Summary
Introduction
As reported in Chapter 1, more than 400 years ago the first description of a tumor resembling retinoblastoma was reported by a Dutch anatomist named Pieter Pauw. However, it was not until the end of the 19th century that most retinoblastoma patients actually survived the disease. Since then numerous studies have investigated the cause of retinoblastoma and searched for better treatment modalities. In the mid 1900s, a report on the late complications of retinoblastoma patients treated with external beam radiation therapy appeared. This was the start of numerous studies investigating the late adverse events after treatment of retinoblastoma survivors. However, these studies were mostly hospital-based rather than population-based. Furthermore, in most studies numbers were small and follow-up was less than 40 years. For this thesis, long-term and complete follow-up data from the Dutch retinoblastoma registry were available from retinoblastoma patients diagnosed since 1862. These data have made it possible to study incidence and mortality of adverse events and risk factors, and to compare specific events in Dutch retinoblastoma patients to the general Dutch population.

In this final chapter the main results are put into perspective, and methodological considerations, clinical implications, assessment of outcome and recommendations for future research are discussed.

Main findings
This section addresses the aims of the thesis as outlined in the Introduction (Chapter 1).

Second primary malignancies
Although many studies have revealed that survivors of hereditary retinoblastoma have an elevated risk of developing second primary malignancies, data on the risk of second primary malignancies in middle-aged retinoblastoma survivors are scarce.

The Dutch retinoblastoma registry was used to analyze risks of second primary malignancies in retinoblastoma patients diagnosed from 1945 to 2005. After extensive follow-up procedures, complete follow-up data of 668 (89%) retinoblastoma patients was obtained. Information of current health, past diseases, including any occurrence of cancer, medical treatments, and various risk factors for cancer were obtained by means of a mailed questionnaire and confirmed by pathology reports, hospital or physician’s records. For both hereditary and nonhereditary retinoblastoma patients, risks of second primary malignancies were compared with the Dutch general population. No statistically significantly elevated risks of second primary malignancies were found among nonhereditary retinoblastoma survivors (standardized incidence rate (SIR) = 1.86; 95% confidence interval (CI): 0.96-3.24, absolute excess risk (AER) = 0.57 per 1000 person-years). Among hereditary retinoblastoma survivors an overall risk of 20.4 (95% CI: 15.6-26.1; AER = 8.61 per 1000 person-
years) was found, which increased almost with 3-fold when these patients were treated with radiotherapy. Because of the small number of hereditary retinoblastoma patients treated with chemotherapy exclusively, our ability to detect any association of chemotherapy with second solid malignancy was limited. Among hereditary retinoblastoma patients, the cumulative incidence of a second malignancy at 40 years after retinoblastoma diagnosis, accounting for death as a result of other causes as competing risk, was 28% (95% CI: 21.0%-35%). Our results confirmed the strongly increased risks of soft tissue sarcoma, osteosarcoma, and melanoma in hereditary retinoblastoma survivors. However, after more than 40 years of follow-up an emerging excess of epithelial cancers (i.e., breast, lung and bladder) was observed, which had not been reported in other long-term follow-up studies.

In conclusion, the excess risk of epithelial cancers such as bladder, lung and breast in middle-aged retinoblastoma survivors are cause of concern and indicates that lifelong follow-up studies are needed to evaluate the full spectrum of second primary malignancy risk in retinoblastoma survivors.

Cause-specific mortality
In contrast to second malignancy incidence studies, little information is available on long-term excess mortality among retinoblastoma survivors.

This cohort study includes a total of 998 (93%) retinoblastoma patients diagnosed since 1862. Patients who had died before 1901 were excluded, because no cause-specific mortality rates were available before 1901. Of all cohort members the vital status was checked using various approaches (telephone directories, hospital administration, the Central Bureau of Genealogy, and municipal registries). If a cohort member had died, the date and place of death, and the death certificate number were recorded. Information on the cause of death was obtained from Statistics Netherlands for all deceased cohort members up to June 2007. A comparison was made between cause-specific mortality in retinoblastoma survivors and the Dutch population, using age-, sex-, and calendar period-specific mortality rates from Statistics Netherlands, which are available since 1961. For breast cancer and melanoma only, historical mortality reference rates were available since 1901. Of the 998 retinoblastoma patients, a total of 332 deaths were observed. Most individuals (n = 156) had died as a consequence of retinoblastoma itself, followed by death due to any other malignancy (n = 84). For nonhereditary as well as hereditary retinoblastoma patients, no statistically significantly elevated risks of causes other than cancer were observed. Mortality due to cancer other than retinoblastoma was statistically significantly elevated for hereditary retinoblastoma patients only (standardized mortality ratio (SMR) = 12.8; 95% CI: 9.6-16.5). Patients treated with radiotherapy as treatment for retinoblastoma had a nonsignificantly elevated risk of death due to a subsequent malignancy compared to those treated otherwise (hazard ratio (HR) = 1.57; 95% CI: 0.83-2.95). This moderately and nonsignificantly elevated risk in our cohort may be explained as follows: hereditary retinoblastoma survivors treated with radiotherapy died relatively young from bone cancers and soft tissue sarcomas, and
hereditary retinoblastoma survivors not treated with radiotherapy died at older ages because of epithelial cancers located outside the field of radiation.

This study is the first to evaluates mortality among retinoblastoma patients in a nationwide cohort with very long-term follow-up and near complete cause of death information. We conclude that despite the results of early detection and good treatment options for cancers occurring outside the head region, the emerging excess risk of mortality in retinoblastoma survivors is a cause of concern. Therefore patients and their physicians must be made aware of the increased risk of death from subsequent malignancies among hereditary retinoblastoma survivors.

Multiple primary malignancies

Since modern cancer treatment protocols have increased survival of patients who developed second primary malignancies, survivors with a third or subsequent malignancy are also increasingly observed. Until now, only one study specifically reported on the incidence and survival of third and additional malignancies. However, no study has reported on the magnitude of the risk and the survival of a third cancer among retinoblastoma patients.

In this study all patients with complete follow-up from the Dutch retinoblastoma registry (n = 1028) were used to quantify third primary malignancy risk using various measures. The risk of a third primary malignancy was compared with cancer risk in the Dutch population. Cox model analysis with a time-dependent covariate was used to compare subsequent malignancy risk and survival among patients with and without a second malignancy. After a median follow-up of 28.6 years of the 1028 retinoblastoma patients from the Dutch retinoblastoma registry, a total of 129 patients with a second primary malignancy were observed. Among those with a second primary malignancy, 11 were observed with a third primary malignancy. In patients with a second primary malignancy the risk of a third primary malignancy was 8-fold (standardized incidence ratio (SIR) = 8.19; 95% confidence interval (CI): 4.09-14.7) increased, with an excess of 234 malignancies per 10,000 person-years. Subsequent cancer risk after a second primary malignancy was more than 7-fold (HR = 7.56; 95% CI: 3.87-14.83) increased compared to the risk of a second primary malignancy after retinoblastoma, adjusted for heredity and treatment. A third malignancy modeled as a time-dependent multivariable covariate, was associated with worse survival compared with patients only diagnosed with a second malignancy (HR = 5.02; 95% CI: 1.66-15.2).

This study is the first to examine whether retinoblastoma survivors who developed a second primary malignancy have a greater risk of a subsequent primary malignancy. Our study shows that having had a second primary malignancy increases the risk of a subsequent malignancy by 7-fold. Therefore, we conclude that treating physicians should be aware of the fact that survivors of retinoblastoma who already have developed a second malignancy have an even higher risk of
subsequent malignancies than retinoblastoma survivors without a second malignancy. Finally, ionizing radiation should be avoided in the treatment of retinoblastoma itself as well as in the treatment for subsequent malignancies.

**RB1 mutations and second primary malignancies**

In this study the *RB1* genotype in relation to second malignancy risk in subjects with hereditary retinoblastoma has been investigated. Since the discovery of the *RB1* gene in 1986, no large cohort studies of retinoblastoma patients have been published investigating whether specific *RB1* mutations might be associated with greater risk of second malignancy.

Eligible subjects for this study included all hereditary retinoblastoma patients from the Dutch retinoblastoma registry (1862-2005), in whom a germline *RB1* mutation was documented. Both living and deceased retinoblastoma patients were eligible. Since the beginning of the 1990s, DNA analysis is part of the diagnosis of retinoblastoma. Patients who were diagnosed before the 1990s, but wanted to participate in the study, were invited to undergo DNA-testing and were offered genetic counselling. DNA analysis included direct sequencing (exons 1, 15 and the *RB1* promoter), Denaturing Gradient Gel Electrophoresis (DGGE) analysis (all other exons), flanking intronic sequences, Multiplex Ligation-dependent Probe Amplification (MLPA) analysis (duplications and deletions), and karyotyping (chromosomal rearrangements). In total, data were available of 199 unilateral and bilateral retinoblastoma patients with a *RB1* mutation. Among 44 carriers of a *RB1* mutation from 31 different families a second primary malignancy was observed. A statistically significantly elevated risk of second malignancies for a certain type of mutation was not found, nor did any type of mutation seem to predispose for a specific tumor type. On the other hand, a trend towards higher second malignancy risk among hereditary retinoblastoma subjects with a nonsense or frameshift mutation compared to other *RB1* mutations was observed (HR = 1.58; 95% CI: 0.82-3.06; *P* = .17, adjusted for age and treatment). Also, secondary tumors were observed more often in patients who had received ionizing radiation for retinoblastoma treatment.

Possibly, the group we studied may have been too small to detect genotype-phenotype correlations between documented *RB1* mutations and second malignancy risk. Therefore, larger international collaborative studies will be needed to follow more retinoblastoma survivors where molecular testing can be combined with data on second malignancy occurrence and possible risk modifiers, such as smoking.

**In vitro fertilization and risk of retinoblastoma**

Between 1995-2002 a statistically significantly increased risk on retinoblastoma among children conceived by IVF was found in the Netherlands. However, two IVF register-based studies did not find a confirmation of this increased risk of retinoblastoma after IVF. Therefore, the objective of
this report was to investigate the incidence of retinoblastoma by estimating nationwide numbers of live births conceived by IVF.

We first obtained nationwide numbers on ongoing pregnancies (an intrauterine pregnancy >10 weeks after embryo replacement confirmed by ultrasound) after IVF and ICSI. Based on these numbers, we estimated numbers of live births conceived by IVF in the Netherlands (n = 40,330) in the period 1995-2007. Subsequently, we estimated the expected numbers of retinoblastoma patients conceived by IVF in that same period. To obtain the actual observed number of retinoblastoma patients in children conceived by IVF we sent questionnaires to the parents of retinoblastoma patients diagnosed between 1995-2005. For patients diagnosed after 2005, and for those who did not respond to the questionnaire, information was available through medical files. Of all eligible retinoblastoma patients diagnosed between 1995 and 2007 (n = 162), 7 patients were conceived by IVF. Of these 7 patients, 3 were non-familial bilateral cases, and 4 non-familial unilateral cases without a detectable RB1 mutation. For the total study period (1995-2007), we found a statistically significantly elevated risk of retinoblastoma among children conceived by IVF (RR = 2.54; 95% CI: 1.02-5.23). However, in the expanded study period (2002-2007), no significantly elevated risk (RR = 1.29; 95% CI: 0.16-4.66) was found. Based on the literature, only in the Netherlands an elevated risk of retinoblastoma was found among children conceived by IVF. An association between retinoblastoma and IVF is difficult to explain. Explanations might be clustering or a chance finding, an association of retinoblastoma and IVF. But it may also be that the same genetic factors are involved in infertility and the occurrence of retinoblastoma or maybe changes in the IVF procedure itself in the course of time.

We conclude that there is a statistically significantly increased risk of retinoblastoma in children conceived by IVF in the period 1995-2007. However, the increased risk in the total study period is mostly based on the much stronger risk increase for the period 1995 to 2002. Caution and awareness on the one hand and avoiding unnecessary worries on the other hand are mandatory at this stage of our knowledge.

Reference List


