retinoblastoma. J Natl Cancer Inst, 2009:101:581-91.

6.1 Introduction and rationale

Although the risk of dying from second malignancies has been established in this cohort (Eng et al., 1993) and other sources (Acquaviva et al., 2006, Fletcher et al., 2004) mortality data was limited among long-term survivors of RB treated with radiation. New information on mortality could indicate additional risks for these survivors and provide data for follow-up guidelines.

6.2 Methods

We investigated cause-specific mortality in cohort 1 (n=1599) diagnosed 1914-1984 and cohort 2 (n=255) who were treated more recently from 1985-1996 at one institution. The combined cohort was comprised of 1854 one-year survivors of RB. This analysis excluded 3 subjects from cohort 1 that had been included in earlier analyses, due to two survivors with resided outside the US and one subject who was subsequently found to not have RB. For cohort 2, we included two subjects in this analysis who were subsequently excluded from later analyses due to being consultation only patients.

Survivors were classified as either hereditary (bilateral RB or unilateral with family history of RB) or non-hereditary (unilateral with no history of RB). *RB1* mutation testing was not available to verify which patients had a germline mutation.

Various tracing sources were used to determine the vital status of the cohorts, and the cohorts were submitted to the NDI in 2005. The most recent date of death available at that time of this study was 2003. The NDI provided coded causes of death according to International Classification of Diseases Eighth revision (ICD-8), in which tumours are coded according to organ site rather than histology. All reported causes of death in the cohort prior to this search were converted to ICD-8 to be compatible for analysis.

Person-years began one-year after RB diagnosis and ended on the date the survivor was last known alive, date of death or end of follow-up (December 31, 2003), whichever occurred earliest. Survivors last known to be alive as of January 1, 1979, lived in the U.S. and not found in the NDI were presumed to be alive as of December. 31, 2003. There were 1380 (74%) survivors alive, 423 (22.8%) had died and 51 (2.8%) were lost to follow-up. For survivors last known to be alive since 1979 when the NDI started, we made the assumption that if a survivor was not matched with the NDI, then we could consider them alive as of the date of NDI linkage.

We estimated the relative risk for each cause of death compared with death in the general population by calculating a SMR and exact Poisson confidence intervals. The expected number of deaths was calculated by applying the US mortality rates from 1925 onwards (by 5-year age, 5 calendar year, and sex specific categories) to

the person-years at risk calculated for the RB cohort. To measure the overall burden of disease, we also calculated EARs. In addition, we compared SMRs for all survivors who were at least 25 years of age with SMRs from a UK study by Fletcher et al. (Fletcher et al., 2004) using the chi-square test of homogeneity.

In order to investigate the influence of multiple factors on the SMRs, we estimated the relative rates of each factor by fitting Poisson regression models. The likelihood ratio test was used to test the statistical significance of each factor. The main factors of interest were hereditary status, age at RB diagnosis (≤12 months, >12 months), attained age, (<25 yrs and ≥25 yrs), time since RB diagnosis by decade, and calendar year of RB diagnosis (<1960 and ≥1960). Cut points were determined a priori by the changes in radiotherapy treatment in 1960 and past study that suggested greater sensitivity to radiation for children ≤12 months of age (Moll et al., 2001). We tested the interaction of hereditary status and radiotherapy using a product term.

Cause-specific cumulative mortality was calculated by treating other causes as competing risks using the R statistical software (Gray, 1988).

6.3 Main Findings

The median age of the combined cohorts at the end of follow-up was 30 years (range 1-79 years). The median duration of follow up was similar for the hereditary and non-hereditary survivors (28.5 yrs and 29.6 yrs, respectively). At the end of follow-up, there were 423 deaths (346 in cohort 1 and 77 in cohort 2). This analysis identified 70

additional deaths in cohort 1 since the last follow-up, and this was the first mortality analysis in cohort 2.

We observed 151 deaths due to second malignant neoplasms other than RB in 1092 hereditary survivors (SMR=35, 95%CI 30-41) compared with 12 deaths in 762 non-hereditary survivors (SMR=2.5, 95%, 95%CI 1.3-4.4) (see Table 6.1).

Table 6.1 Causes of death other than retinoblastoma in 1-Year survivors by hereditary status

	All			Hereditary			Nonhereditary		
No. of persons followed up	1854			1092			762		
Person-years	49924			28250			21674		
Cause of Death* (ICD-8 code)	O	SMR (95% CI)	EAR	O	SMR (95% CI)	EAR	O	SMR (95% CI)	EAR
Malignant and benign neoplasms other than Rb†	172	19 (16, 22)	32.6	160	37 (31, 43)	55.1	12	2.5 (1.3, 4.3)	3.3
Malignant neoplasms other than Rb	163	18 (15, 21)	30.8	151	35 (30, 41)	51.9	12	2.5 (1.3, 4.4)	3.3
Bone (170)	56	332 (251, 431)	11.2	56	595 (449, 773)	19.8	0	0 (0, 49)	0.0
Connective tissue (171)	31	175 (119, 248)	6.2	31	329 (223, 467)	10.9	0	0 (0, 44)	0.0
Melanoma (172)	13	44 (24, 76)	2.5	13	89 (47, 151)	4.5	0	0 (0, 25)	-0.1
Brain and other parts of nervous system (191-192)	12	16 (8.4, 28)	2.3	10	25 (12, 46)	3.4	2	5.9 (0.7, 21)	0.8
Brain (191)	8	13 (5.5, 25)	1.5	6	18 (6.5, 39)	2.0	2	6.7 (0.8, 24)	0.8
Other parts of nervous system (192)‡	4	36 (9.6, 91)	0.8	4	61 (16, 155)	1.4	0	0 (0, 80)	0.0
Lung and trachea (162)	8	4.9 (2.1, 9.7)	1.3	8	12 (5.3, 24)	2.6	0	0 (0, 3.8)	-0.5
Corpus uteri (182)§	6	81 (30, 177)	1.2	5	154 (50, 359)	1.8	1	24 (0.3, 134)	0.4
Nasal cavities (160)	5	392 (126, 914)	1.0	5	790 (254, 1843)	1.8	0	0 (0, 570)	0.0
Breast (174)	5	5.1 (1.6, 12)	0.8	2	4.4 (0.5, 16)	0.5	3	5.7 (1.2, 17)	1.1
Buccal cavity and pharynx (140-149)**	2	15 (1.7, 55)	0.4	2	34 (3.8, 123)	0.7	0	0 (0, 51)	-0.03
Leukemia (204-207)††	2	1.9 (0.2, 6.9)	0.2	1	1.7 (0.02, 9.5)	0.1	1	2.2 (0.03, 12)	0.2
Thyroid (193)	1	55 (0.7, 305)	0.2	0	0 (0, 436)	0.0	1	102 (1.3, 568)	0.5
Bladder (188)	1	15 (0.2, 85)	0.2	1	40 (0.5, 224)	0.3	0	0 (0, 90)	0.0
Benign tumors (210-239)‡‡	9	38 (17, 73)	1.8	9	72 (33, 137)	3.1	0	0 (0, 33)	-0.1

Table 6.1 Continued

		All			Hereditary			Nonhereditary	
No. of persons followed up		1854			1092			762	
Person-years		49924			28250			21674	
Cause of Death* (ICD-8 code)	O	SMR (95% CI)	EAR	O	SMR (95% CI)	EAR	O	SMR (95% CI)	EAR
Other known causes of death	39	1.0 (0.7, 1.3)	-0.4	19	0.9 (0.5, 1.4)	-0.9	20	1.0 (0.6, 1.6)	0.2
Infections (000-139)	3	1.1 (0.2, 3.3)	0.1	1	0.7 (0.01, 3.9)	-0.1	2	1.7 (0.2, 6.0)	0.4
Endocrine and Metabolic (240-279)	2	1.5 (0.2, 5.4)	0.1	1	1.5 (0.02, 8.4)	0.1	1	1.5 (0.02, 8.3)	0.2
Mental disorders (290-315)	3	4.7 (0.9, 14)	0.5	0	0 (0, 11)	-0.1	3	9.7 (2.0, 28)	1.2
Neurologic (320-389)	2	1.5 (0.2, 5.5)	0.1	2	2.9 (0.3, 10)	0.5	0	0 (0, 6.0)	-0.3
Circulatory (390-458)	7	1.0 (0.4, 2.0)	-0.05	4	1.3 (0.3, 3.2)	0.3	3	0.7 (0.2, 2.2)	-0.5
Arteriosclerotic heart disease (410-414)	4	1.1 (0.3, 2.8)	0.1	3	2.0 (0.4, 5.7)	0.5	1	0.5 (0.01, 2.6)	-0.5
Cerebrovascular accidents (430-438)	1	1.0 (0.01, 5.3)	-0.01	1	2.1 (0.03, 11)	0.2	0	0 (0, 6.5)	-0.3
Respiratory (460-519)	2	1.1 (0.1, 3.9)	0.03	2	2.3 (0.3, 8.2)	0.4	0	0 (0, 3.7)	-0.5
Digestive system (520-579)	5	2.7 (0.9, 6.2)	0.6	3	3.3 (0.7, 9.5)	0.7	2	2.1 (0.2, 7.6)	0.5
External causes (800-998)	15	0.7 (0.4, 1.1)	-1.6	6	0.5 (0.2, 1.0)	-2.4	9	0.9 (0.4, 1.7)	-0.5
Ill-defined condition (796)	10	11.4 (5.5, 21)	1.8	6	13 (4.7, 28)	2.0	4	9.9 (2.7, 25)	1.7

*Death certificate not available for 13 hereditary and 3 non-hereditary RB survivors.

†Cancer sites not listed for herediitary RB include retroperitoneal (2), colon (1), non-melanoma skin cancer (2), ovary (1), kidney (1), pineal gland (1), lymphoid tissue (1), cancer, NOS (7); and for non-hereditary, cancer, NOS (4).

‡Neuroblastoma (4)

§Hereditary: LMS (3), carcincoma (1), Mullerian mixed tumor (1); non-hereditary: cancer of corpus uteri

**Nasopharynx (2)

††Hereditary: Acute lymphocytic leukemia (1) and non-hereditary: acute myeloid leukemia (1)

‡‡Benign tumors: meningioma of the spine (1), pituatary gland andcraniopharyngeal duct (1) and brain

The most common subsequent malignant neoplasms in hereditary survivors were sarcomas of bone and connective tissue, melanoma and cancers of the brain and other central nervous system tumours. For the non-hereditary survivors, elevated mortality was noted for only cancer of the breast and thyroid. Among the non-hereditary survivors, the SMRs were only significantly elevated for those who received radiotherapy for RB (6 deaths, SMR=7.3, 95%CI 2.7-15.8) but not for those who did not receive radiotherapy (6 deaths, SMR=1.5, 95%CI 0.6-3.3). We did not observe elevated mortality due to non-cancer causes in hereditary nor non–hereditary survivors.

The SMRs differed significantly by attained age for the hereditary survivors, with higher SMR for <25 yrs vs. ≥ 25 yrs (SMR=76, 95%CI 61-95 and SMR=22, 95%CI 17-28) (Table 3). Sarcomas accounted for 76.5% of deaths prior to age 25, whereas, they accounted for only 35.7% of deaths 25 years and older. Excess mortality in the older age group was mainly attributed to deaths from melanoma, lung cancer, uterine cancer and digestive organs.

Similar to the second cancer incidence data, radiation conferred a 3.4 times higher risk of death from all second cancers compared with the SMR for all second cancers in non-irradiated hereditary patients. Among the hereditary survivors who died of a second cancer, the median age at death for the 140 irradiated survivors was younger than the median age at death for the 11 non-irradiated survivors (20.5 years, range 1-67 years versus 44 years, range 10-64 years). In addition, hereditary survivors irradiated ≤12 months of age (SMR=59, 95%CI 48-73) were 2.2 times more likely to die of a second

cancer than those survivors who were irradiated >12 months of age (SMR=27, 95%CI 20-35).

Comparison of SMRs for all second cancers by gender did not reveal any statistically significant differences (Males, SMR=38, 95%CI 29-48 and females, SMR= 46, 95% CI 36-57). However, the risk of death from cancer of the brain and other parts of the nervous system was greater in females compared with males (SMR for females=67, 95%CI 30-127, 9 cancers compared with SMR for males= 5.1, 95%CI 0.07-28, 1 cancer; *P*=0.001).

Radiotherapy for RB was related to increased RRs for second cancers among both hereditary and non-hereditary survivors. We observed higher RR for all second cancers combined in non-hereditary patients (RR=7.19, 95%CI 2.2-23 based on 6 second cancers) compared with hereditary patients (RR=2.46, 95% CI 1.4-4.8 based on 140 second cancers). Table 6.2 shows the results of a multivariable Poisson model that evaluated the relative rates of death from second cancers in relation to possible modifiers of risk in hereditary and non-hereditary survivors.

Table 6.2. Multivariate Poisson regression model of relative rate of mortality from subsequent malignant neoplasms in 1-year survivors of retinoblastoma, by hereditary status

	Hereditary			Non-hereditary		
Risk factor	**O**	**RR (95%CI)**	**P***	**O**	**RR (95% CI)**	**P***
Radiation			0.001			0.002
No	11	1.0 (referent)		6	1.0 (referent)	
Yes	140	2.46 (1.39, 4.84)		6	7.19 (2.21, 23.37)	
Sex			0.03			0.42
Male	67	1.0 (referent)		4	1.0 (referent)	
Female	84	1.41 (1.03, 1.95)		8	1.63 (0.51, 6.21)	
Age at Rb diagnosis			0.06			0.63
0-12 months	95	1.37 (0.99, 1.93)		3	1.40 (0.31, 4.79)	
>12 months	56	1.0 (referent)		9	1.0 (referent)	
Calendar year of RB diagnosis			0.05			0.10
1914-1959	78	1.44 (1.00, 2.05)		9	3.85 (0.77, 21.02)	
1960+	73	1.0 (referent)		3	1.0 (referent)	
Latency, years			<0.001			0.05
1-9	19	1.0 (referent)		1	1.0 (referent)	
10-19	52	3.40 (2.05, 5.90)		3	3.28 (0.41, 66.62)	
20-29	25	2.01 (0.91, 4.22)		2	0.94 (0.02, 40.50)	
30-39	30	3.59 (1.33, 9.56)		0	NA	
40+	25	5.82 (2.09, 15.95)		6	2.79 (0.07, 170.38)	
Attained age			0.76			0.37
1-24 years	81	1.0 (referent)		4	1.0 (referent)	
25+ years	70	1.13 (0.52, 1.56)		8	4.16 (0.22, 74.39)	

Abbreviations: Rb, retinoblastoma; O, observed; RR, relative rate; CI, confidence interval; NA, not applicable

*P-value from likelihood ratio test

We evaluated the interaction between hereditary status and radiotherapy but found that the interaction between these two variables was not statistically significant (Table 6.3).

Table 6.3. Risk of mortality from second cancers in retinoblastoma patients by radiotherapy and hereditary status

Type of retinoblastoma	No Radiotherapy	Radiotherapy
Hereditary	RR=7.12 (95%CI 2.7-21)	RR=17.9 (95%CI 8.6-46)
Non-hereditary	RR=1.00	RR=7.20 (95%CI 2.3-23)

Likelihood ratio test for interaction *P*=0.12

The cumulative mortality from second cancers at 50 years after RB after adjustment for competing risk of death from other causes differed for hereditary and non-hereditary survivors (25.5%, 95% CI 21-30 and 1.0%, 95%CI 0.2-1.8). Further investigation of the cumulative mortality among hereditary survivors revealed differences by radiotherapy, but the confidence intervals overlapped (radiotherapy, 26.8%, 95%CI 22-32 and no radiotherapy, 17.2%, 95% CI 5.4-29).

6.4 Significance

Compared with the general population, we demonstrated that hereditary RB survivors had increased risks of deaths from second cancers (Yu et al., 2009). These risks greatly exceeded those from RB and from other non-cancer causes. The most common causes of death were from sarcomas, melanomas, and cancers of the brain and other parts of the nervous system. Deaths attributable to second cancers occurred beyond 40 years after RB diagnosis. The study revealed for the first time a previously unreported increased risk of death due to uterine corpus cancer (mainly sarcomas) and confirmed the elevated risk of lung cancer that we reported previously (Kleinerman et al., 2000) (see Chapter 3). Among both hereditary and non-hereditary survivors, the relative rates of mortality from second cancers were higher in those treated previously with radiotherapy compared with those who had not received radiation. It should be noted that RB survivors were not at increased risk of death from non-cancer causes compared with the general population.

This mortality analysis differed from the previous mortality study in this cohort (Eng et al., 1993) by increasing the number of person years with an additional 13 years of follow up as well as including survivors from cohort 2 (n=255). The previous mortality analysis had classified survivors according to laterality whereas the current analysis used hereditary status. This had the effect of combining unilateral patients with a family history of RB who presumably had a germline mutation with bilateral survivors in the hereditary group. This new analysis also stratified the SMRs by attained age and treatment-related variables, evaluated the effects of possible risk factors on the risk of mortality from second cancers by hereditary status, modelled the interaction between radiotherapy and hereditary status and investigated risks by gender.

Although these data indicate an increased risk of dying from a second cancer among those survivors who were treated with radiation, regardless of hereditary status, the majority of the second cancer deaths occurred in the hereditary patients who were treated with radiation. Interestingly, the relative rate of dying of a second cancer related to radiotherapy was lower among hereditary survivors compared with non-hereditary survivors. This could reflect a higher background rate for second cancers in hereditary survivors due to their genetic susceptibility to second cancers, which would result in a lower relative rate of second cancers due to radiotherapy. The interaction between hereditary status and radiation did not reach statistical significance.

Among the hereditary survivors the other risk factors in addition to radiation that were statistically significantly associated with dying from a second cancer were female gender, and increasing risk with increasing time since RB diagnosis (latency).

Overall, the cumulative mortality risk of dying from a second cancer indicated a risk of 1 in 4 hereditary survivors dying 50 years after RB diagnosis compared with only 1 in 100 non-hereditary survivors over the same time period.

One notable limitation is that cause of death recorded on death certificates may be inaccurate in some cases. When we investigated the nine causes of death attributed to benign tumours, we found that four of these persons who had died had been diagnosed with a soft tissue sarcoma of the cranial or facial area, one had a malignant glioma, and another had a sebaceous carcinoma of the eyelid. Additionally, mortality is not a good measure of non-fatal cancers such as breast or thyroid cancer.

Although we had data up to 60 years after RB diagnosis, the number of survivors followed 50 years and more was only 72 in both the hereditary and non-hereditary groups. Age 50 is when usually when epithelial cancers start to increase and although SMRs for many epithelial cancers were elevated, the number of observed cancer deaths was small for some sites. We were not able to reliably estimate risks of dying from specific epithelial causes, such as bladder cancer, which had been reported by a UK study (Fletcher et al., 2004) and Dutch study (Marees et al., 2010).

Interestingly, there was a substantial increase in the EARs over time in hereditary patients from EAR=21 at 1-9 years after RB up to EAR=132 at $\geq$40 years and more after RB diagnosis, indicating that the burden of cancer deaths increased with time since diagnosis. For the non-hereditary patients, the EARs increased from EAR=1.1 at 1-9 years to EAR=18 at $\geq$40 years.

Although it was not the focus of this paper, it is of interest to follow up the cohort with another mortality analysis as the cohort ages in order to learn more about the risk of non-cancer causes that have been linked to radiation such as heart disease (Aleman et al., 2014).

These mortality data combined with the incidence data provide information about risks of second cancers that can help guide health care providers and hereditary survivors as to what risks they need to be aware of as they age as well as inform development of screening programs for the early detection and treatment of some cancers

6.5 Public Health Message

Hereditary RB survivors are more likely to die of a second cancer than RB. These survivors should be followed for their lifetime for the possible development of second cancers and encouraged to be aware of these risks to permit early identification of second cancers. Non-hereditary survivors are at no greater risk of dying from a second cancer than the general population.

6.6 Role in study

Role: I initiated the idea for the study. Study team: Dr Yu analysed the study and drafted the manuscript. Dr Yu was a post-doctoral fellow whom I mentored. Dr Furukawa provided statistical consultation to Dr Yu. Drs Abramson and Seddon were the clinical collaborators; Dr Stovall provided dose data; and Drs Tucker and Fraumeni were the senior study investigators. My contribution: 60%.

6.7 Publication

Chapter 7. Family history of retinoblastoma

Kleinerman RA, Yu CL, Little MP, Li Y, Abramson DH, Seddon JM, Tucker MA. Variation of second cancer risk by family history among long-term survivors of retinoblastoma. J Clin Oncol, 2012;30:950-957.

7.1 Introduction and rationale

The increased risk of second cancers diagnosed in hereditary retinoblastoma patients is well established, but studies have not previously assessed how this risk varies according to whether a germline *RB1* mutation was inherited from a parent or occurred *de novo.* This information could provide an insight into biological mechanisms and influence surveillance recommendations for hereditary survivors of RB. Although this cohort does not have mutation testing data for germline *RB1* mutations, we estimated the risk of second cancers according to laterality and family history as surrogate measures for mutation status (inherited or *de novo*). Survivors are inferred to have a germline mutation based on the presence of tumours in both eyes (bilateral) or in one eye (unilateral) with a family history of RB.

The question that we asked was: Are RB survivors who inherit a germline gene mutation from their parent at higher risk of another cancer than survivors with a *de novo* germline mutation? The aim of this analysis was to estimate the risk of second cancers in long-term survivors of RB according to the classification of germline mutation, using surrogate measures based on family history and laterality of the RB.

7.2 Methods

We assembled a cohort of 1852 eligible one-year survivors of RB from two hospital centres in the US. Based on data abstracted from medical records, we classified survivors as either bilateral (n=1036, 55.9%) or unilateral (n=816, 44.1%). We defined a positive family history as either a first- or second-degree relative with RB. We excluded having a child with RB as evidence of family history, because not all subjects would have had children nor would it have been mentioned in the original medical record. We then cross-classified survivors into four groups for analysis (Table 7.1).

Table 7.1 Classification by laterality and family history of retinoblastoma

Group	Bilateral	Positive family history of RB	Number and %
1	Yes	Yes	199 (10.7)
2	Yes	No or unknown	837 (45.2)
3	No	Yes	36 (2.0)
4	No	No or unknown	780 (42.1)

We confirmed second cancers by pathology reports (62.8%), hospital records (15.2%), autopsy reports (3.4%) or death certificates (18.6%). We included pineoblastoma, an intracranial tumour often referred to as trilateral RB, as a second cancer. It occurs in less than 10% of bilateral RB patients and is usually diagnosed at least 20 months after the bilateral RB (Rodriguez-Galindo et al., 2015). We excluded all in-situ cancers (except bladder), benign tumours and non-melanoma skin cancers, because general population incidence rates for these tumours are not available in the US.

Accrual of person years began one year after entry into the cohort and ended on the date of second cancer diagnosis, date of death, date last known alive or end of study

(December 31, 2001 for survivors diagnosed prior to 1985 (Cohort 1) and December 31, 1998 for survivors diagnosed with RB ≥ 1985 (Cohort 2), whichever occurred earliest.

Three types of analysis were conducted that focused on the risk of all second cancers and specifically bone cancer, soft tissue sarcomas and melanomas. These cancers were selected because they were the most common type of second cancers. We calculated SIRs compared with general population expected rates for all four groups of survivors. Univariate and multivariate Poisson regression models were conducted taking into account other risk factors but were restricted to bilateral survivors only, because the focus of the paper was on the difference between modes of transmission of germline mutations. The cumulative incidence of second cancers up to 50 years after RB diagnosis was determined for all four groups.

7.3 Main Findings

Overall, 13% of all RB survivors had a positive family history of RB (11% bilateral and 2% unilateral). Table 7.2 presents selected characteristics of the 4 groups. The median follow-up was less for the bilateral survivors with a family history compared with those without (19 yrs versus 26 yrs), whereas the unilateral survivors had similar years of follow up for those with and without a family history (25 yrs versus 28 years). Not unexpectedly, both bilateral and unilateral patients with a family history were significantly more likely to be diagnosed with RB at a younger age.

Table 7.2. Characteristics of 1852 1-year survivors of retinoblastoma,

	Bilateral (n=1036)				Unilateral (n=816)			
	Family history		No family history[a]		Family history		No family history[b]	
Characteristic	No.	%	No.	%	No.	%	No.	%
No. Survivors	199	10.7	837	45.2	36	1.9	780	42.1
Sex								
Male	107	53.8	434	51.8	20	55.6	395	50.6
Female	92	46.2	403	48.2	16	44.4	385	49.4
				P=0.626				P=0.564
Age at Rb								
<12 months	153	76.9	454	54.2	19	52.8	173	22.2
12-23 months	29	14.6	251	30.0	10	27.8	224	28.7
24+ months	17	8.5	132	15.8	7	19.4	383	49.1
				P<0.001				P<0.001
Calendar yr RB								
<1960	45	22.6	252	30.1	5	13.9	178	22.8
1960-69	48	24.1	245	29.3	8	22.2	205	26.3
1970-79	45	22.6	195	23.3	12	33.3	196	25.1
1980+	61	30.7	145	17.3	11	30.6	201	25.8
Median year	1970		1966		1975		1970	
				P<0.001				P=0.448
Radiation								
Yes	184	92.5	747	89.3	20	55.6	138	17.7
No	14	7.0	87	10.4	16	44.4	635	81.4
Unknown	1	0.5	3	0.3	0	0	7	0.9
				P=0.344				P<0.001
Chemotherapy								
Yes	72	36.2	349	41.7	5	13.9	101	12.9
No	123	61.8	474	56.6	31	86.1	669	85.8
Unknown	4	2.0	14	1.7	0	0	10	1.3
				P=0.334				P=0.784
Attained age								
<10 years	47	23.6	183	21.9	3	8.3	113	14.5
10-19 years	55	27.6	139	16.6	5	13.9	101	12.9
20-29 years	29	14.6	136	16.2	13	36.1	169	21.7
30-39 years	46	23.1	224	26.8	11	30.6	186	28.9
40+ years	22	11.1	155	18.5	4	11.1	211	27.0
				P=0.002				P=0.077
Median follow up yrs and range	19 (1-55)		26 (1-69)		25 (1-56)		28 (1-77)	

[a] includes 653 no and 184 unknown family history for bilateral survivors

[B] includes 641 no and 139 unknown family history for unilateral survivors

We observed significantly elevated SIRs for all second cancers combined in bilateral survivors with a family history (SIR=36) compared with those without a family history (SIR=19) (*P*=<0.001). We noted a similar pattern in unilateral survivors with a family history, based on much smaller number of second cancers (SIR=7.1 versus SIR=1.5, *P*=0.004). SIRs for melanoma were also significantly higher for bilateral survivors with a family history compared with those with no family history (*P*=0.004). Risks were increased for cancers of the bone, soft tissue, eye/orbit and nasal cavities independent of family history. (See table 7.3).

Table 7.3 Risk of second cancer in 1,852 1-year survivors of retinoblastoma

Retinoblastoma		Bilateral		Unilateral	
		Family history	No family history*	Family history	No family history*
No. survivors		199	837	36	780
Person-years		4,065	19,739	898	20,504
Cancer Site (ICD-O classification)					
All cancers (140-172,174-207)	O†	56[a]	188[b]	3[c]	22[d]
	Exp	1.6	10.1	0.4	14.5
	SIR	35.8	19	7.1	1.52
	95%CI	(27-46)	(16-22)	(1.5-21)	(0.9-2.3)
	AER	133	90	29	3.7
Bone (170)	O	15	62	0	0
	Exp	0.03	0.16	0.01	0.17
	SIR	459	388	0	0
	95%CI	(259-757)	(297-497)	(0-460)	(0-21)
	AER	37	35	-0.09	-0.08
Soft tissue (171, 192.4-.5)	O	10	25	1	0
	Exp	0.04	0.21	0.01	0.23
	SIR	233	118	106	0
	95%CI	(112-428)	(76-174)	(2.7-594)	(0.0-16)
	AER	24	13	11	-0.11
Cutaneous Melanoma (173, M8720-8790)	O	8	15	1	0
	Exp	0.12	0.77	0.03	0.99
	SIR	65.5	19.6	35	0
	95%CI	(29-129)	(11-32)	(0.9 -193)	(0-3.6)
	AER	19	7.2	6.3	-0.5
Eye/orbit (190)	O	2	8	0	0
	Exp	0.01	0.05	0	0.05
	SIR	177	155	0	0
	95%CI	(21-640)	(67-305)	(0->1000)	(0-73)
	AER	4.9	4.0	-0.02	-0.02
Nasal cavities (160)	O	7	22	0	0
	Exp	0.01	0.02	0	0.03
	SIR	2000	1041	0	0
	95%CI	(803->1000)	(652->1000)	(0->1000)	(0-132)
	AER	17	11	-0.01	-0.01
Brain, CNS (191-192)	O	2	9	0	2
	Exp	0.12	0.57	0.03	0.63
	SIR	16.7	15.7	0.0	3.2
	95%CI	(2.0-60)	(7.2-30)	(0-146)	(0.4-11)
	AER	4.6	4.3	-0.28	0.7

Pineoblastoma (194.4)	O	6	2	0	0
	Exp	0.01	0.04	0.0	0.04
	SIR	584	44.5	0.0	0.0
	95%CI	(214->1000)	(5.4-161)	(0->1000)	(0-82)
	AER	14.7	1.0	-0.02	-0.02

* No family history includes unknown family history

†O=observed no. of cancers; Exp=expected no. of cancers, SIR=Standardised incidence ratio; CI=confidence interval; AER=Absolute excess risk (Obs-Exp/person years x 10,000)

[a] Other cancers include 2digestive (1colon,1 small intestine), 1 other respiratory, 1 acute lymphocytic leukaemia, and 2 unknown site

[b] other cancers include 2 tongue, 2 salivary gland, 2 nasopharynx, 2 colon, 3 lung, 3 other respiratory, 8 female breast, 1 male breast, 5 corpus uteri, 1 testis, 1 kidney, 3 bladder, 2 thyroid, 1 NHL, 2 Hodgkin lymphoma, 1 lymphocytic leukaemia NOS, and 6 unknown primary site

[c] other cancer includes one Hodgkin lymphoma

[d] other cancers include 7 female breast cancers, 2 thyroid, 2 uterine corpus , 1 rectum, 1 prostate, 1 kidney, 1 Hodgkin lymphoma, 1 acute myeloid leukaemia, and 4 unknown primary sites.

We noted a modestly elevated relative risk (RR) for all second cancers combined associated with family history in bilateral survivors (RR=1.37, 95%CI 1.00-1.86, *P*=0.05) adjusted for treatment, age and length of follow-up. A stronger association was noted for melanoma (RR=3.08, 95%CI 1.23-7.16, *P*=0.02), but not for bone cancer or soft tissue sarcoma. Aside from family history, the other risk factors associated with increased risk of second cancers included radiotherapy (*P*=0.001) and older attained age (*P*<0.001). Both risk factors were also significantly increased for bone cancer and soft tissue sarcomas, whereas risks for melanoma were associated with older attained age (>25 years) and earlier calendar year of diagnosis (<1970) but not radiotherapy.

Cumulative incidence of all second cancers at 50 years after RB diagnosis was higher for bilateral survivors with a family history of RB compared with those without a family history (47%, 95%CI 35%-59% compared with 38%, 95%CI 32%-44%, *P*=0.004) (Figure 7.1). A similar pattern was observed among the unilateral survivors with and

without a family history of RB, based on small numbers of second cancers (18%, 95% CI 0.0%-42% and 8%, 95% CI 3%-13%). Consistent with the multivariate analyses, the cumulative incidence did not differ by family history for bone or soft tissue sarcoma, but did differ for melanoma (9%, 95%CI 3%-15% compared with 2%, 95% CI 0.9%-4.0%).

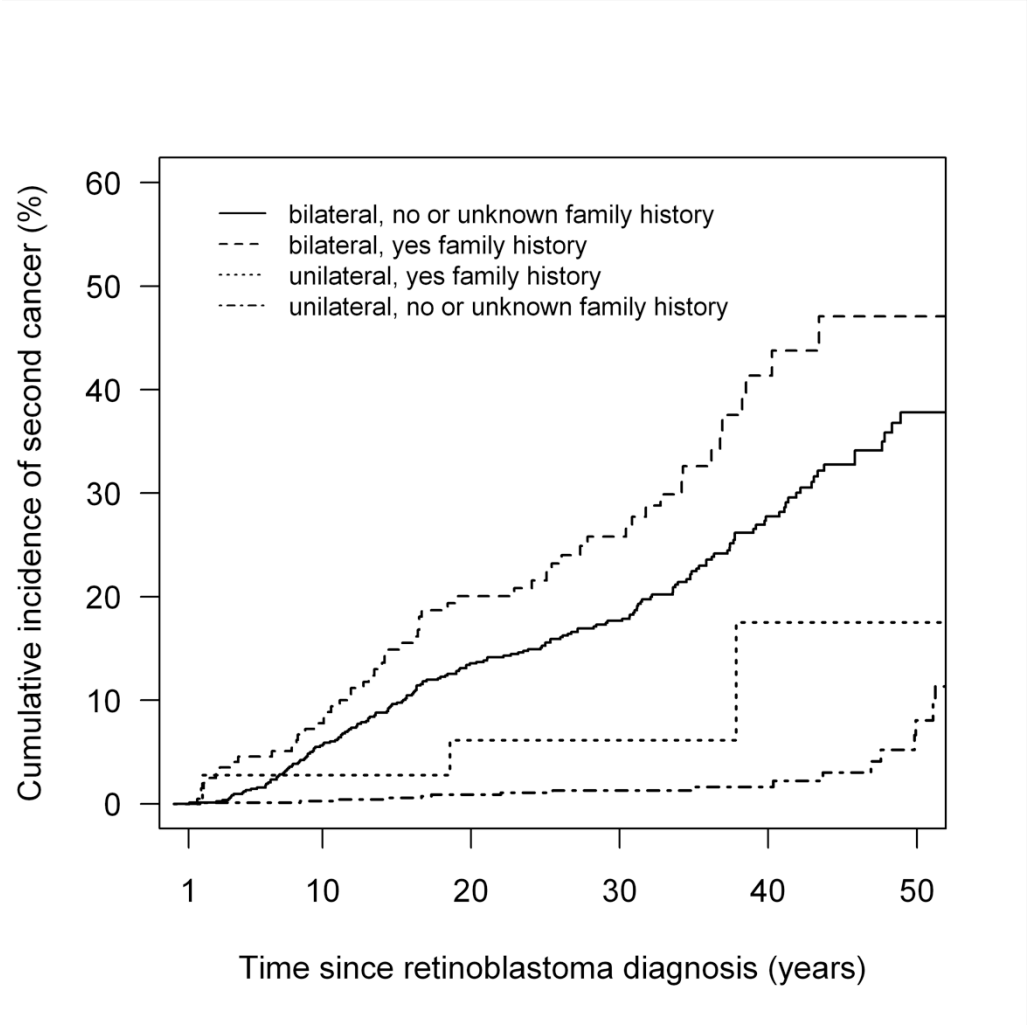


Figure 7.1. Cumulative incidence percent of second cancers by decade up to 50 years after retinoblastoma diagnosis in 1,852 1-year survivors of retinoblastoma by family history and laterality

7.4 Significance

This was the first report to estimate the risk of second cancers in long-term survivors of RB by the presence of an inherited or *de novo* germline mutation taking treatment and other risk factors into account (Kleinerman et al., 2012). Using laterality of and family history of RB as surrogate measures of mutation status, we showed that a presumed inherited germline mutation confers a 37% increased risk for a second cancer, adjusted for treatment, age and length of follow-up compared with survivors with a *de novo* germline mutation. An even stronger association with family history was noted for melanoma, but not for bone cancer or soft tissue sarcoma. Consistent with these findings, the cumulative incidence of a second cancer 50 years after RB diagnosis was highest for bilateral survivors with a family history, followed by bilateral survivors without a family history, unilateral survivors with a family history and unilateral survivors with no family history.

Among the three most common tumours after RB that we investigated, melanoma was the only one to be consistently related to family history of RB. Not unexpectedly, melanoma was also increased in survivors older than age 25 and those treated prior to 1970. Melanoma typically starts to increase in incidence in the 20s (Bradford et al., 2010) consistent with the trend that we observed. In contrast to bone cancer and soft tissue sarcoma, increased risk for melanoma was not associated with radiotherapy. These findings indicate that having an inherited germline *RB1* mutation may predispose to melanoma and that there is a strong genetic component.

Treatment exerted a stronger influence for the development of bone cancer and soft tissue sarcoma. This was of interest but not too surprising since a radiation dose response has been demonstrated for both of these tumours (Hawkins et al., 1996, Tucker et al., 1987, Wong et al., 1997).

Table 7.4 Relative risks for the three most common cancers in bilateral retinoblastoma survivors.

Risk Factor	Bone	STS	Melanoma
	RR 95%CI	RR 95%CI	RR 95%CI
Family History RB (yes/no)	0.92(0.5-1.6)*	0.99 (0.5-1.9)	3.08 (1.2-7.2)
Age at RB (≥24 months)	0.71 (0.3-1.4)	0.19 (0.03-0.6)	0.45(0.1-1.6)
Calendar Yr RB (<1970)	0.93 (0.6-1.6)	0.87 (0.4-1.8)	5.99 (1.2-110)
Sex (Female)	0.92 (0.6-1.5)	0.76 (0.5-1.3)	1.92 (0.8-4.7)
Radiotherapy (yes/no)	7.05 (1.6-125)	7.16 (1.6-127)	1.46 (0.4-9.3)
Chemotherapy (yes/no)	1.71 (1.1-2.8)	1.32 (0.8-2.2)	0.83 (0.4-2.0)
Attained Age (≥40 years)	0.33 (0.02-1.6)	5.01 (1.6-14)	6.28 (2.6-18)¶

*Relative risks adjusted for all of the other risk factors other than one of interest.
¶ Attained age ≥25 years

This analysis was conducted differently than previous analyses in order to address the question of family history and risk. We did not assign survivors to hereditary and non-hereditary categories. Although it was clear that there was an increased risk of second cancers among unilateral survivors with a family history reported, we decided not to combine these 36 survivors with the bilateral survivors. These unilateral survivors were phenotypically different from the bilateral patients and their germline mutations may represent mosaicism with incomplete penetrance of the *RB1* mutation.

A weakness of the analysis was the reliance on surrogate measures of the mutation status rather than mutation testing data. Although laterality is a reliable

measure, family history is subject to misclassification if it is not recorded in the medical record. It is likely that family history could have been under-reported in the medical records, which would bias the results towards the null. We had restricted knowledge of family history to the original medical record rather than rely on subsequent reporting by a survivor on a questionnaire. Using additional data from questionnaires could bias the results in the other direction if those with a family history were more likely to answer the questionnaire. The proportion of survivors in our study with a positive family history was consistent with other population-based studies (Houdayer et al., 2004, Marees et al., 2010).

Moll et al. (Moll et al., 2012) in a letter to the editor raised the possibility that mosaicism in those hereditary survivors with a de novo *RB1* germline mutation could account for a lower risk of second cancers compared with survivors with an inherited germline mutation. Due to mosaicism, not all cells carry the *RB1* mutation and this could explain the lower risk of second tumours (Kaye and Harbour, 2004). Moll et al (Moll et al., 2012) point out that between 6-10% of de novo mutations may be mosaic.

In response to their concern (Kleinerman et al, 2012, Reply to AC Moll), we estimated what the relative risk of a second cancer would be if the rate of mosaicism was 10% in our cohort as suggested by Moll. At a rate of 10%, we were able to detect significant differences in overall risk of second cancers. We then estimated that the rate of mosaicism would have to be as high as 30% in the *de novo* survivors in order to no longer detect a significance difference in risk of second cancers. We agreed that the difference in risk could be attributed partially to mosaicism, although we have no

information on the prevalence of mosaicism in our study, however, the difference could also be attributed to the penetrance of the *RB1* mutations or perhaps modifier genes in the families.

Interestingly, two recent studies on the frequency of mosaicism in *RB1* sporadic patients indicate that low-level mosaic variants ranged from 8-24% in 40 RB patients (Amitrano et al., 2015) and 10% in 140 RB patients (Dommering et al., 2014). Unfortunately we do not have mutation testing available for our entire cohort in order to estimate the level of mosaicism.

7.5 Public Health Message

Hereditary RB survivors with an inherited germline mutation should undergo annual skin examinations starting in adolescence to identify early signs of melanoma.

7.6 Role in study

Role: I initiated the idea for the analysis, reviewed the literature and drafted the manuscript. Study team: Dr Little was the consulting statistician, Dr Yu, a post-doctoral fellow whom I mentored, conducted the analysis with consultation from Dr Li. Drs Abramson and Seddon were the clinical collaborators and provided the patients; Dr Tucker was the senior study investigator. My contribution: 80%

7.7 Publication

Chapter 8. Chemotherapy and Radiotherapy

Wong JR, Morton LM, Tucker MA, Abramson DH, Seddon JM, Sampson JN, Kleinerman RA. Risk of subsequent malignant neoplasms in long-term retinoblastoma survivors following chemotherapy and radiotherapy. J Clin Oncol, 32:3284-3290, 2014.

8.1 Introduction and rationale

Hereditary retinoblastoma survivors are at risk of developing second cancers due to radiotherapy, but patients are increasingly being treated with chemotherapy and little is known about risks of second cancers related to chemotherapy. Previous analyses in this cohort suggested increased risks for those treated with chemotherapy compared with those not treated with chemotherapy. However, no detailed analyses exploring chemotherapy on the risk of second cancers have previously been conducted.

8.2 Methods

For this analysis, we focused on the risk of second cancers in 5-year survivors of hereditary RB (n=906) in cohorts 1 and 2, because treatment effects were not likely to occur earlier. The majority of these survivors were treated with radiotherapy (n=813, 89.7%) and 43% (n=336) of those also received chemotherapy. Only 80 survivors (n=10%) received surgery alone and very few received only chemotherapy (n=13, <0.1%).

We estimated risks of all second cancers combined, for the three most common second cancers (bone cancer, soft tissue sarcoma and melanoma) and for epithelial tumours combined (breast, lung, thyroid, bladder, colorectal, kidney, nasal cavity, prostate, tongue and uterus) compared with the general population rates for these

cancers. SEER data were used to generate expected rates of these cancers from the US general population in order to calculate SIRs. Person-years began being counted 5 years after RB diagnosis and ended on the date of second cancer diagnosis, death, lost to follow up or last contact, whichever occurred earliest. Follow-up for this analysis was up through 2009 based on the date of the last contact with study subjects.

We used a Cox proportional hazard regression model with age as the time scale to evaluate the risk of second cancers among those treated with chemotherapy and radiotherapy and for different chemotherapy subgroups relative to those treated with radiotherapy alone. Because we had not collected treatment for any of the second cancers, we included a time-dependent indicator variable for those survivors who developed a second cancer other than the second cancer of interest. We also stratified models by calendar year of diagnosis to account for changes in treatment practices over time. Most patients were treated with TEM prior to 1970, whereas, many other alkylating agents were used after that time.

Other exploratory analyses included risks by SIRs by attained age (<25, ≥25 years at RB diagnosis), location of the second cancer in relation to the radiotherapy field (in or outside), and an estimate of the cyclophosphamide equivalent dose for specific chemotherapies.

Cumulative incidence for all of the second cancer groups and specific subtypes were calculated taking into account the competing risk of death and loss to follow up.

8.3 Main findings

Among the 813 survivors treated with either radiotherapy with or without chemotherapy, 33% (n=265) developed at least one second cancer and 6% (n=46) developed more than one. The median follow-up was 26.3 years (0.6-63 years).

SIRs for the risk of second cancers significantly differed for those treated with radiotherapy compared with radiotherapy plus chemotherapy (Obs=135, SIR=20.4, 95% CI 17.1-24.2 and Obs=130, SIR=26.4, 95%CI 22.0-31.3; *P*=0.04) (See Table 8.1). SIRs were significantly greater for those treated with chemotherapy and radiotherapy compared with radiotherapy for bone cancers (*P*=0.03) and LMS (*P*<0.001). We did not observe similar differences for SIRs for by treatment other soft tissue sarcomas, melanomas or epithelial tumours.

The results of the hazards ratio models indicated similar findings as the SIR analyses. Chemotherapy and radiotherapy were related to a significantly increased risk for all second cancers combined (HR=1.31, 95% CI 1.02-1.68) compared with radiotherapy alone. Risks for bone cancer (HR=1.73, 95% CI 1.13-2.67) and leiomyosarcoma (HR=2.61, 95% CI 1.19-5.70) were significantly increased. Estimating risks separately for alkylating agents and radiotherapy and for TEM and radiotherapy indicated increased risks for bone tumours associated with alkylating agents (HR=1.60, 95% CI 1.03-2.49) and for LMS with both alkylating agents (HR=2.67, 95%CI 1.22-5.85) and TEM (HR=3.21, 95%CI 1.40-7.39).

Table 8.1. Risk of subsequent cancer by treatment received by 813 5-year survivors of hereditary retinoblastoma[1]

	RT[2]	CT+RT		AA+RT		TEM+RT		Other AA+RT	
Outcome	**N**	**N**	**HR (95% CI)**	**N**	**HR (95% CI)**	**N**	**HR (95% CI)**	**N**	**HR (95% CI)**
All SMN	135	130	**1.31 (1.02-1.68)**	124	1.27 (0.99-1.63)	101	1.27 (0.96-1.68)	23	1.18 (0.77-1.80)
Bone	44	48	**1.73 (1.13-2.67)**	44	**1.60 (1.03-2.49)**	32	1.48 (0.88-2.47)	12	1.70 (0.88-3.28)
Soft tissue sarcoma	46	46	1.29 (0.84-1.97)	45	1.30 (0.84-1.99)	38	1.40 (0.88-2.25)	7	1.05 (0.46-2.39)
Leiomyosarcoma	10	22	**2.61 (1.19-5.70)**	22	**2.67 (1.22-5.85)**	20	**3.21 (1.40-7.39)**	2	1.65 (0.31-8.80)
Other/unspecified soft tissue sarcoma	36	24	0.89 (0.52-1.52)	23	0.87 (0.51-1.51)	18	0.85 (0.46-1.57)	5	0.94 (0.36-2.44)
Melanoma	19	12	0.72 (0.35-1.50)	12	0.74 (0.36-1.55)	11	0.83 (0.38-1.78)	1	–
Epithelial	25	21	0.94 (0.51-1.73)	20	0.89 (0.48-1.64)	16	0.78 (0.40-1.52)	4	1.07 (0.34-3.36)

RT= radiotherapy without chemotherapy, CT+RT= chemotherapy and radiotherapy, AA+RT= any alkylating agent and radiotherapy, TEM+RT= TEM only and radiotherapy, Other AA+RT= other alkylating agent, with or without TEM, and radiotherapy, N= number of subjects, HR= hazard ratio, 95% CI=: 95% confidence interval, SMN= subsequent malignant neoplasm. Subjects who received surgery (80) or chemotherapy only (13) were excluded since few or no subsequent neoplasms were reported in this treatment group. Bold indicates statistical significance at $p < 0.05$

[1] adjusted for sex, age at retinoblastoma diagnosis (<1, 1+ years), calendar year of Rb diagnosis (1914-59, 1960-69, 1970-79, 1980-1996), and time-dependent covariate for prior SMN diagnosis

[2] reference group for all hazard ratio calculations

Cumulative incidence of second cancers at 50 years after RB diagnosis for survivors treated with radiotherapy compared with radiotherapy and alkylating agents indicated significant differences only for leiomyosarcoma (*P*=0.02).

Table 8.2. Cumulative incidence of subsequent cancers after hereditary retinoblastoma by treatment received

	Radiotherapy Cumulative incidence (%) and 95% confidence intervals at attained age of:		**Alkylating Agent with Radiotherapy** Cumulative incidence (%) and 95% confidence intervals at attained age of:		
Type of second cancer	**25 years**	**50 years**	**25 years**	**50 years**	*P*
Bone	8.4 (5.2-11.6)	13.0 (2.1-23.9)	11.7 (7.4-16.0)	14.7 (3.9-25.5)	0.16
Leiomyosarcoma	0.5 (0.0-1.3)	6.3 (0.0-14.3)	0.7 (0.0-1.8)	8.7 (0.0-17.4)	0.02
Other/unspecified soft tissue sarcoma	4.8 (2.3-7.3)	15.5 (3.5-27.5)	3.3 (0.9-5.7)	9.2 (0.4-18.0)	0.35
Melanoma	1.0 (0.0-2.2)	7.1 (0.0-15.5)	1.0 (0.0-2.3)	4.9 (0.0-11.7)	0.34

Exploratory analyses by age at RB diagnosis indicated that there was no difference in risk for all second cancers combined for those who received alkylating agents before and after one year of age. However, the association of risk with age was stronger for leiomyosarcoma in survivors who were treated with alkylating agents at less than one year compared with over one year (HR=5.2 vs. HR=1.8, *P*=0.08).

Risks for individual second cancers (leiomyosarcoma and melanoma) were not related to location of the tumour in relation to the radiotherapy field. However, risk for bone cancers did differ by location in relation to the radiation field (HR= 2.28, 95%CI 1.02-5.11 for infield and HR=1.44, 95%CI 0.82-2.52 for outside the field).

8.4 Significance

We had previously evaluated risks for soft tissue sarcomas in relation to chemotherapy but not as detailed as the current analyses. This report provided quantitative evidence that the risks for both bone tumours and leiomyosarcoma are higher for hereditary survivors who had been treated with alkylating agents and radiation compared with those who were treated with radiation alone. Our data are consistent with a previous UK case-control study of bone cancers in RB survivors that reported a non-significant 2.1-fold risk of bone cancers in those who received chemotherapy and radiation compared with radiation alone (Hawkins et al., 1996). The findings for leiomyosarcoma are also in agreement with another UK study of childhood cancer survivors that found a positive dose-response for chemotherapy (alkylating agents) for soft tissue sarcomas (Jenkinson et al., 2007). Unfortunately, we had too few survivors treated with chemotherapy alone to evaluate the risk of second cancers in that group and we lacked dose data to demonstrate a dose-response. These data provide further evidence of risks of bone cancer and leiomyosarcomas in RB survivors who were previously treated with both alkylating agents and radiation. Although these survivors were treated mainly with TEM, which is no longer used, current chemotherapies include

other alkylating agents that have similar toxicity (Rodriguez-Galindo et al., 2015). This makes these data relevant to the current use of chemotherapy used to treat RB patients.

8.5 Public Health Message

The higher risks for bone tumours and leiomyosarcomas associated with alkylating agents and radiation treatment should increase awareness of the potential chemotherapy-related risks for second cancers in long-term survivors.

8.6 Role in study

Role: I initiated the idea for the study and contributed to the interpretation of the data and preparation of the manuscript. Study team: Ms Wong was a pre-doctoral student whom I mentored and she conducted the analyses and wrote the manuscript. Drs Morton and Tucker were senior study investigators, Dr Sampson was the statistician and the clinical collaborators were Drs Abramson and Seddon. My contribution: 55%.

8.7 Publication

Appendix

Table A1. SIRs for SMNs by Treatment Received and Follow-Up Among 5-Year Survivors of Hereditary Rb (n = 813)

	RT*				CT Plus RT†				
Outcome (years of follow-up)	Observed	SIR	95% CI	EAR‡	Observed	SIR	95% CI	EAR‡	*P*
All SMNs									
< 25	75	53.1	41.7 to 66.5	98.3	64	62.9	48.5 to 80.3	119.1	.33
≥ 25	60	11.6	8.8 to 14.9	166.8	66	16.9	13.0 to 21.5	230.4	**.03**
Bone tumor									
< 25	36	452.2	316.7 to 626.1	46.9	40	788.0	563.0 to 1,073.0	74.8	**.01**
≥ 25	8	324.9	140.3 to 640.1	21.7	8	397.0	171.4 to 782.3	25.9	.69
Soft tissue sarcoma									
< 25	25	218.3	141.3 to 322.3	32.5	13	165.2	87.9 to 282.4	23.9	.32
≥ 25	21	81.4	50.4 to 124.4	57.9	33	138.4	95.3 to 194.4	110.6	**.05**
Leiomyosarcoma									
< 25	2	391.5	47.4 to 1,414.2	2.6	2	513.9	62.2 to 1,856.5	3.7	.34
≥ 25	8	291.5	125.9 to 574.4	21.7	20	982.7	600.2 to 1,517.6	65.7	**< .001**
Other/unspecified soft tissue sarcoma									
< 25	23	209.9	133.0 to 314.9	29.9	11	146.5	73.1 to 262.2	20.2	.25
≥ 25	13	55.3	29.4 to 94.5	35.2	13	56.9	30.3 to 97.3	41.6	.91
Melanoma									
< 25	4	37.6	10.2 to 96.1	5.0	4	49.3	13.4 to 126.2	7.2	.65
≥ 25	15	31.2	17.4 to 51.4	40.1	8	18.9	8.2 to 37.3	25.1	.25
Epithelial tumor									
< 25	8	30.9	13.3 to 60.8	10.0	4	19.3	5.3 to 49.5	7.0	.43
≥ 25	17	4.5	2.6 to 7.2	37.0	17	5.9	3.4 to 9.4	45.8	.44

NOTE. Survivors who underwent surgery (n = 80) or received CT only (n = 13) were excluded, because few or no subsequent neoplasms were reported in these treatment groups. Bold font indicates statistical significance at $P < .05$.
Abbreviations: CT, chemotherapy; EAR, excess absolute risk; Rb, retinoblastoma; RT, radiotherapy; SIR, standardized incidence ratio; SMN, subsequent malignant neoplasm.
*Total of 477 survivors and 10,769.9 person-years at risk.
†Total of 336 survivors and 7,981.9 person-years at risk.
‡Per 10,000 persons.

Table A2. Risk for SMNs by Treatment Received and Tumor Location Among 5-Year Survivors of Hereditary Rb (n = 813)

	RT*	CT Plus RT		
Outcome	No.	No.	HR	95% CI
Bone				
In field	25	27	1.44	0.82 to 2.52
Out of field	12	15	**2.28**	**1.02 to 5.11**
Leiomyosarcoma				
In field	4	8	2.50	0.73 to 8.58
Out of field	6	13	2.57	0.91 to 7.27
Other/unspecified soft tissue sarcoma				
In field	27	17	0.82	0.44 to 1.54
Out of field	3	3	1.50	0.28 to 7.99
Melanoma				
In field	5	4	0.87	0.23 to 3.26
Out of field	9	8	1.04	0.39 to 2.78
Epithelial tumor				
In field	11	7	0.59	0.20 to 1.79
Out of field	15	14	0.86	0.37 to 1.97

NOTE. Survivors who underwent surgery (n = 80) or received CT only (n = 13) were excluded, because few or no subsequent neoplasms were reported in these treatment groups. Adjusted for sex, age at retinoblastoma diagnosis (< 1 *v* ≥ 1 year), calendar year of Rb diagnosis (1914-1959, 1960-1969, 1970-1979, or 1980-1996), and time-dependent covariate for prior SMN diagnosis. Bold font indicates statistical significance at $P < .05$.
Abbreviations: CT, chemotherapy; HR, hazard ratio; Rb, retinoblastoma; RT, radiotherapy; SMN, subsequent malignant neoplasm.
*Reference group for all HR calculations.

Table A3. Cumulative Incidence of Subsequent Malignant Neoplasms After Retinoblastoma by Treatment Received

	Radiotherapy						Alkylating Agent With Radiotherapy						
	20 Years			40 Years			20 Years			40 Years			
Cancer Site	No.	Cumulative Incidence (%)	95% CI	No.	Cumulative Incidence (%)	95% CI	No.	Cumulative Incidence (%)	95% CI	No.	Cumulative Incidence (%)	95% CI	*P*
Bone	345	6.5	3.9 to 9.1	109	9.0	3.6 to 14.4	227	11.0	6.9 to 15.1	105	12.7	6.3 to 19.1	.07
Leiomyosarcoma	349	0.4	0.0 to 1.1	109	1.6	0.0 to 4.0	237	0.0	0.0 to 0.0	103	5.8	1.3 to 10.3	**.01**
Other/unspecified soft tissue sarcoma	343	4.4	2.2 to 6.6	109	6.0	1.5 to 10.5	235	2.5	0.5 to 4.5	103	6.9	2.0 to 11.8	.58
Melanoma	349	0.4	0.0 to 1.1	110	4.0	0.3 to 7.7	236	0.6	0.0 to 1.6	103	3.3	0.0 to 6.7	.57
Epithelial	347	0.8	0.0 to 1.7	108	3.8	0.2 to 7.4	237	0.6	0.0 to 1.6	105	3.4	0.0 to 6.9	.87

NOTE. Bold font indicates statistical significance at $P < .05$.

Table A4. CED Estimates for Typical Chemotherapies Received for Rb

Dose	Cyclophosphamide[53]	Ifosfamide[53]*	Carboplatin[53]†	Nitrogen Mustard[53]‡	TEM[17]§
Equivalent dose	100 mg/m^2	409 mg/m^2	29 mg/m^2	1 mg/m^2	0.3 mg/m^2
Equivalent dose factor‖	1.0	0.244	3.448	100	333
Typical dose for Rb	120 mg/m^2	1,600 mg/m^2 × 5 days or 3,000 mg/m^2 × 2 days	200 mg/m^2	12 mg/m^2	0.2 mg/m^2
CED¶	**120 mg/m^2**	**1,464 to 1,952 mg/m^2**	**689.6 mg/m^2**	**1,200 mg/m^2**	**66.6 mg/m^2**

NOTE. Bold font indicates statistical significance at $P < .05$.
Abbreviations: CED, cyclophosphamide equivalent dose; Rb, retinoblastoma; TEM, triethylenemelamine.
*Pratt CB et al: Med Pediatr Oncol 13:330-333, 1985; Pratt CB et al: Cancer Treat Rep 71:131-135, 1987; and Schwartzman E et al: Cancer Chemother Pharmacol 24:S11-S12, 1989 (suppl 1).
†Kingston JE et al: Arch Ophthalmol 114:1339-1343, 1996.
‡Mrazek RG Jr et al: J Am Med Assoc 159:160-163, 1955 and Diamond HD: Ann N Y Acad Sci 68:974-978, 1958.
§Mrazek RG Jr et al: J Am Med Assoc 159:160-163, 1955; Diamond HD: Ann N Y Acad Sci 68:974-978, 1958; Reese AB et al: AMA Arch Ophthalmol 60:897-906, 1958; and Hyman GA et al: Arch Ophthalmol 80:744-746, 1968.
‖CED/dose of drug of interest.
¶Equivalent dose factor × typical dose for Rb.

Chapter 9. Summary

9.1 Summary

In this series of studies conducted by the author in a hospital-based retrospective cohort of 1852 long-term survivors of retinoblastoma, survivors with a *RB1* germline mutation have an increased risk for second cancers mainly due to the occurrence of three types of cancers: soft tissue sarcomas, bone cancers and melanoma. Second cancer risks persist for decades, and by age 50, one in three hereditary survivors and one in 20 non-hereditary survivors will develop a second cancer.

The two major contributing causes to second cancers in this cohort were having a germline *RB1* mutation and treatment of RB with radiation. Over 85% of hereditary patients received radiation treatment and this clearly increased the risk of many second cancers, primarily bone and soft tissue sarcomas that were previously shown to be dose-related (Wong et al., 1997). Although chemotherapy in combination with radiotherapy increased the risk of some second cancers, notably bone sarcomas, the role of chemotherapy was not as clear due to small number of survivors treated only with chemotherapy.

This series of studies presented data for the first time on the risk of soft tissue sarcomas by histologic subtype, which highlighted the greatest risks for leiomyosarcomas in this population (Kleinerman et al., 2007). In particular, an increased risk of uterine leiomyosarcoma in female hereditary survivors was identified that was followed up by a more in-depth evaluation of the risk (Francis et al., 2012).

Risks for individual epithelial tumours of adulthood following treatment for hereditary RB were not as clear in these data likely due to small numbers. Lung and breast cancers were the most frequently reported epithelial cancers in these survivors. Somatic mutations in the *RB1* gene have been reported in non-small cell lung cancer (Harbour et al., 1988) and in breast cancer (Bosco and Knudsen, 2007). Lung cancer mortality was significantly elevated in hereditary survivors, but risk was not associated with past radiotherapy for RB (Kleinerman et al., 2000). All of the lung cancer cases were smokers suggesting an interaction with the *RB1* gene or perhaps enhanced susceptibility to the effects of tobacco. Interestingly, breast cancer risk was elevated in both hereditary and non-hereditary survivors. There are a number of other epithelial cancers that have somatic *RB1* mutations identified in their pathways, such as, bladder, ovary and prostate, but our data were too limited to be able to show significantly increased risks of cancer of these organ sites.

Genetic predisposition, i.e., *RB1* germline mutation, also contributes to these increased risks of second cancers. We provided data that showed for the first time that melanoma risks appear to be higher for those bilateral survivors who inherit a *RB1* germline mutation from a parent compared with those bilateral survivors with a *de novo* mutation (Kleinerman et al., 2012). There was a suggestion in our data that the risk for all second cancers combined was higher for those with an inherited *RB1* germline mutation, but not for bone or soft tissue sarcoma individually.

My colleagues and I currently have genetic studies underway with this cohort to identify genetic variants of the *RB1* germline mutations that may be related to the risk of

specific second cancers in hereditary survivors. Identification of specific *RB1* germline mutations would signal those survivors at highest risk of a second cancer. Future work would also include detecting molecular changes that characterize second cancers in relation to radiotherapy or chemotherapy.

9.2 Limitations of the data

The source of the population was hospital-based rather than population-based and included only two institutions that may potentially limit the generalisability of the findings to the general population. The cohort was not representative of the true proportion of hereditary patients in the general population, because it included a much larger proportion of hereditary compared with non-hereditary survivors typically found in the general population. However, loss to follow-up was similar in both groups and each group was independently compared with the general population. In addition, RB is a rare cancer and second cancers are rare, so the inclusion of more hereditary survivors increases the value of the population to identify risks of second cancers. Treatment differed greatly by hereditary status with almost all hereditary patients receiving radiotherapy (85%) compared with a very small proportion of non-hereditary patients treated with radiotherapy (15%). This could have confounded the results, but when we stratified the risks by radiation dose in three categories (high, moderate and low), we observed the highest risks for organ sites in the head region that received the highest doses of scatter radiation.

Because we did not conduct mutation testing on all of the survivors to determine the presence of a germline *RB1* mutation, we did not know whether all of the unilateral

patients were classified correctly as non-hereditary. Approximately 10%-15% of unilateral survivors have a germline mutation that is less penetrant causing only one eye to be affected. We relied on laterality of RB and mention of family history of RB in the medical record to determine whether a survivor was likely to be hereditary. It is likely that some unilateral RB survivors may have been misclassified as non-hereditary, due to unknown family history of RB or a less penetrant form of a germline *RB1* mutation (mosaicism). This would have biased the risk upward for second cancers in the non-hereditary survivors, and influenced the ratio of risks of hereditary: non-hereditary towards the null. But given the very large increase in second cancer risk in the hereditary patients, the misclassification would likely have had a very small effect. Interestingly, when we restricted the unilateral patients with a family history whom we had classified as hereditary, their second cancer risk resembled that of the bilateral patients.

For the incident analyses, we had to rely on validated self-reports of incident cancers in this cohort, because there is not national cancer registry in the US. We were unable to contact 100% of the cohort in the most recent survey that we conducted and therefore we likely missed a number of incident cancers or conversely, we may have bias in that survivors with second cancers were more likely to respond to the survey. In the future, we will conduct sensitivity analyses to evaluate the extent of the bias in the cohort. We can also make the assumption that all non-responders developed the second cancer of interest and calculate a SIR and compare it to the SIR if none of the non-responders developed the cancer of interest and see how it differs. A virtual cancer

registry in the US is in development and in a few years when it is up available, we can match our cohort to ascertain incident cancers more efficiently on a nation-wide level.

9.3 Disentangling the role of genetic susceptibility and treatment

All of these studies that I have described in this dissertation have been focused primarily on hereditary survivors and radiotherapy, because the majority of the hereditary survivors were treated with radiation and almost all of the second cancers were diagnosed in hereditary survivors. There were too few hereditary survivors who were treated by surgery alone to be able to quantify the contribution of genetic susceptibility in the absence of radiation. We formally tested an interaction between radiotherapy and genetic susceptibility for increased mortality due to second cancers, but it was not statistically significant (Yu et al., 2009). An on-going analysis of risk related to the location of the bone and soft tissue sarcoma in proximity to the radiotherapy field may yield some data on this issue (Kleinerman et al, in preparation, 2016). In the current analysis I am finding that 25% of the bone cancers are diagnosed in the lower leg are diagnosed only up to age 25, whereas the other 75% of bone sarcomas are diagnosed in the head, which was in the radiotherapy field, up to age 55 years. The diagnosis of bone sarcoma in the lower leg is clearly not related to radiotherapy received by the eye. Similarly, the increased risk of uterine leiomyosarcoma indicates a likely genetic predisposition, because the scatter dose to the uterus was quite small, about 0.2 Gray.

9.4 Changes in treatment and implications for cancer screening

Over the past 10 years, treatments for RB indicate much less use of radiotherapy and replacement with intra-arterial or intravitreal chemotherapy (Abramson et al., 2015b). To date, there are no formal screening guidelines for young adult or adult survivors of RB. Based on data from our cohort and other cohorts in the UK and Netherlands, we know that there is a pool of survivors treated with radiotherapy in the past who will need to be followed for future bone and soft tissue sarcomas of the head. Chemotherapy (alkylating agents) was related to the incidence of bone sarcomas and leiomyosarcomas, and may still pose a risk in the future for survivors. Although not related to radiotherapy, hereditary survivors will need to aware of increased risks melanoma and lung cancer. The risks for breast cancer related to treatment are not clear, but survivors should be aware of possible risks.

9.5 Cancer prevention and screening recommendations

I had evaluated the cancer screening behaviour of the survivors in our cohort based on responses to a telephone questionnaire in 2000 (Sheen et al., 2008). We found that 87% of females had a Pap test within the past 2 years, 76% of females age >40 years reported having a mammogram within the past 2 years, and 17.4% of males had performed monthly testicular self-examinations. A significantly higher proportion of hereditary compared with non-hereditary survivors reported having undergone an MRI or CT scan in the past 5 years, likely due to second cancers. Higher education, greater contact with the medical care system, and having a second cancer were found to be associated positively with most screening practices. We found that cancer screening

behaviour reported by RB survivors was similar to national screening rates for breast, cervical, and testicular cancer. As the first report of cancer screening practices of Rb survivors, we concluded that survivors of hereditary RB should be encouraged to maintain, if not increase, their current screening practices to ensure early detection of second cancers in this high-risk population.

Unfortunately, there are no widely used screening procedures for sarcomas other than possibly using whole body Magnetic Resonance Imaging (WB-MRI) that does not use radiation (Friedman et al, 2012) or F-18 Fluorodeoxyglucose-Positron Emission Computed Tomography (PET) screening, which does includes exposure to ionizing radiation (Masciari et al., 2008) . Both Friedman et al and Masicari et al performed pilot studies to evaluate the utility of WB-MRI and PET scans, respectively, to detect sarcomas in childhood cancer survivors. Neither study was able to demonstrate a clear advantage to using either method to detect sarcomas. Based on these studies presented in this dissertation, we recommend the following for prevention of second cancers:

Table 9.1 Public health recommendations to prevent second cancers based on published studies.

Recommendation	Rationale	Source
Avoid smoking or if a smoker, make an effort to quit.	Increased mortality from lung cancer in hereditary patients	(Kleinerman et al., 2000, Fletcher et al., 2004)
Reduce sun exposure and avoid tanning beds. Begin regular skin clinical examinations in adolescence.	Increased risk of melanoma starting at age 20 in hereditary survivors	(Kleinerman et al., 2012).

9.6 Risk factors

The following chart lists the relative strength of risk factors related to the main second cancers occurring after hereditary RB that we were able to evaluate.

Table 9.2 Risk factors for selected second cancers after hereditary retinoblastoma

Risk Factor	Bone	Soft Tissue Sarcoma	Melanoma	Uterine Leiomyosarcoma	Lung
Family History RB (yes/no)	--	--	+++	NE	NE
Age at RB	--	++	--	NE	NE
Calendar Yr RB	--	--	++	NE	NE
Sex	--	--	--	+++	++
Radiotherapy (yes/no)	+++	+++	--	+	--
Chemotherapy (yes/no)	+++	++	--	+	NE
Older Attained Age (>25 yrs)	++	++	+++	+++	+++

+++=strongly associated, ++=moderately associated, +weakly associated, -- =not associated; NE=not evaluated

9.7 What we still need to learn about retinoblastoma and second cancers

The role of chemotherapy in the risk of second cancers in retinoblastoma survivors needs to be clarified further and is becoming an important issue due to several factors:

1) **Increasing use of chemotherapy is replacing radiotherapy** (Shinohara et al., 2014). One study of RB survivors that evaluated the risk of second cancers after systemic chemotherapy only followed 187 germline patients and 58 non-germline patients for a mean of 7 years did not report an increased risk of second cancers, however that study did not have enough person time to have adequate power to evaluate this risk (Turaka et al., 2012). More recently, intra-arterial and intravitreal delivery of chemotherapy using interventional radiation therapy to direct the procedure is becoming more widely used (Abramson et al., 2015b). It is not known what the long-term effects of this type of

delivery will be in terms of second cancers or other outcomes. However, it is thought that the avoidance of systemic chemotherapy would reduce the risk of second cancers in these survivors.

2) **Increased incidence of acute myeloid leukaemia following specific systemic chemotherapies such as alkylating agents and epidophyllotoxins** (Morton et al., 2014b). Leukaemia has been reported in a case series of RB survivors treated with chemotherapy (Gombos et al., 2007) that suggested that acute myeloid leukaemia might be increased in survivors who received chemotherapy. However, acute myeloid leukaemia is rare and a large number of survivors would need to be assembled in order to detect a risk.

3) **Reports of risk of sarcomas in relation to specific systemic chemotherapies (anthracylines) in other paediatric cancer survivors** (Jenkinson et al., 2007, Henderson et al., 2012)

We have developed a small cohort of RB survivors treated between 1995 and 2006 at one institution to evaluate the risk associated with chemotherapy, but they will have to be followed for many more years and combined with other cohorts to have the statistical power to detect increased risks of second cancers. This remains an important question to be answered as more and more children are being treated with chemotherapy.

Because RB patients have excellent survival but face an increased risk of sarcomas and melanoma due to their germline mutation and past therapy, it is important to develop survivorship guidelines for these survivors.

9.8 Future efforts

Through this series of analyses, my colleagues and I have followed a unique cohort of almost 1800 RB survivors treated as early as 1914, and have shown the increased risk of second cancers progresses as the cohort ages. My colleagues and I at the NCI are continuing to follow these survivors in order to describe their lifetime risk of second cancers through surveys and death certificate notifications. We have recently conducted a new NDI search, have launched a new study follow-up using an on-line survey to update information on cancer incidence, and are also collecting saliva samples from survivors in order to sequence their *RB1* genetic mutation. We expect the field effort to be completed in Spring 2017.

We think that this next phase of study incorporating genotype-phenotype analyses will move us to the next level of understanding of the genetic predisposition of these survivors to second cancers and how treatment may influence this predisposition on the molecular level.

An important next step would be the pooling of epidemiologic and genetic data from other large cohorts that have long-term follow-up in the UK, the Netherlands and Germany would allow further exploration of the roles of genetic susceptibility and treatment to second cancer risk in hereditary survivors. By pooling the data, we would be able to address the following aims: a) quantify second cancer risks associated with chemotherapy (without radiotherapy) for patients treated in the current era, b) identify specific *RB1* mutations associated with sarcomas and melanomas, c) identifying other genetic variants that are associated with second cancer risk, and d) evaluate the

molecular profile of second tumors occurring in RB survivors compared with sporadic second tumor cases and comparing characteristics of irradiated and unirradiated tumors.

Appendix 1 – Data collection instruments, newsletters

A1.1 – medical record abstract form

A1.2 – telephone questionnaire

A1.3 – newsletters (2001, 2005)

SECOND MALIGNANT NEOPLASMS IN SURVIVORS OF RETINOBLASTOMA

ABSTRACTOR: _____

DATE ABSTRACTED: _____

SOCIAL HISTORY

1. DATE OF BIRTH: .....|__|__|-|__|__|-|__|__|__|__| (26-27)
   MO DA YR

2. SEX: 1 = Male 2 = Female 9 = Not Mentioned .....|__| (28)

3. RACE: .....|__| (29)
   1 = White (Not Hispanic) 3 = Asian/Pacific Islander 8 = Other (SPECIFY) _____
   2 = Black (Not Hispanic) 4 = Hispanic 9 = Not Mentioned

RETINOBLASTOMA (RB) DIAGNOSTIC HISTORY

4. RB DIAGNOSTIC STATUS:

   4a. DEFINITIVE RB DIAGNOSIS MADE: .....|__| (30)
   0 = No -- non-Rb diagnosis (e.g., Coat's Disease) [STOP ABSTRACTING]
   1 = Yes
   7 = Unclear/Discrepant Information [STOP ABSTRACTING]
   9 = Not Mentioned [STOP ABSTRACTING]

   4b. STUDY CENTER INVOLVEMENT: .....|__| (31)
   0 = Subject Never Seen -- Consultation Only [STOP ABSTRACTING]
   1 = Subject Examined after December 31, 1984 [STOP ABSTRACTING]
   2 = Subject Examined and Treated
   3 = Subject Examined Only -- Referred Elsewhere for Treatment

5. LATERALITY OF RB:.....|__| (32)
   1 = Right eye (OD) 3 = Bilateral (OU) 9 = Not Mentioned
   2 = Left eye (OS) 4 = Unilateral, Eye Unspecified

6h

6. DESCRIPTION OF RB DIAGNOSIS(ES):

	RIGHT EYE (OD) OR "N" UNSPECIFIED	LEFT EYE (OS)	
6a. Date of First Sign or Symptom	____ - ____ ___ (MO / YR)	____ - ____ ___ (MO / YR)	(35-46)
6b. Date of First Diagnosis	____ - ____ ___ (MO / YR)	____ - ____ ___ (MO / YR)	(47-60)
6c. Place First Diagnosed HOSPITAL/PHYSICIAN: Name:	_____ ___	_____ ___	(61-66)
Address:	_____	_____	
	_____	_____	
Hospital Record #	_____	_____	(67-82)
6d. Date First Seen at Study Centre	____ - ____ ___ (MO / YR)	____ - ____ ___ (MO / YR)	(83-96)
6e. Most Definitive Method of Diagnosis (See table below)	__	__	(97-98)
6f. Surgical Pathology Report of Diagnosis, Photocopy Enclosed? 0 = Not in Record 1 = Yes	__	__	(99-100)
6g. Number of Tumors 96 = Multiple/Mass 99 = Not Mentioned	___	___	(101-104)
Initial Diagram of Eye Tumors, Photocopy Enclosed? 0 = Not in Record 1 = Yes	__	__	(105-106)
6i. Year Last Treated for RB (of any site)	____ (YR)	____ (YR)	(107-110)

Method of Diagnosis:

1 = Enucleation
2 = Ocular Biopsy/Tapping anterior chamber
5 = Exam without obtaining tumor specimen
7 = Unclear/Discrepant
8 = Other (SPECIFY)
9 = Not Mentioned

TREATMENT FOR RB

Case ID

7. RADIATION THERAPY FOR RB: 0 = No 1 = Yes / = Unclear/Discrepant 9 = Not Mentioned ..... |__| (12)
Include both External Beam and Brachytherapy.
(If yes, record treatment details below; otherwise, skip to Item 8)

	DATE FIRST TREATMENT MO YR	DATE LAST TREATMENT MO YR	METHOD 1=External 2=Brachy (Plaque)* 9=Not Mentioned	PHOTOCOPY ENCLOSED 0=Not in Record 1=Yes	TOTAL DOSE (RADS)	SITE TREATED 91=Right Eye (OD) 92=Left Eye (OS) 93=Both Eyes (OU) 94=Eye Unspecified 96=Non-ocular site (SPECIFY)	HOSPITAL/PHYSICIAN (Name/Address)	
a.	|__|__|-|__|__|	|__|__|-|__|__|	|__|	|__|	|__|__|__|__|__|	|__|__|		(13-32)
b.	|__|__|-|__|__|	|__|__|-|__|__|	|__|		|__|__|__|__|__|				(33-52)
c.	|__|__|-|__|__|	|__|__|-|__|__|	|__|	|__|	|__|__|__|__|__|	|__|__|		(53-72)
d.	|__|__|-|__|__|	|__|__|-|__|__|	|__|	|__|	|__|__|__|__|__|	|__|__|		(73-92)
e.	|__|__|-|__|__|	|__|__|-|__|__|	|__|			|__|__|__|__|__|	|__|__|		(93-112)
f.	|__|__|-|__|__|	|__|__|-|__|__|	|__|	|__|	|__|__|__|		|__|__|		(113-132)
g.	|__|__| |__|__|	|__|__| |__|__|	|__|	|__|				Card 05 (12-31)
h.	|__|__| |__|__|	|__|__|-|__|__|	|__|	|__|	|__|__|__|__|__|		|__|__|		(32-51)

* Photocopy must be enclosed.

8. ENUCLEATION FOR RB: 0 = No 1 = Yes 7 = Unclear/Discrepant 9 = Not Mentioned
(Code status for each eye/If yes, record date of enucleation below; otherwise, skip to Item 9)

	RIGHT EYE (OD) OR EYE UNSPECIFIED			LEFT EYE (OS)		
	STATUS	MO	YR	STATUS	MO	YR
	|__|	|__|__|-|__|__|		|__|	|__|__|-|__|__|	

THERAPY, PHOTOCOAGULATION, AND OTHER ~~SURGICAL TREATMENT FOR RB:~~ .....
0 = No 1 = Yes 7 = Unclear/Discrepant 9 = Not Mentioned
(If yes, record dates of surgery below; otherwise, skip to Item 10)

9. CRYO

	SURGERY (Specify Type)	EYE TREATED 1 = Right Eye (OD) 2 = Left Eye (OS) 3 = Both Eyes (OU) 4 = Eye, Unspecified	DATE BEGAN MO	DATE BEGAN YR	DATE ENDED MO	DATE ENDED YR
a.		|__|	|__|__|	|__|__|	|__|__|	|__|__|
b		|__|	|__|__|	|__|__|	|__|__|	|__|__|
c		|__|	|__|__|	|__|__|	|__|__|	|__|__|
d		|__|	|__|__|	|__|__|	|__|__|	|__|__|
e		|__|	|__|__|	|__|__|	|__|__|	|__|__|
f		|__|	|__|__|	|__|__|	|__|__|	|__|__|
g		|__|	|__|__|	|__|__|	|__|__|	|__|__|
h		|__|	|__|__|	|__|__|	|__|__|	|__|__|

\|__\|__\|-\|__\|__\| (13-22)
\|__\|__\|-\|__\|__\| (23-32)
\|__\|__\|-\|__\|__\| (33-42)
\|__\|__\|-\|__\|__\| (52-61)
\|__\|__\|-\|__\|__\| (43-52)
\|__\|__\|-\|__\|__\| (62-71)
\|__\| (12)
\|__\|__\|-\|__\|__\| (63-72)
\|__\|__\|-\|__\|__\| (73-82)
\|__\|__\|-\|__\|__\| (83-92)

d

(49-60)

REC NO

10. CHEMOTHERAPY FOR RS: 0 = No 1 = Yes 7 = Unclear/Discrepant 9 = Not Mentioned..... |__| (12)
(If yes, record details in table; otherwise, skip to item 11)

PROTOCOL	DATE FIRST TREATMENT MO YR	DATE LAST TREATMENT MO YR	HOSPITAL/PHYSICIAN (Name/Address)	
a. _____ _____	|_|_| |_|_|	|_|_|-|_|_|	_____ _____	(13-24)
b. _____ _____	|_|_|-|_|_|	|_|_|-|_|_|	_____ _____	(25-36)
c. _____ _____	|_|_|-|_|_|	|_|_|-|_|_|	_____ _____	(37-48)
d. _____ _____	|_|_|-|_|_|	|_|_|-|_|_|	_____ _____	
e. _____ _____	|_|_|-|_|_|	|_|_|-|_|_|	_____ _____	(61-72)
f. _____ _____	|_|_|-|_|_|	|_|_|-|_|_|	_____ _____	(73-84)

NEOPLASMS

RECORD

11. MALIGNANT AND BENIGN NEOPLASMS OTHER THAN BCC: 0 = No 1 = Yes 7 = Unclear/Discrepant 9 = Not Mentioned..... |__| (17)
(If yes, record details in table below; otherwise, skip to Item 12)

NEOPLASM TABLE (Exclude BCC metastases or recurrences) DIAGNOSIS (Type/Site)	DATE OF DIAGNOSIS MO YR	PLACE DIAGNOSED (Hospital/Physician Address)	CONFIRMATION SOURCE	PHOTOCOPY OF CONFIRMATION 0 = Not in Record 1 = Yes	
a.	|__|__|-|__|__|		|__|	|__|	(1-16) (17-34)
b.	|__|__|-|__|__|		|__|	|__|	(35-38) (39-56)
c.	|__|__|-|__|__|		|__|	|__|	(57-60) (61-78)

CONFIRMATION SOURCE: 1 = Surgical Pathology Report 3 = Radiology Report 5 = Clinical Only 8 = Other (SPECIFY)
2 = Autopsy Summary Report 4 = Death Certificate Only 6 = Correspondence Only 9 = Not Mentioned

12. OTHER MEDICAL ILLNESSES/CONDITIONS: 0 = No 1 = Yes 7 = Unclear/Discrepant 9 = Not Mentioned..... |__| (79)
(If yes, record details below; otherwise, skip to Item 13)

TYPE OF ILLNESS/CONDITION	DATE OF DIAGNOSIS MO YR	
a.	|__|__|-|__|__|	(80-87)
b.	|__|__|-|__|__|	(88-95)
c.	|__|__|-|__|__|	(96-103)
d.	|__|__|-|__|__|	(104-111)
e.	|__|__|-|__|__|	(112-119)

REC 21

b

FAMILY HISTORY

13. FAMILY PEDIGREE, PHOTOCOPY ENCLOSED: 0 = Not in Record 1 = Yes..... |___| (12)

14. FAMILY HISTORY OF RB: 0 = No 1 = Yes 7 = Unclear/Discrepant 9 = Not Mentioned..... |___| (13)
(If yes, complete table for each relative diagnosed; otherwise, skip to Item 15)

|___| (51-6

	Relationship to Subject (Specify gender and maternal/paternal relationship)	Laterality 1 = Unilateral 2 = Bilateral 9 = Unspecified	Vital Status 1 = Alive 2 = Deceased 9 = Unknown	
a.		|___|	|___|	(14-18
b.		|___|	|___|	(19-23
c.		|___|	|___|	(24-28
d.		|___|	|___|	(29-33
e.		|___|	|___|	(34-38

15. FAMILY HISTORY OF CANCER OTHER THAN RB: 0 = No 1 = Yes 7 = Unclear/Discrepant 9 = Not Mentioned..... |___| (39)
(If yes, complete table for each relative diagnosed; otherwise, skip to Item 16)

	Relationship to Subject (Specify gender and maternal/paternal relationship)	Cancer Type (Specify type and site)	Age at Diagnosis	Vital Status 1 = Alive 2 = Deceased 9 = Not Mentioned	
a.					
b.					
c.					
d.				|___|	(73-

FOLLOW-UP INFORMATION

16. DATE LAST SEEN AT STUDY CENTER: ..... |__|__|-|__|__|-|__|__| (84-89)
MO DA YR

17. LAST KNOWN VITAL STATUS AND DATE: ..... |__|-|__|__|-|__|__|-|__|__| (90-96)
1 = Alive 2 = Deceased
MO DA YR

18. CAUSE OF DEATH DOCUMENTATION, PHOTOCOPY ENCLOSED: ..... |__| (97)
0 = Not in Record 1 = Yes + = Inapplicable, Subject Alive

UNDERLYING CAUSE OF DEATH: |__|__|__|__| OTHER CAUSES: |__|__|__| |__|__|__|__| |__|__|__|__| SOURCE: |__| (98-114)

COMMENTS: |__| (115)

December 1985

SECOND MALIGNANT NEOPLASMS IN SURVIVORS OF RETINOBLASTOMA STUDY

FOLLOW-UP INFORMATION

PLEASE PRINT

PATIENT STUDY NUMBER |___|___|___|___|___|___|-|__|

LAST STATUS: Living Deceased

DATE OF LAST STATUS: _____

PLACE OF DEATH: _____
City State

DEATH CERTIFICATE RETRIEVAL REQUIRED? [ ]
1 = Yes 2 = No + = Patient Alive, Inapplicable

PATIENT NAME _____
Childhood Last First Middle Married Other

BIRTHDATE ____-____-____ SOCIAL SECURITY NUMBER _____
MO DA YR

_____ RACE: _____ CITIZENSHIP: USA OTHER (Specify)_____ _____

MARITAL STATUS: Single Married Divorced/Seperated Widowed Unknown NUMBER OF MARRIAGES: _____

NAME of MOST RECENT SPOUSE _____ SPOUSE'S BIRTHDATE: _____

SPOUSE'S OCCUPATION/EMPLOYER _____ YR. _____

PATIENT'S OCCUPATION/EMPLOYER _____ YR. _____

LAST KNOWN ADDRESS _____

_____ TELEPHONE #: _____ YR. _____

PREVIOUS ADDRESS AT DIAGNOSIS _____

_____

_____ TELEPHONE #: _____ YR. _____

LAST KNOWN CONTACT (Form and date) _____

DATE FIRST OCULAR VISIT _____ DATE LAST CLINIC VISIT _____

RECONTACT STATUS: Is there any person subject or family should not be contacted? YES NO
(If yes, explain)

_____

_____

FATHER:
Name: _____ Social Security Number: ___-___-___

Date of Birth: _____ Place of Birth: _____ Vital Status: Living Dead Yr. _____

Last Known Address: _____

_____ Yr. _____

Occupation: _____ Employer (Name/Address): _____ Yr. _____

MOTHER:
Name (Including Maiden): _____ Social Security Number: ___-___-___

Date of Birth: _____ Place of Birth: _____ Vital Status: Living Dead Yr. _____

Last Known Address: _____ Yr. _____

Occupation: _____ Employer (Name/Address): _____ Yr. _____

RELATIVES/FRIENDS/ NEIGHBORS: NAME	ADDRESS	TELEPHONE #	DOB or AGE (note yr)	BIRTHPLACE	LIVING OR DECEASED	YEAR NOTED

OTHER PHYSICIANS/OTHER HOSPITALS/AGENCIES/INSTITUTIONS WITH RECORD OF PATIENT: (Specify name, address, and date rendered.)

1. _____ Yr. _____

2. _____ Yr. _____

3. _____ Yr. _____

4. _____ Yr. _____

5. _____ Yr. _____

CATI

RETINOBLASTC STUDY
TELEPHONE FOLLOW-U. SURVEY III 2000
SUBJECT QUESTIONNAIRE

DATE: _____ [illegible]

INTERVIEWER INITIALS: _____

Show INFOSCRN before AUTODIAL

INFOSCRN:
SUBJECT IDENTIFICATION SCREEN
SUBJECT NAME
DESIGNATED RESPONDENT (IF SUBJECT < 18)
ADDRESS 1
ADDRESS 2
ADDRESS 3
CITY
STATE ZIP
TELEPHONE NUMBER

CURRENT RESULT
APPOINTMENT TIME
DATE OF BIRTH
AGE
SEX

DESIGNATED RESPONDENT =
SUBJECT < 18 = PARENT/GUARDIAN

INTRO?
[Hello, may I speak with [SUBJECTYDESIGNATED RESPONDENT]?

[My name is (INTERVIEWER NAME). I'm calling on behalf of the New York Hospital-Cornell Medical Center and the National Institutes of Health. We are conducting a follow up study of patients treated for a tumor of the eye called retinoblastoma.]

1. SUBJECT SPEAKING / COMING TO PHONE
2. SUBJECT LIVES HERE – NEEDS APPOINTMENT
3. SUBJECT KNOWN, LIVES AT ANOTHER NUMBER
4. NEVER HEARD OF SUBJECT
5. TELEPHONE COMPANY RECORDING
6. SUBJECT DECEASED

final– 12/09/992:24 PM

AM. A' 'ERING MACHINE
RT. RE.. / DIALING
GT. GO TO RESULT

2/08/992:24 M

1 GO TO INTRO3
2 GO TO CALLBACK
3 GO TO INTRO2OV (COLLECT PHONE NUMBER) SEND CASE TO 15
4 GO TO FOLLOW CATI STANDARD
5 GO TO FOLLOW CATI STANDARD
6 GO TO END1
AM GO TO ANSWERING MACHINE MESSAGE (NEED TO ASK K. CHIMES)
RT GO TO AUTODIAL
GT GO TO RESULT SCREEN

INTRO3
[My name is (INTERVIEWER NAME). I'm calling on behalf of the New York Hospital-Cornell Medical Center and the National Institutes of Health. We are conducting a follow up study of patients treated for a tumor of the eye called retinoblastoma.]

Because you are one of our former patients, you are being invited to participate in this health survey. The purpose of this study is to obtain follow-up medical history on patients treated in early childhood for the eye disease, retinoblastoma. You may remember participating in an interview in [FILL IN YEAR(S) OF EARLIER INTERVIEWS].

This is an important health survey, and we are therefore making every effort to obtain current information for everyone selected. All personal information you provide will be confidential and will be used only for medical, statistical purposes. Although your cooperation is important to us, your refusal to participate or answer any questions will not affect your future medical care or any other benefits to which you may be entitled.

At some later date we may be interested in obtaining more detailed information from your doctor, or hospital where you were treated for certain medical conditions reported to us during this interview. In that event we will contact you again to ask your consent before requesting these documents.

There is no risk to you in participating in the interview and your answers to these questions will provide us with valuable information that can further our knowledge regarding those afflicted with the eye disease, retinoblastoma.

1. CONTINUE
2. NEED NEW DESIGNATED RESPONDENT
GT. GO TO RESULT

1 GO TO VERIFY1
2 GO TO CLCTDR
GT GO TO RESULT

VERIFY1

Before I begin, I'd just like to verify some information to be sure I have reached the correct person. Your/The subject's name is [DISPLAY SUBJECT'S FNAME, MNAME, LNAME], IS THAT CORRECT?

176

1. Y CORRECT EXACT MATCH
2. YEs, CORRECT – MATCH WITH QUALIFICATIONS
3. NO, INCORRECT – DOES NOT MATCH
DT. GO TO RESULT

! GO TO VERIFY2
2 COLLECT NAME WITH VERIFY10V THEN GO TO VERIFY2
3 FOLLOW CATI STANDARD
GT GO TO RESULT

VERIFY2
Yeso/SUBJECT'S current address is....

ADDRESS1 _____

ADDRESS2 _____

ADDRESS3 _____

CITY _____ STATE _____ ZIP _____

RETURN THROUGH FIELDS AND/OR ENTER NEW INFORMATION
GO TO Q1

END1
I'm sorry to learn of his/her death. I'm [INTERVIEWER NAME] and I was calling you/SUBJECT about a health study of patients treated for the eye cancer called retinoblastoma.) Would you be able to tell me exactly when and where SUBJECT died?

()
1. YES (GO TO END10V)
2. NO (GO TO THANK)

END10V

DATE OF DEATH:

PLACE OF DEATH:
CITY: _____ STATE: _____

final - 12/08/92:24 PM

____/____/_____

ACCE: K OR RF IN ALL FIELDS OF END10V
GO TO THANK1

CLCTDR

Who would know the most about SUBJECT'S medical history?

FIRST NAME:

LAST NAME:

PHONE NUMBER: (_____) _____ -

ALLOW DK OR RF IN ALL FIELDS
GO TO ADDITIONAL INFORMATION SCREEN
IF SAME PHONE NUMBER AS THE ONE BEING CALLED, UPDATE DESIGNATED RESPONDENT NAME AND GO TO INTOR2
IF DK OF RF IN ANY FIELD CODE 10 ELSE CODE 15

|_|_| - |_|_| - |_|_|_|_|
MO DA YEARS
NO.

SECTION 1

First, I would like to ask you some general questions about yourself.

1. In [FILL IN YEAR [YYYY] OF PREVIOUS INTERVIEW] we recorded that you were born on [FILL IN DATE OF BIRTH [MMDDYYYY]]. Did we record that correctly?

YES.....1 (GO TO Q2)
NO 2

1.a Can you please give me your correct date of birth?

2. Which of the following categories best describes your race? Would you say.....? [CODE ALL THAT APPLY]

White.....1
Black or African American.....2
Asian.....3
American Indian or Alaskan Native, or.....4
Native Hawaiian or Other Pacific Islander.....5
OTHER.....91 (Please specify)
SPECIFY_____

|_| |_|_|

3. Do you consider yourself Hispanic or Latino?

.....1
.....2

4. What is your current weight without shoes?

FT. IN.

RANGE: IF LESS THAN 3 FT TALL AND GREATER THAN 8 FT TALL, HAVE INTERVIEWER CONFIRM.

|_|_|_|_|
LBS.

5. What is your current weight without shoes?

RANGE: IF LESS THAN 90 LBS AND GREATER THAN 300 LBS, HAVE INTERVIEWER CONFIRM

SECTION H

HOSPITALIZATIONS

Now, I have some questions about your medical history and treatment experience.

6. Have you been hospitalized any time in the last five years for any reason [FOR FEMALE SUBJECTS ADD: other than for childbirth]?

YES.....1
NO.....2 (GO TO Q8)

7. IF HOSPITALIZED ASK THE FOLLOWING QUESTIONS:

a. Why were you hospitalized, please be specific.	b. On what date were you admitted to the hospital?	c. What was the name of the hospital?	d. Were there any other hospitalizations?
REASON: _____ _____ 1 - 60 CHAR LINES	_ _ / _ _ / _ _ _ _ MO DA YEAR EDIT: DATE SHOULD BE LESS THAN CURRENT DATE AND YEAR SHOULD NOT BE LESS THAN 1995. IF LESS THAN '95 HAVE INTERVIEWER CONFIRM DATE.	_____ HOSPITAL/FACILITY NAME 1 – 60 CHAR LINE	YES.....1 NO.....3 (GO TO Q8)
REASON: _____ _____	_ _ / _ _ / _ _ _ _ MO DA YEAR	_____ HOSPITAL/FACILITY NAME	YES.....1 NO.....2 (GO TO Q8)
REASON: _____ _____	_ _ / _ _ / _ _ _ _ MO DA YEAR	_____ HOSPITAL/FACILITY NAME	YES.....1 NO.....2 (GO TO Q8)

180

Now I would like to ask you some questions about other medical procedures you may have had in the past five years.

8. In the last five years, have you ever received a CAT (computerized axial tomography) scan or a MRI (magnetic resonance imaging)?

YES.....1
NO.....3 (GO TO Q11)

9. How many total CAT scans and or MRI's have you received in the last five years?

|_|_|_|_|
NUMBER

RANGE: IF MORE THAN 10, INTERVIEWER SHOULD CONFIRM NUMBER.

10 a. What was the reason for your CAT scan or MRI?

_____ 2 - 60 CHAR LINES
SPECIFY REASON(S)

10b. What was the date of your last CAT scan or MRI?

|_|_| - |_|_| - |_|_|_|_|
MO DA YR

EDIT: DATE SHOULD BE LESS THAN CURRENT DATE AND YEAR SHOULD NOT BE LESS THAN 1995. IF LESS THAN '95 HAVE INTERVIEWER CONFIRM DATE.

11. Have you ever had cataracts removed?

1.....YES
2.....NO (GO TO Q12)

11a. How old were you when you had the last cataract removed?

|_|_|
AGE

RANGE: AGE SHOULD NOT BE GREATER THAN SUBJECT'S CURRENT AGE.

11.b. Can you tell me in which eye or eyes you had the cataract removed?

	YES	NO
RIGHT EYE	1	2 **(GO TO Q12)**
LEFT EYE	1	2 **(GO TO Q12)**
BOTH EYES	1	2 **(GO TO Q12)**

Don't Know/Refused **(GO TO Q12)**

11.b.1. On what date did you have the cataract(s) removed from your [right/left or each] eye?

	DATE REMOVED MO YR
RIGHT EYE	__|__ - 19 __|__
LEFT EYE	__|__ - 19 __|__

RANGE: YEAR SHOULD BE LESS THAN CURRENT YEAR AND NOT EQUAL TO DATE OF BIRTH.

SECT III

SKIN EXAM

12. Do you receive routine skin examinations from a physician?

YES	1
NO	2

13. Have you ever had a skin biopsy?

YES	.1
NO	2 **(GO TO BOX A)**

14. I'd like to ask you some questions about each skin biopsy you have had.

chemotherapy?

a. Thinking about the last biopsy you received, on what part of your body was the skin biopsied? Please be specific.	b. What was the date of that biopsy?	c. What was the diagnosis of the skin biopsy you had on DISPLAY DATE OF BIOPSY FROM Q14a?	d. What is the name of the clinic or hospital where you had the skin biopsy on DISPLAY DATE OF BIOPSY FROM Q14a?	e. What is the address of the clinic or hospital where you had the skin biopsy DISPLAY DATE OF BIOPSY FROM Q14a?	f. Were there any other skin biopsies?
1 CHAR LINES	MO DA YEAR EDIT: DATE SHOULD BE LESS THAN CURRENT DATE, HAVE INTERVIEWER CONFIRM DATE.	SPECIFY REASON 1 - 60 CHAR LINES	NAME 1 - 60 CHAR LINES	ADDRESS 1 - 45 CHAR LINES 1 – 20 CHAR LINES 1 - 3 CHAR LINES 1 – 6 CHAR LINES	YES NO....1 GO TO Q15
	MO DA YEAR	SPECIFY REASON	NAME	ADDRESS 1 - 45 CHA LINES 1 – 20 CHAR LINES 1 - 3 CHAR LINES 1 – 6 CHAR LINES	YES 1 NO.... 2 GO TO Q15

SECTION IV
OTHER CANCERS

The following questions are in regard to any tumors or cancers you may have had, other than resinoblastoma and skin cancer.

BOX A
PRELOAD

NOT INTERVIEWED PREVIOUSLY/INTERVIEWED W/ NO CANCER..... .....1 (GO TO Q15.a)
INTERVIEWED PREVIOUSLY W/ CANCER..... ..... 2 (GO TO 15.b)

15.a. Have you ever been examined or treated for tumors or cancers other than Rb and skin cancer. Treatment may have included a biopsy, surgery, radiation treatment or

YES..... ..1 (GO TO Q16)
NO..... 2 (GO TO Q17)

15.b. In [FILL IN YEAR OF LAST INTERVIEW], you told us that you had been diagnosed with [FILL IN CANCER TYPE(S)]. Since then have you been diagnosed with any cancers other than Rb or skin cancer?

YES..... ..1
NO..... ..2 (GO TO Q17)

|__|__|-
|__|__|__|__|
MO
YEAR

NAME

I'd like to get some information on each cancer you have been diagnosed with since your last interview] or other than reticuloblastoma or skin cancer.

a. What type of cancer were you diagnosed with? Please specify the site. THE DETAIL PROVIDED FOR 'TYPE' AND 'SITE' WILL BE USED TO IDENTIFY THE APPROPRIATE ICD CODE. THESE SHOULD BE ASKED AS ONE QUESTION AND THE RESPONSE CAN BE ENTERED INTO 2 45 CHAR LINES.	b. On what date were you diagnosed with this cancer?	c. What is the name of the physician who originally diagnosed this cancer?	d. What is the name and address of the physician who originally diagnosed this cancer?	e. Was this a recurrence or a new cancer?	f. Did you receive any of the following types treatment for this cancer? [CODE ALL THAT APPLY]	g. What is the name of the facility where you received treatment for your [CANCER]	h. What is the address of the facility where you received treatment for your "CANCER]	i. Have you been diagnosed with any other cancers?
_____ TYPE/SITE	EDIT: DATE SHOULD BE LESS THAN CURRENT DATE, AND GREATER THAN DOB. HAVE INTERVIEWER CONFIRM DATE.	_____ NAME 1 – 60 CHAR LINE	ADDRESS 1 - 45 CHAR LINES 1 – 20 CHAR LINES 1 - 3 CHAR LINES 1 – 6 CHAR LINES	Recurrence 1 New cancer 2	No Treatment 0 CHEMOTHERAPY 1 RADIATION 2 SURGERY 3 OTHER 4 [IF 1,2,OR 4 ARE CODED ASK Q, IF NO TREATMENT (0) SKIP TO Q.]	_____ 1 – 60 CHAR LINE	ADDRESS 1 - 45 CHAR LINES 1 – 20 CHAR LINES 1 - 3 CHAR LINES 1 – 6 CHAR LINES	YES.....1 NO.....2 (GO TO Q17)
_____ TYPE/SITE	|__|__|- |__|__|__|__| MO YEAR	_____ NAME	ADDRESS 1 - 45 CHAR LINES 1 – 20 CHAR LINES 1 - 3 CHAR LINES 1 – 6 CHAR LINES	Recurrence 1 New cancer 2	No Treatment 0 CHEMOTHERAPY 1 RADIATION 2 SURGERY 3 OTHER 4 [IF 1,2,OR 4 ARE CODED ASK Q. IF NO TREATMENT (0) SKIP TO Q.]	_____ NAME	ADDRESS 1 - 45 CHAR LINES 1 – 20 CHAR LINES 1 - 3 CHAR LINES 1 – 6 CHAR LINES	YES ..... 1 NO..... 2 (GO TO Q17)

185

FAMI HISTORY

|_|_|_| - |_|_| - |_|_|_|_|

MO DA YEAR

|_|_| - |_|_| - |_|_|_|_|

MO DA YEAR

BOX B

1

2

3

|__|__|

AGE

IF INTERVIEWED PREVIOUSLY AND SUBJECT REPORTED HE/SHE HAD RELATIVES WITH RB GO TO 17a.

IF NOT INTERVIEWED PREVIOUSLY OR NO RELATIVES WITH RB REPORTED IN EARLIER INTERVIEWS THEN GO TO 17b.

17 a. Now I would like to verify information we recorded in [FILL IN YEAR] about some of your first-degree relatives, that is your parents, siblings and children who were diagnosed with RB and ask you a few more questions about these family members In [FILL IN YEAR] we recorded your [FILL IN RELATIONSHIP] had been diagnosed with RB. [ONCE VERIFIED, INTERVIEWER WILL START BY ASKING QUESTION C AND CONTINUE TO VERIFY AND ASK NEW QUESTIONS THROUGH TO Qj. THEN CONTINUE TO VERIFY INFORMATION FOR THE NEXT RELATIVE LISTED.] Now I would like to ask you about the rest of your first-degree relatives. [IF PARENTS NOT LISTED PREVIOUSLY START BY SAYING Now tell me about your Mother |. Father |. ELSE ASK Do you have any (other) siblings |. any (other) children. ASK ALL QUESTIONS FOR ALL FIRST DEGREE RELATIVES]

|_|_|_| |_|_|_| |_|_|_|_|

MO DA YEAR

|_|_|

AGE

|__|__|

AGE

17 b. Now I would like to ask you to tell us about your first-degree relatives, by first degree relatives I am referring to your biological parents, siblings and children. Let's start with your parents first,

what is your mother's date of birth? ASKING EACH FOR EACH BE SURE TO ASK IF HALF-SIBLINGS ARE MATERNAL OR PATERN

a. INTERVIEWER RECORD THE SEX OF THE RELATIVE	B What is/(Verify) [HIS/HER] relationship to you?)	c. What is [HIS/HER] date of birth? [CONTINUE QUESTION RELATIVE, |_|_| - |_|_| - |_|_|_|_| MO DA YEAR	d. Is [HE/SHE] still living? [IF KNOWN TO BE DECEASED FROM PREVIOUS INTERVIEW VERIFY VITAL STATUS AND ASK E.]	IF DECEASED ASK: e. Can you tell me when [HE/SHE] died? |_|_| - |_|_| - |_|_|_|_| MO DA YEAR	Was/(Verify) [RELATIVE] ever diagnosed with RB?	g. IF YES, In which eye was your [RELATIVE]'s RB diagnosed?	h. At what/(Verify) age was your [RELATIVE diagnosed?	i. In [YEAR] you told us your [RELATIVE] had [CANCER]. Has your [RELATIVE] ever been diagnosed with any other cancer? IF YES, Can you describe the type of condition or cancer your [RELATIONSHIP] had?	j. In [YEAR] you told us your [RELATIVE] was [AGE] when (he/she) was diagnosed with cancer. How old was [RELATIONSHIP] when [HE/SHE] was diagnosed with [NEW CANCER]?
MALE.....1 FEMALE...2	SEE ATTACHED	_ DOB MUST NOT BE EQUAL TO CURRENT DATE	LIVING.....1 DECEASED....2			YES.....1 NO.....2	Right eye..... Left eye..... Both eyes.....		_	_	 AGE	YES.....1 NO.....2 SPECIFY:_____	
MALE .....1 FEMALE...2		_	LIVING. ....1 DECEASED 2			YES.....1 NO.....2	Right eye.....1 Left eye.....2 Both eyes.....3			YES.....1 NO.....2 SPECIFY:_____	
MALE.....1 FEMALE...2		_	LIVING.....1 DECEASED....2			YES.....1 NO.....2	Right eye.....1 Left eye.....2 Both eyes.....3	|_|_| AGE	YES.....1 NO.....2 SPECIFY:_____	|_|_| AGE

186

AGE

BOX C

MO DA

INTERVIEWER: DID THE SUBJECT MENTION ANY CHILDREN AT Q1? IF SO ASK Q18, IF NO YEARTO Q19.

(SKIP TO SECTION VII - Q 29 IF SUBJECT UNDER 18)

18 Thinking about the children you have told us have been diagnosed with a cancer, please tell me about <CHILD'S NAME>'S other biological parent

PLEASE CIRCLE THE SEX OF THE OTHER PARENT	a. What is [HIS/HER] date of birth?	c. Is [HE/SHE] still living?	d. Can you tell me [HIS/HER] date of death?	e. Has [HE/SHE] ever been diagnosed with a cancer.	f. Please describe the cancer [HE/SHE] was diagnosed with.	g. What was [HIS/HER] age at the time the cancer was first diagnosed.	h. Was [HE/SHE] diagnosed with any other cancers?	i. Is this <NEXT CHILD'S NAME>'S parent also?
MALE.....1 FEMALE...2	__/__/__ MO DA YEAR DOB SHOULD NOT EQUAL CURRENT DATE. INTERVIEWER SHOULD CONFIRM	LIVING.....1 DECEASED ..2 [IF '1' GO TO Q18e.]	DOD SHOULD NOT EQUAL DOB (Q18A). INTERVIEWER SHOULD CONFIRM	YES.....1 (Q19) NO.....2	_____ _____ _____ _____ TYPE THIS SHOULD ALLOW FOR 2 – 60 CHAR LINES THEN REPEAT UP TO 4 TIMES IF R. ANSWERS '1' AT H.	THIS SHOULD REPEAT UP TO 4 TIMES IF R. ANSWERS '1' AT H.	YES...1 (Q18f) NO ..2 (Q19)	YES...1 NO...2

187

SECTION V

SMOKING

19. Have you smoked at least 100 cigarettes in your entire life?

YES
NO. 2 **(GO TO Q24)**
.....1

19.a. About how old were you when you first started smoking cigarettes fairly regularly? **[IF R. SAYS "NEVER SMOKED REGULARLY" ASK: WHEN DID YOU FIRST START SMOKING?]**

|_|_|
AGE

RANGE: AGE SHOULD BE LESS THAN CURRENT AGE AND NO LESS THAN AGE 14, IF UNDER AGE 14 HAVE INTERVIEWER VERIFY RESPONSE.

20. Do you smoke cigarettes now?

YES .1 **(GO TO Q22)**
.....NO..... .2
.....

21. How old were you when you last smoked? |_|_|
YEARS

|_|_|
AGE

RANGE: AGE SHOULD BE GREATER THAN AGE AT Q19.a

22. On average, how many cigarettes a day [do/did] you smoke?

|_|_|_|
# of Cigarettes a day

RANGE: NUMBER OF CIGARETTES SHOULD NOT BE GREATER THAN 80, ELSE HAVE INTERVIEWER VERIFY RESPONSE.

23. How many years, in total, have you smoked?

RANGE: THE NUMBER OF YEARS SMOKED SHOULD NOT BE GREATER THAN THE SUBJECT'S AGE.

Now I would like to ask you a few questions about other types of tobacco products you have ever used.

	Chewing Tobacco	Snuff Tobacco	c. Pipes	d. Cigars
24. Thinking about the following tobacco products, chewing tobacco, snuff tobacco, a pipe, or cigar, would you say you never used [FILL IN TOBACCO], yes used, but no longer use [TOBACCO], yes, occasionally use, or yes regularly use [TOBACCO]?	Never Used.....1 (NEXT) Yes, no longer use.....2 (Q25) Yes, occasionally use.....3(Q25) Yes, regularly use.....4(Q25)	Never Used.....1 (NEXT) Yes, no longer use.....2(Q25) Yes, occasionally use.....3(Q25) Yes, regularly use.....4(Q25)	Never Used.....1 (NEXT) Yes, no longer use.....2(Q25) Yes, occasionally use.....3(Q25) Yes, regularly use.....4(Q25)	Never Used.....1(Q26) Yes, no longer use.....2(Q25) Yes, occasionally use.....3(Q25) Yes, regularly use.....4(Q25)
25. How long (did you/or have you) (use/used).....	Less than 1 Year.....0 1-2 Years.....1 3-4 Years.....2 5-10 Years.....3 11or more years.....4	Less than 1 Year.....0 1-2 Years.....1 3-4 Years.....2 5-10 Years.....3 11 or more years.....4	Less than 1 Year.....0 1-2 Years.....1 3-4 Years.....2 5-10 Years.....3 11or more years.....4	Less than 1 Year.....0 1-2 Years.....1 3-4 Years.....2 5-10 Years.....3 11or more years.....4

SECTION VI

ALCOHOL

26. Are you a social drinker?

YES.....1
NO.....2 (GO TO Q29)

26.a. How old were you when you first started drinking (not counting small tastes or sips of alcohol)?

|_|_|
AGE

RANGE: AGE SHOULD BE LESS THAN OR EQUAL TO CURRENT AGE. IF AGE IS LESS THAN 9, HAVE INTERVIEWER VERIFY AGE.

27. In the past 2 years, on average, how many drinks did you have of the following: (IF NONE ENTER '000')

	Unit	Time
a. Wine?	___	Day.....1 Week.....2 Months.....3 Years.....4
b. Beer?	___	Day.....1 Week.....2 Months.....3 Years.....4
c. Liquor?	___	Day.....1 Week.....2 Months.....3 Years.....4

28. During the past two years, on average, how many drinks do you have on the days that you drink?

ONE DRINK/DAY.....1
TWO DRINKS/DAY.....2
THREE DRINKS/DAY.....3
FOUR DRINKS/DAY.....4
FIVE DRINKS/DAY.....5
SIX OR MORE DRINKS/DAY.....6

SECTION VII

SUN EXPOSURE

29. When you were **[FILL IN FIRST/NEXT AGE RESPONSE -- IF SUBJECT IS LESS THAN THE MAXIMUM AGE RANGE, SAY, "FROM THE AGE OF [MINIMUM AGE] UNTIL TODAY...]** did you usually wear protective clothing when you were in the sun, such as a hat or a long-sleeved shirt? **[IF SUBJECT'S AGE IS LESS THAN THE AGE CATEGORY, ENTER '9' AND GO TO NEXT QUESTION.]**

	YES	NO	NOT APPLICABLE
a. Under 13 years old?	1	2	9
b. 13 to 19 years old?	1	2	9
c. 20-39 years old?	1	2	9
d. Age 40 or older ?	1	2	9

RANGE: IF SUBJECT'S AGE IS LESS THAN AGE 20, THEN 29c. SHOULD = '9', IF SUBJECT IS LESS THAN AGE 40, THEN 29d. SHOULD = '9'.

The n.. iew questions are about sunscreen lotions, which became available in the 1970 s. These are lotions with ANYONE BORN IN 1970 OR LATER BEGIN ART AT 'c'.]

are designed to protect the skin from the sun.

YES

30. Now I would like to ask you some questions about your sunscreen lotion usage during different times in your life. **[FOR ASKING AT 'a', FOR ANYONE BORN BETWEEN 1957 AND 1969 BEGIN ASKING 'b', ALL OTHERS SHOULD ST**

GROUP	a. Under 13	b. 13 to 19	c. 20 to 39 years old	d. A 40 to ge today
AGE 30.1 When you were **[FILL IN AGE GROUP]**, did you ever use sunscreen lotions?	YES, years old .1 NO.. .2 **(NEXT)**	YES 1 2 **(NEXT)**	)to 39 years old ..1 NO..2 **(NEXT)**	YES .1 NO.. .2 **(NEXT)**
30.2 When you were **[FILL IN AGE GROUP]**, what SPF or sun protection factor number did you usually use? **(CAN RECORD SINGLE DIGIT OR RANGE OF SPF NUMBERS.)**	SPF NUMBER TO __|__| SPF NUMBER	SPF NUMBER TO __|__| SPF NUMBER	— — SPF NUMBER TO __|__| SPF NUMBER	SPF NUMBER TO __|__| SPF NUMBER
30.3 When you were **[FILL IN AGE GROUP]**, during the periods when you used any sunscreen lotions, did you usually use them.....	Most of the time,.....1 About half of the time,..2 Once in awhile?.....3 **(NEXT)**	Most of the time,.....1 About half of the time,..2 Once in awhile?.....3 **(NEXT)**	Most of the time,.....1 About half of the time,..2 Once in awhile?.....3 **(NEXT)**	Most of the time,.....1 About half of the time,..2 Once in awhile.....3 **(Q31)**

RANGES FOR SPF '03 TO 45'

191

Now I have some questions about how your skin would react at the age you are now if you were exposed to strong sunlight for one half hour without protective sunscreen. By strong sunlight, I mean noonday sunlight on the brightest, clearest day in the summer where you currently live.

31. Let's start with how your skin would react if you had no tan on the first time you are exposed to strong sunlight in the summer. What would happen, would you.....[IF RESPONDENT SAYS THEY HAVE NO CHANGE IN SKIN COLOR ENTER A '0']

Get a severe sunburn with blisters.....1
Get a painful sunburn but no blisters.....2
Get a mild sunburn followed by some suntan.....3
Or, become tan without any sunburn.....4

32. After your skin has been exposed to the sun for a week, how would your skin react after being in strong sunlight? What would happen, would you.....[IF RESPONDENT SAYS THEY HAVE NO CHANGE IN SKIN COLOR ENTER A '0']

Get a severe sunburn with blisters.....1
Get a painful sunburn but no blisters.....2
Get a mild sunburn followed by some suntan.....3
Or, become tan without any sunburn.....4

33. After repeated and prolonged exposure to sunlight, at the age you are now, would your skin become.....

Not tan at all.....0
Lightly tanned.....1
Moderately tanned, or.....2
Very brown and deeply tanned?.....3

192

RESIDENTIAL HISTORY

34. Now I have a few questions about places you have lived for six months or longer throughout your entire life. I am only interested in the city or town you lived in or near, not the actual address of each place. Let's begin with the first place you lived for six months or more.....now, then prior to that.....

a. In what city or town did you (last/next) live for six months or longer?	b. In what year did you start living there?	c. If you're unsure of the year can you remember your age at the time you first moved there?	d. For how many months or years did you live there? (ROUND TO THE NEAREST EVEN NUMBER)	e. Did you live anywhere else for six months or longer?
_____ CITY/STATE _____ COUNTRY **130 CHAR FIELD/12 CHAR/130 CHAR FIELD**	☐☐☐☐ (GO TO 34d) YEAR DON'T KNOW.....8888 (GO TO Q34c) **RANGE: YEAR CANNOT = 2000**	☐☐ AGE **RANGE: AGE MUST BE LESS THAN CURRENT AGE, IF AGE IS = TO CURRENT AGE, INTERVIEWER SHOULD ASK SUBJECT IF HE/SHE HAS LIVED THERE FOR MORE THAN 6 MONTHS**	☐☐ # MONTHS.....1 YEARS.....2	YES.....1 (NEXT) NO.....2 (GO TO Q35)
_____ CITY/STATE _____ COUNTRY	☐☐☐☐ (GO TO 34d) YEAR DON'T KNOW.....8888 (GO TO Q34c)	☐☐ AGE	☐☐ # MONTHS.....1 YEARS.....2	YES.....1 (NEXT) NO.....2 (GO TO Q35)
_____ CITY/STATE _____ COUNTRY	☐☐☐☐ (GO TO 34d) YEAR DON'T KNOW.....8888 (GO TO Q34c)	☐☐ AGE	☐☐ # MONTHS.....1 YEARS.....2	YES.....1 (NEXT) NO.....2 (GO TO Q35)

SECTION VIII

The next questions are about medicines or drugs you have taken in the past five years. We are interested in medicine or drugs, which you have taken consistently for more than one month, or for a total of 30 days in one year. Please include only those drugs prescribed by your doctor and filled by a pharmacist. Include all prescribed pills, syrups, injections, patches and creams. Please do not include medicine or drugs that you buy off the shelf at the drug store, sometimes called "over the counter drugs".

35. Has a doctor prescribed and have you taken _____, for more than one month or for a total of 30 days in the past five years?

	YES	NO
a. Antibiotics	1	2
b. Birth Control Pills	1	2
c. Estrogens or Progesterones (female hormones)	1	2
d. Testosterones (male hormones)	1	2
e. Thyroid Medications	1	2
f. Other Medicines to Replace Body Hormones	1	2
g. Medication for Diabetes	1	2
h. Muscle Relaxants	1	2
i. Prescribed Pain Medicines	1	2
j. Prescribed Nutritional Supplements	1	2
k. Anti-Epileptic (Anti-Seizure) Drugs	1	2
l. Drugs for High Blood Pressure or for your Heart	1	2
m. Prescribed Antacids	1	2
n. Chemotherapy/Immune Suppressants	1	2
o. Antidepressants or other Prescribed Drugs for depression or other mood disorders	1	2
p. Other Prescribed Drugs (Please Specify)	1	2
SPECIFY_____		

HRT

e.

The next series of questions relate to medical conditions that <u>have ever occurred in your lifetime</u>. Please tell me if a doctor or other health care professional has told you that you have any of the following conditions. In addition, please give me your approximate age at the time you were first told about this condition. If you had the condition more than once please tell me your age when you were <u>first</u> told by a doctor that you had the condition.

URINARY SYSTEM

26. Have you ever been told by a doctor or other health care professional that you have, or have had

a. Kidney stones? YES.....1 (GO TO Q36a.1) NO.....2 (NEXT)	b. Repeated kidney infections? YES.....1 (GO TO Q36b.1) NO.....2 (NEXT)	c. Repeated bladder infections? YES.....1 (GO TO Q36c.1) ____ NO.....2 (NEXT) AGE	d. Dialysis? YES.....1 (GO TO Q36d.1) NO.....3 (NEXT)	Any other kind of kidney or urinary tract disorder? YES.....1 (GO TO Q36e.1) NO.....7 (NEXT)
a.1 At what age were you first told you had kidney stones? ____ AGE RANGE: AGE SHOULD NOT BE GREATER THAN CURRENT AGE	b.1 At what age were you when you first began to have repeated kidney infections? ____ AGE	c.1 At what age were when you began to have repeated bladder infections?	d.1 At what age were you when you had your first dialysis? ____ AGE	e.1 At what age were you when you first had this condition?

____
AGE

195

NER' 'S SYSTEM

					AGE

37. Have you ever been told by a doctor or other health care professional that you have.....

a. Epilepsy?		b. Repeated seizures, convulsions, or blackouts?	c. Can you please describe this problem.	d. Migraine?		e. Other frequent headaches?
YES.....1 **(GO TO Q37a.1)** NO.....2 **(NEXT)**		YES.....1 **(GO TO Q37b.1)** NO.....2 **(GO TO Q37d)**	1– 60 Char spaces	YES.....1 **(GO TO Q37d.1)** NO.....2 **(NEXT)**	.....	YES .1 **(GO TO Q37e.1)** NO. .2 **(NEXT)**
a.1 At what age were you first told you had epilepsy? | || | _ _ AGE **RANGE: AGE SHOULD NOT BE GREATER THAN CURRENT AGE.**		b.1 At what age were you when you first began to have seizures, convulsions, or blackouts? | || | _ _ AGE		d.1 At what age were when you began to have migraines? | || | _ _ AGE		e.1 At what age were you when you began to have frequent headaches? _ _

196

HOR 4AU SYSTEM

38. Have you ever been told by a doctor or other health care professional that you have, or have had...

38.a. An overactive thyroid gland (hyperthyroid)? YES.....1 (GO TO Q38.a.1) NO.....2 (NEXT)	38.a.1 At what age were you first diagnosed with an overactive thyroid gland or hyperthyroid? __ __ RANGE: AGE SHOULD NOT BE GREATER THAN CURRENT AGE.
38.b. An underactive thyroid gland (hypothyroid)? YES.....1 (GO TO Q38.b.1) NO.....2 (NEXT)	38.b.1 At what age were you first diagnosed with an underactive thyroid gland or hypothyroid? __ __ AGE
38.c. Thyroid nodules? YES.....1 (GO TO Q38.c.1) NO.....2 (NEXT)	38.c.1 At what age were you first diagnosed with thyroid nodules? __ __
38.d. Other thyroid enlargement? YES.....1 (GO TO Q38.d.1) NO.....2 (NEXT)	38.d.1 At what age were you first diagnosed with other thyroid enlargement?
38.e. Deficiency of growth hormones? YES.....1 (GO TO Q38.e.1) NO.....2 (NEXT)	38.e.1 At what age were you first diagnosed with a deficiency of growth hormones? __ __ AGE
38.f. Have you ever received injections of growth hormones (Protropin or Humatrope)? YES.....1 (GO TO Q38.f.1) NO.....2 (NEXT)	38.f.1 At what age did you first receive injections of a growth hormone, like Protropin or Humatrope? __ __ AGE
38.g. Osteoporosis, brittle, weak or fragile bones? YES.....1 (GO TO Q38.g.1) NO.....2 (NEXT)	38.g.1 At what age were you first diagnosed with osteoporosis, brittle, weak or fragile bones?

__ __
AGE

__ __
AGE

__ __
AGE

__ __
AGE

38.h. ' you need medication to go into puberty? YES.....1 (GO TO Q38.h.1) NO.....2 (NEXT)*	i8.h.1 At what age did yo ; begin taking medication to start puberty? ___1___ AGE
38.i. Any other hormonal problems? YES.....1 (GO TO Q38.i.1) NO.....2 (GO TO 39)	38.i.1 At what age were you first diagnosed with your other hormonal problem? ___1___ AGE
38.j Please describe this hormonal problem. _____ _____ 2 ..DK Other specify	

198

ASK MALE SUBJECTS ONLY: (MALE SUBJECTS SKIP TO Q44)

started menstruating?

39. Did you ever menstruate?

YES.....1
NO.....2 **(GO TO Q40)**

39a. How old were you when you

|_|_|
AGE BEGAN

MENSES

RANGE: AGE SHOULD NOT BE LESS THAN 9 AND GREATER THAN 14.

. Have

39b you ever taken female hormones, including birth control pills (oral contraceptives) to have your period?

.....

YES. ..1 **(GO TO 39.b.1)**
NO..... .2 **(GO TO 39.c.1)**

How

39.b.1 old were you when you started taking the pill to have your period?

— —

| | | |
AGE BEGAN

RANGE: AGE SHOULD NOT BE GREATER THAN CURRENT AGE.

(SKIP TO SECTION XI - Q.45 IF SUBJECT UNDER 18)

39c. Are you currently having menstrual periods?

YES.....1 **(GO TO Q40)**
NO.....2

39c.1. At what age was your last menstrual period?

|_|_|_|
AGE

RANGE: AGE SHOULD NOT BE GREATER THAN CURRENT AGE.

39d. Have you ever taken Premarin, estrogen, or other hormone replacement therapy like Provera? Please include pills as well as patches.

YES.....1
NO.....2 **(GO TO Q40)**

39d. I... .e you currently taking hormone replacement therapy?

YES.....1
NO.....2

40. When was the last time you had a Pap smear, a test for cancer of the cervix, would you say it was....**[ENTER '0' IF NEVER HAD A PAP SMEAR.]**

NEVER.....0
Less than 1 year ago,.....1
1-2 years ago,.....2
3-4 years ago, or.....3
5 or more years ago?.....4

41. How often do you perform monthly breast self-examination would you say.....

Regularly,.....1
Occasionally,.....2
Rarely or never?... 3

42. When was the last time you had a breast examination by a doctor or a health care professional would you say it was..... **[ENTER '0' IF NEVER HAD A BREAST EXAM.]**

NEVER..... 0
Less than 1 year ago,.....1
1-2 years ago,.....2
3-4 years ago, or.....3
5 or more years ago?.....4

43. Have you ever had a mammogram?

YES.....1
NO.....2 **(GO TO Q45)**

43a. What age were you when you had your last mammogram would you say.....

___
AGE

RANGE: AGE SHOULD NOT BE GREATER THAN CURRENT AGE.

ASK MALE SUBJECTS ONLY FEMALE SUBJECTS GO TO Q45

44 How often do you perform monthly testicular self-examination?

Regularly,..... 1
Occasionally,.....2
Rarely or never?.....3

SECTION IX

SCHOOLING AND RELIGIOUS PREFERENCE

Now I have a few more general questions.

45. What is the highest grade or level of schooling that you have completed, was it ..?

1-8 years in grade school,.....1
9-12 years of high school, but did not graduate,.....2
Completed high school,.....3
Training after high school, other than college,.....4
Some college,.....5
College graduate, or.....6
Post graduate level?.....7

46. Which of the following represents your religious preference? Would you say you were.....(CODE ALL THAT APPLY)

None,.....0
Protestant.....1
Catholic.....2
Jewish.....3 (GO TO 46.a)
Islam/Muslim.....4
OTHER.....9

46.a Do you consider yourself to be an Ashkenazi Jew?

YES.....1
NO.....2

CLOSING

In order to maintain a complete record of your treatment history it is useful for us to know your Social Security Number. This information is helpful in locating you should we wish to contact you again in a few years or to link you with vital statistics records in the future. The disclosure of your Social Security Number is voluntary. Providing or refusing to provide this information will in no way affect any rights, benefits, or privileges that you may be receiving now or in the future.

BOX D	
SSN IS ALREADY KNOWN	1 (ASK Q47.a)
SSN IS NOT KNOWN	2 (ASK Q47.b)

47.a. **IF SSN IS ALREADY KNOWN, SAY:** In [YEAR OF INTERVIEW] we recorded your Social Security Number as, [SSN], is that correct?
YES.....1 **(ASK THE FOLLOWING)**
NO.....2 **(LEAVE SSN BLANK)**

47.b **IF NOT CORRECT OR NOT KNOWN, ASK:** Can you tell me your Social Security Number? **(FILL IN SOCIAL SECURITY NUMBER)**
YES.....1 **(FILL IN SSN)**
NO.....2) **(LEAVE SSN BLANK))**

_ _ _ - _ _ - _ _ _ _
SOCIAL SECURITY NUMBER

48. In case we wish to contact you again in the future, it would be a great help to us if you could provide us with the names and addresses of one or two people who could give us your new address should you move. We would only contact these persons if we were unable to reach you at this address. We would prefer the name of someone who does not live with you.

a. Can you give us the name of someone who we could contact? NAME: _____ [1 – 60 CHAR LINES]

b. **What is [HIS/HER] address?**

STREET ADDRESS: [1 – 60 CHAR]
CITY: [1-30 CHAR]
STATE: [1-2 CHAR] ZIP: [1-5 CHAR]
PHONE: ( ) [1-10 CHAR]

c. **How is [FILL IN NAME] related to you?** [SEE ATTACHED CODES]

d. **Under what first and last name is his/her telephone number likely to be listed?**
e. FIRST NAME: _____ LAST NAME: _____

[1-60 CHAR lines]

CONCLUSION

Thank you so much for participating in this interview. Your answers will help us in our efforts to understand more about the treatment and effects of the Retroblastoma. Please be assured that your participation will continue to make a significant contribution to this study.

Appendix 2 – Supplemental publications

A.2 Publications

A number of additional publications, a book chapter and invited presentations to academic bodies have resulted from the associated research contained in this thesis.

Table A.2 Number of citations of publications described in this dissertation.

Chapter No.	Publication	No. of citations*
3	Kleinerman RA, Tarone RE, Abramson DH, Seddon JM, Li FP, Tucker MA. Hereditary retinoblastoma and risk of lung cancer. J Natl Cancer Inst 92;2037-39, 2000	37
4	Kleinerman RA, Tucker MA, Tarone RE, Abramson DH, Seddon JM, Stovall M, Li FP, Fraumeni, JF, Jr. Risk of new cancers in long-term survivors of retinoblastoma: An extended follow-up. J Clin Oncol 2005;23:2272-9	189
5	Kleinerman RA, Tucker MA, Abramson DH, Seddon JM, Tarone RE, Fraumeni JF, Jr. Risk of soft tissue sarcomas by individual subtype in survivors of hereditary retinoblastoma. J Natl Cancer Inst, 2007;99:24-31.	72
6	Yu CL, Tucker MA, Abramson DH, Furukawa K, Seddon JM, Stovall M, Fraumeni JF Jr, Kleinerman RA. Cause-specific mortality in long-term survivors of retinoblastoma. J Natl Cancer Inst, 2009:101:581-91.	43
7	Kleinerman RA, Yu CL, Little MP, Li Y, Abramson DH, Seddon JM, Tucker MA. Variation of second cancer risk by family history among long-term survivors of retinoblastoma. J Clin Oncol, 2012;30:950-957.	21
8	Wong JR, Morton LM, Tucker MA, Abramson DH, Seddon JM, Sampson JN, Kleinerman RA. Risk of subsequent malignant neoplasms in long-term retinoblastoma survivors following chemotherapy and radiotherapy. J Clin Oncol, 2014;32:3284-3290.	7

*Web of Science accessed April 24, 2016

A.2.1 Supplemental publications

Wong FL, Boice JD Jr, Abramson DH, Tarone RE, Kleinerman RA, Stovall M, Goldman MB, Seddon JM, Tarbell N, Fraumeni JF Jr, Li FP. Cancer incidence after retinoblastoma: radiation dose and sarcoma risk. JAMA 1997; 278:1262-7.

Kleinerman RA. Second cancers after treatment for Retinoblastoma: Incidence and mortality in long-term survivors. Proceedings of the American Society for Clinical Oncology Education Book: 596-598, 1999

Kleinerman RA. Cancer risks following diagnostic and therapeutic radiation exposure in children. Paediatric Radiol 2006; 36 (Suppl 14):121-5.

Cebulla CM, Kleinerman RA, Alegret A, Kulak A, Dubovy SR, Hess DJ, Murray TG. Rapid appearance of rhabdomyosarcoma after radiation and chemotherapy for retinoblastoma: A clinicopathologic correlation. Retinal Cases and Brief Reports, 2009;3:343-346.

Schefler AC, Kleinerman RA, Abramson DH. Genes and environment: Effects on the development of second malignancies in retinoblastoma survivors. Expert Rev Ophthalmology, 2008;3:51-61.

Francis JH, Kleinerman RA, Seddon JM, Abramson DH. Increased risk of secondary uterine leiomyosarcoma in hereditary retinoblastoma. Gynecol Oncol, 2012;124:254-9.

Liu J, Givi B, Wolden S, Kleinerman RA, Dunkel IJ, Lee N, Shah JP, Abramson DH, Kraus DH. Secondary skull base malignancies in survivors of retinoblastoma: The Memorial Sloan Kettering Cancer Center experience. Skull Base Journal, 2011;21:103-107.

Little MP, Kleinerman RA, Stiller C, Li G, Kroll M, Murphy MFG. Analysis of retinoblastoma age incidence data using a fully stochastic cancer model. Int J Cancer, 2012; 130:631-640.

Bhagia P, Colanta A, Abramson DH, Carlson D, Kleinerman RA, Kraus D, Dunkel I. Sinonasal adenocarcinoma: A rare second malignancy in long term retinoblastoma survivors. Pediatr Cancer Blood, 2011; 57:693-695.

Mills MB, Balise RR, Hudgins L, Kleinerman RA. Mutation risk associated with paternal and maternal age in a cohort of retinoblastoma survivors. Hum Mutat, 2012; 131:1115-1122.

Kleinerman RA, Schonfeld SJ, Tucker MA. Sarcomas in hereditary retinoblastoma. Clinical Sarcoma Review, 2:15;1-7, 2012.

Wong JR, Tucker MA, Kleinerman RA, Devesa SS. Retinoblastoma incidence patterns in the U.S. Surveillance, Epidemiology, and End Results program. JAMA Ophthalmology, 132:478-83, 2014.

Little MP, Schaeffer ML, Reulen RC, Abramson DH, Stovall M, Abramson DH, Weathers R, de Vathaire F, Diallo I, Seddon JM, Hawkins MM, Tucker MA, Kleinerman RA. Breast cancer risk after radiotherapy for hereditary and non-hereditary retinoblastoma: a US-UK study. Brit J Cancer, 110:2623-32, 2014.

Ford JS, Chou JF, Sklar CA, Oeffinger KC, Friedman DN, McCabe M, Robison LL, Kleinerman RA, Li Y, Marr BP, Abramson DH, Dunkel IJ. Psychosocial outcomes in adult survivors of retinoblastoma, J Clin Oncol, 2015, in press.

Friedman DN, Chou JF, Oeffinger KC, Ford JS, Sklar CA, McCabe M, Robison LL, Kleinerman RA, Marr BP, Abramson DH, Dunkel IJ. Chronic medical conditions and general health of adult survivors of retinoblastoma. In press, Cancer, 2015.

A.2.2 Book Chapter

Kleinerman RA, Morton LM, Wong JR, Tucker MA. Second tumours in retinoblastoma survivors. In Abramson DH, Francis JH eds. Recent advances in retinoblastoma treatment. Essentials in Ophthalmology, 105-112, Springer, New York, 2015.

A.2.3 Abstracts published

Kleinerman RA. Cancer after radiotherapy for hereditary retinoblastoma: Genetic susceptibility and radiation exposure. Proceedings of the Am. Stat. Assoc. Conference on Radiation and Health. Radiat Res 1999;151:92-117.

Kleinerman RA, Stovall M, Tarone RE, Tucker MA. Gene-environment interactions in a cohort of irradiated Retinoblastoma patients. Proceedings of the Am. Stat. Assoc. Conference on Radiation and Health. Radiat Res, 2005;163:701-2.

A.2.4 Reply letters

Kleinerman RA, Yu CL, Little MP, Li Y, Abramson DH, Seddon JH, Tucker MA. Reply to AC Moll, et al. J Clin Oncol, 24:3028-29, 2012.

A.2.5 Invited Presentations

Lung cancer risk in hereditary Retinoblastoma patients. International Congress of Ocular Oncology, Amsterdam, Netherlands, June, 2001.

Second cancers in retinoblastoma patients: mortality. An internal collaborative retinoblastoma - research mini-symposium. Ophthalmic Oncology Center, New York Presbyterian Hospital, New York, NY, March, 2002.

Second cancer risk following radiotherapy: Past, present and future. Radiation Research Society, St. Louis, MO, April, 2004.

Gene environment interaction in a cohort of irradiated retinoblastoma patients. American Statistical Association Conference on Radiation and Health. Beaver Creek, CO, June, 2004.

Genetic susceptibility to second cancers in a cohort of irradiated retinoblastoma patients. Workshop on cancer survivorship: Genetic susceptibility and second primary cancers, Rockville, MD, November, 2004.

Risk of new cancers following radiotherapy in long-term survivors of retinoblastoma. International Society of Genetic Eye Diseases and International Retinoblastoma Symposium, Vancouver, Canada, September, 2005.

Second cancers in retinoblastoma: What is the risk with modern radiotherapy and no chemotherapy? International Society of Ocular Oncology, Siena, Italy, June, 2007.

Radiation-sensitive genetically susceptible paediatric sub-populations. Society for Paediatric Radiology, ALARA Concept in Paediatric Imaging: Oncology, Scottsdale, AZ, May, 2008.

Risk of second cancers following treatment for retinoblastoma since 1970, International Society of Ocular Oncology, Cambridge, England, September, 2009

Risk of uterine leiomyosarcoma after retinoblastoma – What is the connection?, Department of Gynecologic Surgery, Memorial Sloan-Kettering Cancer Center, New York, October, 2009.

Risk of second cancers following treatment for retinoblastoma since 1970 (Poster). NCI Translational Science Meeting, Vienna VA, November, 2009.

Variation of second cancer risk by family history of retinoblastoma among long-term survivors. International Society of Ocular Oncology, Buenos Aires, Argentina, November, 2011

Sarcomas after retinoblastoma: Role of genetic susceptibility and radiation dose, ASA Conference on Radiation and Health, Kennebunkport, ME, June, 2012.

Second tumours in retinoblastoma survivors. Symposium "Celebrating 100 years of Our Retinoblastoma Center in New York" Memorial Sloan Kettering Cancer Center, New York, September 18-19, 2014.

Melanoma after retinoblastoma: Clinical, epidemiologic and pathologic characteristics. International Society of Ocular Oncology, Paris, France, June, 2015.

Appendix 3. Verification Letters

As this thesis is based around prior publications, the institutional rules of City University require co-authors to confirm the involvement of the student in all activities associated with the research. This is required to demonstrate originality of the publications and to confirm the contribution of the student to the academic process.

Dr Joseph F Fraumeni Jr: Founding Director and Senior Investigator Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health

Dr Margaret Tucker, Director, Human Genetics Program, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health

Dr Johanna Seddon, Director, Ophthalmic Epidemiology and Genetics Service and Professor of Ophthalmology, Tufts New England Medical Center

Dr David Abramson, Director Ocular Oncology Service, Memorial Sloan Kettering Cancer Center, New York

NATIONAL CANCER INSTITUTE
Division of Cancer
Epidemiology & Genetics

March 8, 2016

I am pleased to confirm that Ms. Ruth Kleinerman was the lead researcher for the publications listed below. I can confirm that she was involved in all areas of the research project and her contributions included but were not limited to the following:

- Conception of the idea for the individual papers
- Literature
- Liaison with statisticians and programmers
- Primary author of the paper (either first or senior)
- Lead on the study management
- Directed the data collection

1. **Kleinerman RA**, Tucker MA, Tarone RE, Abramson DH, Seddon JM, Stovall M, Li FP, Fraumeni, JF, Jr. Risk of new cancers in long-term survivors of retinoblastoma: An extended follow-up. J Clin Oncol 2005;23:2272-9.

2. **Kleinerman RA**, Tucker MA, Abramson DH, Seddon JM, Tarone RE, Fraumeni JF, Jr. Risk of soft tissue sarcomas by individual subtype in survivors of hereditary retinoblastoma. J Natl Cancer Inst, 2007;99:24-31.

3. Yu CL, Tucker MA, Abramson DH, Seddon JM, Stovall M, Fraumeni JF Jr, **Kleinerman RA**. Cause-specific mortality in long-term survivors of retinoblastoma. J Natl Cancer Inst, 2009:101:581-91.

Sincerely,

[redacted]

Joseph F. Fraumeni, Jr, M.D.

[redacted]

NATIONAL CANCER INSTITUTE
Division of Cancer
Epidemiology & Genetics

March 10, 2016

Ruth A. Kleinerman, M.P.H.
[redacted]

Dear Ruth,

I am delighted to confirm that you were the lead researcher for the publications listed below. I confirm that you were involved in all areas of the research project and your contributions included the following:

- Conception of the idea for the individual papers
- Literature review
- Liaison with statisticians and programmers
- Primary author of the paper (either first or senior)
- Lead on the study management and oversight
- Direction of the data collection and cleaning

1. **Kleinerman RA**, Tarone RE, Abramson DH, Seddon J, Li FP, Tucker MA. Hereditary retinoblastoma patients and risk of lung cancer. J Natl Cancer Inst 2000;92:2037-39.

2. **Kleinerman RA**, Tucker MA, Tarone RE, Abramson DH, Seddon JM, Stovall M, Li FP, Fraumeni, JF, Jr. Risk of new cancers in long-term survivors of retinoblastoma: An extended follow-up. J Clin Oncol 2005;23:2272-9.

3. **Kleinerman RA**, Tucker MA, Abramson DH, Seddon JM, Tarone RE, Fraumeni JF, Jr. Risk of soft tissue sarcomas by individual subtype in survivors of hereditary retinoblastoma. J Natl Cancer Inst, 2007;99:24-31.

4. **Kleinerman RA**, Yu CL, Little MP, Li Y, Abramson DH, Seddon JH, Tucker MA. Variation of second cancer risk by family history among long-term survivors of retinoblastoma. J Clin Oncol, 2012;30:950-957.

5. Yu CL, Tucker MA, Abramson DH, Seddon JM, Stovall M, Fraumeni JF Jr, **Kleinerman RA**. Cause-specific mortality in long-term survivors of retinoblastoma. J Natl Cancer Inst, 2009:101:581-91.

6. Wong JR, Morton LM, Tucker MA, Abramson DH, Seddon JM, Sampson JN, **Kleinerman RA**. Risk of subsequent malignant neoplasms in long-term

retinoblastoma survivors following chemotherapy and radiotherapy. J Clin Oncol, 32:3284-3290, 2014.

Your commitment to and direction of this longterm cohort project has been essential for the success of the study of second cancers following treatment for retinoblastoma. This long collaboration would not have been possible without your constant tending of the relationships between the clinicians caring for the cohort members and the staff at NCI. You have also been a source of information for the cohort participants about their medical issues. Your efforts have led to recognition of risks related to therapy for retinoblastoma and have helped change clinical practice, both in primary treatment of bilateral retinoblastoma and in longterm screening and management of their quite complicated medical issues. Your contributions have been transformative for these survivors.

I am happy to answer any questions that may arise.

Best wishes,

[redacted]

Margaret A. Tucker, M.D.
[redacted]
Email: [redacted]

The principal teaching hospital for Tufts University School of Medicine

Tufts Medical Center

800 Washington Street, #450
Boston, Massachusetts 02111
[redacted]
tuftsmedicalcenter.org

March 8, 2016

Johanna M. Seddon, MD, ScM
Director
Ophthalmic Epidemiology & Genetics Service
Etiologic Studies of Age-Related Macular Degeneration
Professor of Ophthalmology
Tufts University School of Medicine
[redacted]

To Whom It May Concern,

I am pleased to confirm that Ms. Ruth Kleinerman was the lead researcher for the publications listed below. I can confirm that she was involved in all areas of the research project and her contributions included but were not limited to the following:

- Conception of the idea for the individual papers
- Literature review
- Liaison with statisticians and programmers
- Primary author of the paper (either first or senior)
- Lead on the study management
- Directed the data collection

1. **Kleinerman RA**, Tarone RE, Abramson DH, Seddon J, Li FP, Tucker MA. Hereditary retinoblastoma patients and risk of lung cancer. J Natl Cancer Inst 2000;92:2037-39.

2. **Kleinerman RA**, Tucker MA, Tarone RE, Abramson DH, Seddon JM, Stovall M, Li FP, Fraumeni, JF, Jr. Risk of new cancers in long-term survivors of retinoblastoma: An extended follow-up. J Clin Oncol 2005;23:2272-9.

3. **Kleinerman RA**, Tucker MA, Abramson DH, Seddon JM, Tarone RE, Fraumeni JF, Jr. Risk of soft tissue sarcomas by individual subtype in survivors of hereditary retinoblastoma. J Natl Cancer Inst, 2007;99:24-31.

4. **Kleinerman RA**, Yu CL, Little MP, Li Y, Abramson DH, Seddon JH, Tucker MA. Variation of second cancer risk by family history among long-term survivors of retinoblastoma. J Clin Oncol, 2012;30:950-957.

5. Yu CL, Tucker MA, Abramson DH, Seddon JM, Stovall M, Fraumeni JF Jr, **Kleinerman RA**. Cause-specific mortality in long-term survivors of retinoblastoma. J Natl Cancer Inst, 2009:101:581-91.

6. Wong JR, Morton LM, Tucker MA, Abramson DH, Seddon JM, Sampson JN, **Kleinerman RA**. Risk of subsequent malignant neoplasms in long-term retinoblastoma survivors following chemotherapy and radiotherapy. J Clin Oncol, 32:3284-3290, 2014.

Sincerely,

[redacted]

Johanna M. Seddon, MD

Memorial Sloan-Kettering Cancer Center

David H. Abramson, MD, FACS
Chief, Ophthalmic Oncology Service

March 8, 2016

To Whom It May Concern:

I am pleased to confirm that Ms. Ruth Kleinerman was the lead researcher for the publications listed below. I can confirm that she was involved in all areas of the research project and her contributions included but were not limited to the following:

- Conception of the idea for the individual papers
- Literature review
- Liaison with statisticians and programmers
- Primary author of the paper (either first or senior)
- Lead on the study management
- Directed the data collection

1. **Kleinerman RA**, Tarone RE, Abramson DH, Seddon J, Li FP, Tucker MA. Hereditary retinoblastoma patients and risk of lung cancer. J Natl Cancer Inst 2000;92:2037-39.

2. **Kleinerman RA**, Tucker MA, Tarone RE, Abramson DH, Seddon JM, Stovall M, Li FP, Fraumeni, JF, Jr. Risk of new cancers in long-term survivors of retinoblastoma: An extended follow-up. J Clin Oncol 2005;23:2272-9.

3. **Kleinerman RA**, Tucker MA, Abramson DH, Seddon JM, Tarone RE, Fraumeni JF, Jr. Risk of soft tissue sarcomas by individual subtype in survivors of hereditary retinoblastoma. J Natl Cancer Inst, 2007;99:24-31.

4. **Kleinerman RA**, Yu CL, Little MP, Li Y, Abramson DH, Seddon JH, Tucker MA. Variation of second cancer risk by family history among long-term survivors of retinoblastoma. J Clin Oncol, 2012;30:950-957.

5. Yu CL, Tucker MA, Abramson DH, Seddon JM, Stovall M, Fraumeni JF Jr, **Kleinerman RA**. Cause-specific mortality in long-term survivors of retinoblastoma. J Natl Cancer Inst, 2009:101:581-91.

[redacted] | [redacted]
Phone: [redacted] | Fax: [redacted]
[redacted] www.mskcc.org
NCI-designated Comprehensive Cancer Center

Memorial Sloan-Kettering Cancer Center

David H. Abramson, MD, FACS
Chief, Ophthalmic Oncology Service

6. Wong JR, Morton LM, Tucker MA, Abramson DH, Seddon JM, Sampson JN, **Kleinerman RA**. Risk of subsequent malignant neoplasms in long-term retinoblastoma survivors following chemotherapy and radiotherapy. J Clin Oncol, 32:3284-3290, 2014.

Respectfully submitted,

[redacted]

David H. Abramson, M.D.
Chief, Ophthalmic Oncology Service, MSKCC
Professor of Ophthalmology
Weill-Cornell Medical School

[redacted] | [redacted]
Phone: [redacted] | Fax: [redacted]
[redacted] | www.mskcc.org
NCI-designated Comprehensive Cancer Center

Appendix 4. Research Funding

Ruth Kleinerman's research funding was supported in full by the Intramural Research Program, National Institutes of Health, National Cancer Institute, Bethesda, Maryland, USA

References

Abramson, DH, Beaverson, K, Sangani, P, Vora, RA, Lee, TC, Hochberg, HM, Kirszrot, J & Ranjithan, M 2003. Screening for retinoblastoma: presenting signs as prognosticators of patient and ocular survival. *Pediatrics,* 112**,** 1248-55.

Abramson, DH, Ellsworth, RM, Kitchin, FD & Tung, G 1984. Second nonocular tumors in retinoblastoma survivors. Are they radiation-induced? *Ophthalmology,* 91**,** 1351-5.

Abramson, DH, Fabius, AW, Issa, R, Francis, JH, Marr, BP, Dunkel, IJ & Gobin, YP 2015a. Advanced Unilateral Retinoblastoma: The Impact of Ophthalmic Artery Chemosurgery on Enucleation Rate and Patient Survival at MSKCC. *PLoS One,* 10**,** e0145436.

Abramson, DH & Frank, CM 1998. Second nonocular tumors in survivors of bilateral retinoblastoma: a possible age effect on radiation-related risk. *Ophthalmology,* 105**,** 573-9; discussion 579-80.

Abramson, DH, Shields, CL, Munier, FL & Chantada, GL 2015b. Treatment of Retinoblastoma in 2015: Agreement and Disagreement. *JAMA Ophthalmol,* 133**,** 1341-7.

Acquaviva, A, Ciccolallo, L, Rondelli, R, Balistreri, A, Ancarola, R, Cozza, R, Hadjistilianou, D, Francesco, SD, Toti, P, Pastore, G, Haupt, R, Carli, M, Santoro, N, Di Cataldo, A, Fiorillo, A, Indolfi, P, Nucci, P, Sandri, A, Porta, F, Porcaro, AB, Tamaro, P & Morgese, G 2006. Mortality from second tumour among long-term survivors of retinoblastoma: a retrospective analysis of the Italian retinoblastoma registry. *Oncogene,* 25**,** 5350-7.

Aleman, BM, Moser, EC, Nuver, J, Suter, TM, Maraldo, MV, Specht, L, Vrieling, C & Darby, SC 2014. Cardiovascular disease after cancer therapy. *EJC Suppl,* 12**,** 18-28.

Amitrano, S, Marozza, A, Somma, S, Imperatore, V, Hadjistilianou, T, De Francesco, S, Toti, P, Galimberti, D, Meloni, I, Cetta, F, Piu, P, Di Marco, C, Dosa, L, Lo Rizzo, C, Carignani, G, Mencarelli, MA, Mari, F, Renieri, A & Ariani, F 2015. Next generation sequencing in sporadic retinoblastoma patients reveals somatic mosaicism. *Eur J Hum Genet,* 23**,** 1523-30.

Bosco, EE & Knudsen, ES 2007. RB in breast cancer: at the crossroads of tumorigenesis and treatment. *Cell Cycle,* 6**,** 667-71.

Bradford, PT, Anderson, WF, Purdue, MP, Goldstein, AM & Tucker, MA 2010. Rising melanoma incidence rates of the trunk among younger women in the United States. *Cancer Epidemiol Biomarkers Prev,* 19**,** 2401-6.

Breslow, NE & Day, NE 1987. Statistical methods in cancer research. Volume II--The design and analysis of cohort studies. *IARC Sci Publ***,** 1-406.

Burkhart, DL & Sage, J 2008. Cellular mechanisms of tumour suppression by the retinoblastoma gene. *Nature reviews. Cancer,* 8**,** 671-82.

Cowper, DC, Kubal, JD, Maynard, C & Hynes, DM 2002. A primer and comparative review of major US mortality databases. *Ann Epidemiol,* 12**,** 462-8.

Damasco, VC & Dire, DJ 2011. A child with leukocoria. *Pediatr Emerg Care,* 27**,** 1170-4.

Derkinderen, DJ, Koten, JW, Wolterbeek, R, Beemer, FA, Tan, KE & Den Otter, W 1987. Non-ocular cancer in hereditary retinoblastoma survivors and relatives. *Ophthalmic Paediatr Genet,* 8**,** 23-5.

Dommering, CJ, Mol, BM, Moll, AC, Burton, M, Cloos, J, Dorsman, JC, Meijers-Heijboer, H & van der Hout, AH 2014. RB1 mutation spectrum in a comprehensive nationwide cohort of retinoblastoma patients. *J Med Genet,* 51**,** 366-74.

Draper, GJ, Sanders, BM & Kingston, JE 1986. Second primary neoplasms in patients with retinoblastoma. *Br J Cancer,* 53**,** 661-71.

Dryja, TP, Mukai, S, Petersen, R, Rapaport, JM, Walton, D & Yandell, DW 1989. Parental origin of mutations of the retinoblastoma gene. *Nature,* 339**,** 556-8.

Eng, C, Li, FP, Abramson, DH, Ellsworth, RM, Wong, FL, Goldman, MB, Seddon, J, Tarbell, N & Boice, JD, Jr. 1993. Mortality from second tumors among long-term survivors of retinoblastoma. *J Natl Cancer Inst,* 85**,** 1121-8.

Fletcher, O, Easton, D, Anderson, K, Gilham, C, Jay, M & Peto, J 2004. Lifetime risks of common cancers among retinoblastoma survivors. *J Natl Cancer Inst,* 96**,** 357-63.

Foster, MC, Kleinerman, RA, Abramson, DH, Seddon, JM, Tarone, RE & Tucker, MA 2006. Tobacco use in adult long-term survivors of retinoblastoma. *Cancer Epidemiol Biomarkers Prev,* 15**,** 1464-8.

Francis, JH, Kleinerman, RA, Seddon, JM & Abramson, DH 2012. Increased risk of secondary uterine leiomyosarcoma in hereditary retinoblastoma. *Gynecologic oncology,* 124**,** 254-9.

Friedman, DL, Whitton, J, Leisenring, W, Mertens, AC, Hammond, S, Stovall, M, Donaldson, SS, Meadows, AT, Robison, LL & Neglia, JP 2010. Subsequent neoplasms in 5-year survivors of childhood cancer: the Childhood Cancer Survivor Study. *J Natl Cancer Inst,* 102**,** 1083-95.

Friend, SH, Horowitz, JM, Gerber, MR, Wang, XF, Bogenmann, E, Li, FP & Weinberg, RA 1987. Deletions of a DNA sequence in retinoblastomas and mesenchymal tumors: organization of the sequence and its encoded protein. *Proceedings of the National Academy of Sciences of the United States of America,* 84**,** 9059-63.

Gobin, YP, Dunkel, IJ, Marr, BP, Brodie, SE & Abramson, DH 2011. Intra-arterial chemotherapy for the management of retinoblastoma: four-year experience. *Arch Ophthalmol,* 129**,** 732-7.

Gombos, DS, Hungerford, J, Abramson, DH, Kingston, J, Chantada, G, Dunkel, IJ, Antoneli, CB, Greenwald, M, Haik, BG, Leal, CA, Medina-Sanson, A, Schefler, AC, Veerakul, G, Wieland, R, Bornfeld, N, Wilson, MW & Yu, CB 2007. Secondary acute myelogenous leukemia in patients with retinoblastoma: is chemotherapy a factor? *Ophthalmology,* 114**,** 1378-83.

Gooley, TA, Leisenring, W, Crowley, J & Storer, BE 1999. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Statistics in medicine,* 18**,** 695-706.

Gray, RJ 1988. A Class of K-Sample Tests for Comparing the Cumulative Incidence of a Competing Risk. *Annals of Statistics,* 16**,** 1141-1154.

Harbour, JW 2001. Molecular basis of low-penetrance retinoblastoma. *Arch Ophthalmol,* 119**,** 1699-704.
Harbour, JW, Lai, SL, Whang-Peng, J, Gazdar, AF, Minna, JD & Kaye, FJ 1988. Abnormalities in structure and expression of the human retinoblastoma gene in SCLC. *Science,* 241**,** 353-7.
Hawkins, MM, Draper, GJ & Kingston, JE 1987. Incidence of second primary tumours among childhood cancer survivors. *Br J Cancer,* 56**,** 339-47.
Hawkins, MM, Wilson, LM, Burton, HS, Potok, MH, Winter, DL, Marsden, HB & Stovall, MA 1996. Radiotherapy, alkylating agents, and risk of bone cancer after childhood cancer. *J Natl Cancer Inst,* 88**,** 270-8.
Henderson, TO, Moskowitz, CS, Chou, JF, Bradbury, AR, Neglia, JP, Dang, CT, Onel, K, Novetsky Friedman, D, Bhatia, S, Strong, LC, Stovall, M, Kenney, LB, Barnea, D, Lorenzi, E, Hammond, S, Leisenring, WM, Robison, LL, Armstrong, GT, Diller, LR & Oeffinger, KC 2015. Breast Cancer Risk in Childhood Cancer Survivors Without a History of Chest Radiotherapy: A Report From the Childhood Cancer Survivor Study. *J Clin Oncol*.
Henderson, TO, Rajaraman, P, Stovall, M, Constine, LS, Olive, A, Smith, SA, Mertens, A, Meadows, A, Neglia, JP, Hammond, S, Whitton, J, Inskip, PD, Robison, LL & Diller, L 2012. Risk factors associated with secondary sarcomas in childhood cancer survivors: a report from the childhood cancer survivor study. *Int J Radiat Oncol Biol Phys,* 84**,** 224-30.
Hisada, M, Garber, JE, Fung, CY, Fraumeni, JF, Jr. & Li, FP 1998. Multiple primary cancers in families with Li-Fraumeni syndrome. *J Natl Cancer Inst,* 90**,** 606-11.
Houdayer, C, Gauthier-Villars, M, Lauge, A, Pages-Berhouet, S, Dehainault, C, Caux-Moncoutier, V, Karczynski, P, Tosi, M, Doz, F, Desjardins, L, Couturier, J & Stoppa-Lyonnet, D 2004. Comprehensive screening for constitutional RB1 mutations by DHPLC and QMPSF. *Hum Mutat,* 23**,** 193-202.
Howlader, NN, A. M.; Krapcho, M. . 2016. *SEER Cancer Statistics Review, 1975-2013* [Online]. [Accessed April 2016].
Jenkinson, HC, Winter, DL, Marsden, HB, Stovall, MA, Stevens, MC, Stiller, CA & Hawkins, MM 2007. A study of soft tissue sarcomas after childhood cancer in Britain. *British journal of cancer,* 97**,** 695-9.
Kansara, M, Teng, MW, Smyth, MJ & Thomas, DM 2014. Translational biology of osteosarcoma. *Nature reviews. Cancer,* 14**,** 722-35.
Kaye, FJ & Harbour, JW 2004. For whom the bell tolls: susceptibility to common adult cancers in retinoblastoma survivors. *J Natl Cancer Inst,* 96**,** 342-3.
Kleinerman, RA 2009. Radiation-sensitive genetically susceptible pediatric sub-populations. *Pediatr Radiol,* 39 Suppl 1**,** S27-31.
Kleinerman, RA, Tarone, RE, Abramson, DH, Seddon, JM, Li, FP & Tucker, MA 2000. Hereditary retinoblastoma and risk of lung cancer. *J Natl Cancer Inst,* 92**,** 2037-9.
Kleinerman, RA, Tucker, MA, Abramson, DH, Seddon, JM, Tarone, RE & Fraumeni, JF, Jr. 2007. Risk of soft tissue sarcomas by individual subtype in survivors of hereditary retinoblastoma. *J Natl Cancer Inst,* 99**,** 24-31.

Kleinerman, RA, Tucker, MA, Tarone, RE, Abramson, DH, Seddon, JM, Stovall, M, Li, FP & Fraumeni, JF, Jr. 2005. Risk of new cancers after radiotherapy in long-term survivors of retinoblastoma: an extended follow-up. *J Clin Oncol,* 23**,** 2272-9.

Kleinerman, RA, Yu, CL, Little, MP, Li, Y, Abramson, D, Seddon, J & Tucker, MA 2012. Variation of second cancer risk by family history of retinoblastoma among long-term survivors. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* 30**,** 950-7.

Knudson, AG, Jr. 1971. Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci U S A,* 68**,** 820-3.

MacCarthy, A, Bayne, AM, Brownbill, PA, Bunch, KJ, Diggens, NL, Draper, GJ, Hawkins, MM, Jenkinson, HC, Kingston, JE, Stiller, CA, Vincent, TJ & Murphy, MF 2013. Second and subsequent tumours among 1927 retinoblastoma patients diagnosed in Britain 1951-2004. *British journal of cancer,* 108**,** 2455-63.

Marees, T, Moll, AC, Imhof, SM, de Boer, MR, Ringens, PJ & van Leeuwen, FE 2008a. Risk of second malignancies in survivors of retinoblastoma: more than 40 years of follow-up. *J Natl Cancer Inst,* 100**,** 1771-9.

Marees, T, Moll, AC, Imhof, SM, de Boer, MR, Ringens, PJ & van Leeuwen, FE 2008b. Risk of second malignancies in survivors of retinoblastoma: more than 40 years of follow-up. *Journal of the National Cancer Institute,* 100**,** 1771-9.

Marees, T, van Leeuwen, FE, Schaapveld, M, Imhof, SM, de Boer, MR, Kors, WA, Ringens, PJ & Moll, AC 2010. Risk of third malignancies and death after a second malignancy in retinoblastoma survivors. *Eur J Cancer,* 46**,** 2052-8.

Maris, JM & Knudson, AG 2015. Revisiting tissue specificity of germline cancer predisposing mutations. *Nat Rev Cancer,* 15**,** 65-6.

Masciari, S, Van den Abbeele, AD, Diller, LR, Rastarhuyeva, I, Yap, J, Schneider, K, Digianni, L, Li, FP, Fraumeni, JF, Jr., Syngal, S & Garber, JE 2008. F18-fluorodeoxyglucose-positron emission tomography/computed tomography screening in Li-Fraumeni syndrome. *JAMA,* 299**,** 1315-9.

Meadows, AT 2007. Retinoblastoma survivors: sarcomas and surveillance. *J Natl Cancer Inst,* 99**,** 3-5.

Moll, AC, Dommering, CJ, Bosscha, MI, de Graaf, P, Kors, WA & van Leeuwen, FE 2012. Risk factors for the incidence of second cancers in survivors of retinoblastoma with a family history. *J Clin Oncol,* 30**,** 3028; author reply 3028-9.

Moll, AC, Imhof, SM, Bouter, LM, Kuik, DJ, Den Otter, W, Bezemer, PD, Koten, JW & Tan, KE 1996. Second primary tumors in patients with hereditary retinoblastoma: a register-based follow-up study, 1945-1994. *Int J Cancer,* 67**,** 515-9.

Moll, AC, Imhof, SM, Schouten-Van Meeteren, AY, Kuik, DJ, Hofman, P & Boers, M 2001. Second primary tumors in hereditary retinoblastoma: a register-based study, 1945-1997: is there an age effect on radiation-related risk? *Ophthalmology,* 108**,** 1109-14.

Monson, RR 1974. Analysis of relative survival and proportional mortality. *Comput Biomed Res,* 7**,** 325-32.

Morton, LM, Onel, K, Curtis, RE, Hungate, EA & Armstrong, GT 2014a. The rising incidence of second cancers: patterns of occurrence and identification of risk factors for children and adults. *Am Soc Clin Oncol Educ Book***,** e57-67.

Morton, LM, Swerdlow, AJ, Schaapveld, M, Ramadan, S, Hodgson, DC, Radford, J & van Leeuwen, FE 2014b. Current knowledge and future research directions in treatment-related second primary malignancies. *EJC Suppl,* 12**,** 5-17.

Ng, AK & Travis, LB 2008. Second primary cancers: an overview. *Hematol Oncol Clin North Am,* 22**,** 271-89, vii.

Preston DL, LJ, Pierce DA, McConney MA 1993. *Epicure: User's Guide,* Seattle, WA, HiroSoft International Corporation.

Preston, DL, Mattsson, A, Holmberg, E, Shore, R, Hildreth, NG & Boice, JD, Jr. 2002. Radiation effects on breast cancer risk: a pooled analysis of eight cohorts. *Radiat Res,* 158**,** 220-35.

Reulen, RC, Frobisher, C, Winter, DL, Kelly, J, Lancashire, ER, Stiller, CA, Pritchard-Jones, K, Jenkinson, HC, Hawkins, MM & British Childhood Cancer Survivor Study Steering, G 2011. Long-term risks of subsequent primary neoplasms among survivors of childhood cancer. *JAMA,* 305**,** 2311-9.

Rodriguez-Galindo, C, Orbach, DB & VanderVeen, D 2015. Retinoblastoma. *Pediatr Clin North Am,* 62**,** 201-23.

Ron, E 2003. Cancer risks from medical radiation. *Health Phys,* 85**,** 47-59.

Rushlow, D, Piovesan, B, Zhang, K, Prigoda-Lee, NL, Marchong, MN, Clark, RD & Gallie, BL 2009. Detection of mosaic RB1 mutations in families with retinoblastoma. *Hum Mutat,* 30**,** 842-51.

Sanders, BM, Jay, M, Draper, GJ & Roberts, EM 1989. Non-ocular cancer in relatives of retinoblastoma patients. *Br J Cancer,* 60**,** 358-65.

Schall, LC, Buchanich, JM, Marsh, GM & Bittner, GM 2001. Utilizing multiple vital status tracing services optimizes mortality follow-up in large cohort studies. *Ann Epidemiol,* 11**,** 292-6.

Sharma, A, Yeow, WS, Ertel, A, Coleman, I, Clegg, N, Thangavel, C, Morrissey, C, Zhang, X, Comstock, CE, Witkiewicz, AK, Gomella, L, Knudsen, ES, Nelson, PS & Knudsen, KE 2010. The retinoblastoma tumor suppressor controls androgen signaling and human prostate cancer progression. *J Clin Invest,* 120**,** 4478-92.

Sheen, V, Tucker, MA, Abramson, DH, Seddon, JM & Kleinerman, RA 2008. Cancer screening practices of adult survivors of retinoblastoma at risk of second cancers. *Cancer,* 113**,** 434-41.

Shennan, MG, Badin, AC, Walsh, S, Summers, A, From, L, McKenzie, M, Goldstein, AM, Tucker, MA, Hogg, D & Lassam, N 2000. Lack of germline CDK6 mutations in familial melanoma. *Oncogene,* 19**,** 1849-52.

Shinohara, ET, DeWees, T & Perkins, SM 2014. Subsequent malignancies and their effect on survival in patients with retinoblastoma. *Pediatr Blood Cancer,* 61**,** 116-9.

Smith, JL & Bedford, MA 1976. Retinoblastoma. *In:* MARSDEN, H. B. & STEWARD, J. K. (eds.) *Tumours in children.* Berlin: Springer-Verlag.

Song, H, Ramus, SJ, Shadforth, D, Quaye, L, Kjaer, SK, Dicioccio, RA, Dunning, AM, Hogdall, E, Hogdall, C, Whittemore, AS, McGuire, V, Lesueur, F, Easton, DF, Jacobs, IJ, Ponder, BA, Gayther, SA & Pharoah, PD 2006. Common variants in RB1 gene and risk of invasive ovarian cancer. *Cancer Res,* 66**,** 10220-6.

Stiller, CA & Parkin, DM 1996. Geographic and ethnic variations in the incidence of childhood cancer. *Br Med Bull,* 52**,** 682-703.

Stovall, M, Weathers, R, Kasper, C, Smith, SA, Travis, L, Ron, E & Kleinerman, R 2006. Dose reconstruction for therapeutic and diagnostic radiation exposures: use in epidemiological studies. *Radiat Res,* 166**,** 141-57.

Swerdlow, AJ, Higgins, CD, Smith, P, Cunningham, D, Hancock, BW, Horwich, A, Hoskin, PJ, Lister, TA, Radford, JA, Rohatiner, AZ & Linch, DC 2011. Second cancer risk after chemotherapy for Hodgkin's lymphoma: a collaborative British cohort study. *J Clin Oncol,* 29**,** 4096-104.

Tucker, MA, D'Angio, GJ, Boice, JD, Jr., Strong, LC, Li, FP, Stovall, M, Stone, BJ, Green, DM, Lombardi, F, Newton, W & et al. 1987. Bone sarcomas linked to radiotherapy and chemotherapy in children. *N Engl J Med,* 317**,** 588-93.

Turaka, K, Shields, CL, Meadows, AT & Leahey, A 2012. Second malignant neoplasms following chemoreduction with carboplatin, etoposide, and vincristine in 245 patients with intraocular retinoblastoma. *Pediatr Blood Cancer,* 59**,** 121-5.

Weinberg, RA 2007. *The Biology of Cancer,* New York, NY, Garland Science Group.

Wong, FL, Boice, JD, Jr., Abramson, DH, Tarone, RE, Kleinerman, RA, Stovall, M, Goldman, MB, Seddon, JM, Tarbell, N, Fraumeni, JF, Jr. & Li, FP 1997. Cancer incidence after retinoblastoma. Radiation dose and sarcoma risk. *JAMA,* 278**,** 1262-7.

Wong, JR, Tucker, MA, Kleinerman, RA & Devesa, SS 2014. Retinoblastoma incidence patterns in the US Surveillance, Epidemiology, and End Results program. *JAMA Ophthalmol,* 132**,** 478-83.

Yu, CL, Tucker, MA, Abramson, DH, Furukawa, K, Seddon, JM, Stovall, M, Fraumeni, JF, Jr. & Kleinerman, RA 2009. Cause-specific mortality in long-term survivors of retinoblastoma. *J Natl Cancer Inst,* 101**,** 581-91.

Zhang, J, Walsh, MF, Wu, G, Edmonson, MN, Gruber, TA, Easton, J, Hedges, D, Ma, X, Zhou, X, Yergeau, DA, Wilkinson, MR, Vadodaria, B, Chen, X, McGee, RB, Hines-Dowell, S, Nuccio, R, Quinn, E, Shurtleff, SA, Rusch, M, Patel, A, Becksfort, JB, Wang, S, Weaver, MS, Ding, L, Mardis, ER, Wilson, RK, Gajjar, A, Ellison, DW, Pappo, AS, Pui, CH, Nichols, KE & Downing, JR 2015. Germline Mutations in Predisposition Genes in Pediatric Cancer. *N Engl J Med,* 373**,** 2336-46.

Zhu, XP, Dunn, JM, Phillips, RA, Goddard, AD, Paton, KE, Becker, A & Gallie, BL 1989. Preferential germline mutation of the paternal allele in retinoblastoma. *Nature,* 340**,** 312-3.