Secondary Malignancies in Retinoblastoma

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A. Introduction

More than 400 years ago, a tumor resembling retinoblastoma was first described by a Dutch anatomist named Pieter Pauw\(^1\). However, it was not until the end of the 19\(^{th}\) century that most retinoblastoma patients actually survived the disease\(^2-4\). Since then, numerous studies have investigated the cause of retinoblastoma and searched for better treatment modalities. In the mid-1900s, a report was published on the late complications of retinoblastoma patients treated with external beam radiation therapy\(^5\). This was the start of numerous studies investigating late adverse events after treatment for retinoblastoma\(^6-8\). However, most of these studies were hospital based rather than population based, and involved a small number of patients who were followed up for less than 40 years. Long-term and complete follow-up data of patients diagnosed with retinoblastoma since 1862 are available from the Dutch retinoblastoma registry. This data has enabled researchers to assess the incidence of adverse events, causes of mortality, and risk factors in retinoblastoma survivors.
Researchers can also compare specific events among Dutch retinoblastoma patients and the general Dutch population.

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

A. References


B. Main Findings

B.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 this risk in middle-aged retinoblastoma survivors is scarce.

Data from the Dutch retinoblastoma registry was used to analyze the risks of second primary malignancies in patients who were diagnosed with
retinoblastoma between 1945 and 2005. After extensive follow-up procedures, complete data were obtained from 668 (89%) retinoblastoma patients. The data consisted of information on current health, past diseases, including cancer, medical treatments, and various risk factors for cancer. The data was obtained by means of mailed questionnaires and confirmed using pathology reports and hospital or physician’s records. For both hereditary and non-hereditary retinoblastoma patients, the risk of developing a second primary malignancy was compared with the risk in the general Dutch population. No statistically significant elevation in risk was found among survivors of non-hereditary retinoblastoma (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 survivors of hereditary retinoblastoma treated with radiotherapy, the overall risk (SIR = 20.4; 95% CI: 15.6–26.1; AER = 8.61 per 1000 person-years) was almost 3-fold that of the rate in the general Dutch population. Because of the small number of patients with hereditary retinoblastoma treated exclusively with chemotherapy, our ability to detect any statistical association between chemotherapy and the development of a second solid malignancy was limited. The cumulative incidence of a second malignancy 40 years after the diagnosis of hereditary retinoblastoma was 28%, (95% CI: 21.0%-35%) accounting for death as a result of other causes as competing risk. Our results confirmed that the risks of developing soft tissue sarcoma, osteosarcoma, and melanoma were markedly increased in survivors of hereditary retinoblastoma. However, after more than 40 years of follow-up, an emerging excess of epithelial cancers (i.e., breast, lung, and bladder) was observed—a finding that has 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 is cause for concern and indicates that lifelong follow-up studies are needed to evaluate the full spectrum of second primary malignancy risk in retinoblastoma survivors.
B.2 *Cause-specific mortality*

Unlike second malignancy risk, which has been studied in detail, little information is available on long-term excess mortality among retinoblastoma survivors.

The Dutch cohort study includes a total of 998 (93%) patients who have been diagnosed with retinoblastoma since 1862. Patients who died before 1901 were excluded from the present study, because no cause-specific mortality rates were available before 1901. The vital status of all cohort members was checked using various approaches (telephone directories, hospital records, Central Bureau of Genealogy data, and municipal registries). For deceased cohort members, the date and place of death, and the death certificate number were recorded. Information regarding the cause of death was obtained from Statistics Netherlands for all deceased cohort members up to June 2007. The cause-specific mortality in retinoblastoma survivors was compared with mortality in the general Dutch population, by 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, 332 died before the end of the study period. The most common cause of death was the retinoblastoma itself (n = 156), followed other malignancies (n = 84).

For both non-hereditary and hereditary retinoblastoma patients, no significantly elevated risks were observed for causes of death other than cancer. The risk of death due to cancers other than retinoblastoma was significantly elevated for patients with hereditary retinoblastoma (standardized mortality ratio = 12.8; 95% CI: 9.66–16.5). Patients treated with radiotherapy had an insignificantly elevated risk of death due to a subsequent malignancy when
compared with patients treated with other therapeutic modalities (hazard ratio (HR) = 1.57; 95% CI: 0.83–2.95). This moderate but non-significantly elevated risk in our cohort may be explained as follows: survivors of hereditary retinoblastoma treated with radiotherapy died of bone cancers and soft-tissue sarcomas at a relatively young age, whereas those not treated with radiotherapy died of epithelial cancers located outside the field of radiation at older ages.

Our study is the first to evaluate mortality among a nationwide cohort of retinoblastoma patients by using long-term follow-up and near-complete data on causes of death. We conclude that despite 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 for concern. Therefore, survivors of hereditary retinoblastoma and their physicians must be made aware of the increased risk of death from subsequent malignancies.

B.3 Multiple primary malignancies

Due to advances in cancer-treatment protocols and the increased survival of retinoblastoma patients who develop second primary malignancies, patients with a third or subsequent malignancy are also increasingly observed. Until now, only 1 study has specifically reported on the incidence of and survival from third and subsequent malignancies in retinoblastoma survivors\(^1\). However, no study has reported the magnitude of the risk of developing a third primary cancer and subsequent survival among retinoblastoma survivors.

In our 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. This risk of a third primary malignancy was compared with the cancer risk in the general Dutch population. A Cox model analysis with a time-dependent covariate was used to compare the risk of
and survival from subsequent malignancies among patients with and without a second malignancy.

After a median follow-up of 28.6 years, 129 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 developed a third primary malignancy. In patients with a second primary malignancy, the risk of developing a third primary malignancy was 8-fold (SIR = 8.19; 95% CI: 4.09–14.7) the risk in patients who did not develop a second malignancy, with an excess of 234 malignancies per 10,000 person-years. The risk of cancer risk after the development of a second primary malignancy was more than 7-fold (HR = 7.56; 95% CI: 3.87–14.83), an increase compared to the risk of the development 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 than that associated with a second malignancy (HR = 5.02; 95% CI: 1.66–15.2).

This study is the first to examine whether retinoblastoma survivors who develop a second primary malignancy have a greater risk of developing 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 made aware of the fact that retinoblastoma survivors with a second malignancy have a higher risk of developing subsequent malignancies than retinoblastoma survivors without a second malignancy. Finally, ionizing radiation should be avoided in both the treatment of retinoblastoma itself as well as in the treatment for subsequent malignancies.

**B.4 RB1 mutations and second primary malignancies**
We assessed the risk of developing a second malignancy in relation to the \textit{RB1} genotype in our cohort of survivors of hereditary retinoblastoma. Since the discovery of the \textit{RB1} gene in 1986, no large cohort studies have investigated whether specific \textit{RB1} mutations are associated with a greater risk of second malignancy in retinoblastoma survivors. Both deceased and living patients who had hereditary retinoblastoma and a documented germline \textit{RB1} mutation, and were included in the Dutch retinoblastoma registry (1862–2005) were eligible for this study. Since the beginning of the 1990s, DNA analysis has been a part of the diagnostic work-up for retinoblastoma. Patients who were diagnosed with retinoblastoma before the 1990s, but who wanted to participate in the study were invited to undergo DNA testing and were offered genetic counseling. DNA analysis included direct sequencing (exons 1, 15, and the \textit{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 for 199 patients who had unilateral or bilateral retinoblastoma and an \textit{RB1} mutation. A second primary malignancy was observed in 44 carriers of a \textit{RB1} mutation from 31 different families. A significantly elevated risk of second malignancies for any specific type of mutation was not found, nor did any type of mutation seem to predispose patients to a specific tumor type. However, a trend towards a higher risk for second malignancy was observed among hereditary retinoblastoma patients with a nonsense or frameshift mutation when compared to patients with other \textit{RB1} mutations (HR = 1.58; 95% CI: 0.82–3.06; \(P = .17\), adjusted for age and treatment). In addition, secondary tumors were observed more often in patients who had received ionizing radiation for the treatment of retinoblastoma. Our study group may have been too small to enable the detection of genotype-phenotype correlations between documented \textit{RB1} mutations and second malignancy risk. Therefore, larger international collaborative studies will
be needed to follow more retinoblastoma survivors for whom molecular testing can be combined with data on second malignancy occurrence and possible risk modifiers such as smoking.

**B.5 In vitro fertilization and risk of retinoblastoma**

Between 1995 and 2002, a significantly increased risk for retinoblastoma was found among children conceived by in vitro fertilization (IVF) in the Netherlands\(^2\). However, 2 IVF register-based studies could not confirm this finding\(^3,4\). Therefore, we aimed to determine the incidence of retinoblastoma in the children by using nationwide estimates for the total number of live births after IVF.

We first obtained nationwide numbers on ongoing pregnancies (defined as an intrauterine pregnancy of >10 weeks duration, resulting from embryo replacement and confirmed using ultrasound) after IVF and intracytoplasmic sperm injection (ICSI). On the basis of these numbers, we estimated the number of live births after IVF in the Netherlands (n = 40,330) from 1995 to 2007. Subsequently, we estimated the expected number of retinoblastoma patients conceived by IVF during the same period. To determine the actual number of retinoblastoma patients among children conceived by IVF, we mailed questionnaires to the parents of children who were diagnosed with retinoblastoma between 1995 and 2005.

For children diagnosed after 2005 and those whose parents did not respond to the questionnaire, we obtained information from medical records. Of all eligible retinoblastoma patients diagnosed between 1995 and 2007 (n = 162), 7 were conceived by IVF. Of these 7 patients, 3 had non-familial bilateral retinoblastoma, and 4 had non-familial unilateral retinoblastoma without a detectable \(RB1\) mutation. For the total study period (1995–2007), we found a significantly elevated risk of retinoblastoma among the children conceived by
IVF (relative risk [RR] = 2.54; 95% CI: 1.02–5.23). However, for the extended study period (2002–2007), no significantly elevated risk (RR = 1.29; 95% CI: 0.16–4.66) was found. A review of the literature revealed that an elevated risk of retinoblastoma among children conceived by IVF has been found only in the Netherlands. The association between retinoblastoma and IVF is difficult to explain but may be attributed to clustering or chance. But it may also be that the same genetic factors are involved in infertility and retinoblastoma development, or certain genetic changes may be introduced during the IVF procedure itself.

We conclude that the risk of the occurrence of retinoblastoma was significantly increased among children conceived by IVF during the period 1995–2007, and most of this increase was derived from the data for the period 1995–2002. Caution and awareness on the one hand and avoiding unnecessary worries on the other hand are mandatory at this preliminary juncture in our research.

B. References


C. Methodological considerations

C.1 Study design

In the 1920s\(^1\), the Dutch retinoblastoma registry was established as a retrospective cohort. In a retrospective cohort study, individuals are identified on the basis of their characteristics in the past and followed up until the present\(^2\). Over the past decades, several investigators have updated the Dutch retinoblastoma registry\(^3-5\). More detailed information on this registry is given in the next section—Study Population.

In the above historical retrospective cohort of Dutch retinoblastoma patients, we studied the occurrence of long-term adverse events, including the occurrence of a second primary malignancy in relation to the presence of an RB1 mutation. The association between IVF and the development of retinoblastoma was examined. Despite the many advantages of this study design, it also has some drawbacks.

First, the effects observed may be attributed to the introduction of new techniques over the years, such as DNA analysis. A potential source of bias is that DNA analysis was not performed in a large part of the cohort, because the cohort members had already died when DNA analysis became available. Consequently, the study comprises a relatively young and small group of patients with hereditary retinoblastoma, and a relatively small number of patients with soft tissue sarcomas and bone cancers compared to the number of patients with epithelial cancers.

Second, due to short follow-up of patients treated with modern treatment modalities, like chemotherapy, our ability to detect long-term adverse events was limited. Also, our long-term follow-up data on patients treated with old treatment modalities, like high-dose radiation therapy, are not applicable to newly diagnosed patients due to changes in treatment over the years. However,
a prospective follow-up would be too short to examine the long-term adverse events after modern treatments.

Third, data on new variables of interest, like smoking habits, were not available for all cohort members. It is an inherent limitation of retrospective cohort studies that outcomes of interest occur in the past. Therefore, it is sometimes difficult to verify additional information or to approach patients in a retrospective cohort study. Information could be obtained from patients who are still alive by using questionnaires, but the outcome measures are prone to misclassification bias and may be influenced by various factors, e.g., the fact that patients know that they are being followed up by their ophthalmologist or that they have developed a second malignancy.

Obtaining medical files from older cohort members was difficult and occasionally impossible because the files had been destroyed. Under Dutch privacy law (Wet Geneeskundige Behandelings Overeenkomst [WGBO], code 7:454), medical files should be maintained for up to 15 years after diagnosis. After 15 years, they must be destroyed, unless the physician has clinical reasons not to do so. In academic centers, some data in medical files, such as pathology reports and surgery reports, must be saved for up to 115 years. Although the WGBO privacy law was introduced only on April 1, 1995, files from before that time were mostly destroyed. During the study, it appeared that some patients in the Dutch retinoblastoma registry did not know that they had been diagnosed with and treated for retinoblastoma as a child. Some of them had searched for the cause of they had lost an eye. In all these cases, medical files in the original treatment center were not available any more. These patients were generally very glad to hear that information on their diagnosis and treatment was available through the Dutch retinoblastoma registry. This indicates how crucial it is to store medical records of patients treated during childhood for a lifetime, even though information about childhood cancer, when first conveyed to the patient, may evoke emotional responses.
To evaluate the results obtained from our study cohort, we used the Dutch general population as the external comparison group. In the Netherlands, several population-based registries are available. Information on cancer incidence in the Netherlands was available from the Eindhoven Cancer Registry (from 1955 onwards) and the Netherlands Cancer Registry (from 1989 onwards). Information about mortality was available from Statistics Netherlands, and some historical mortality data were obtained from Prof. Dr. Johan P. Mackenbach, Department of Public Health, Erasmus Medical Center, Rotterdam, Netherlands. We used population-based estimates of ongoing pregnancies from the Dutch Society of Obstetrics and Gynaecology (NVOG) and the National Infertility Registry (LIR). We will further discuss these registries in the section on assessment of outcomes.

C.2 Study population

To address our study questions we used the Dutch retinoblastoma registry, which is a nationwide cohort and consists of Dutch retinoblastoma patients diagnosed since 1862. This unique nationwide cohort is virtually complete from 1945\textsuperscript{6,7} to the present. The Dutch retinoblastoma registry contains information on demographic characteristics, family history of retinoblastoma, tumor laterality, treatment of retinoblastoma, reports of additional cancers, and cause of death. The registry was set up retrospectively in the 1920s by Hemmes\textsuperscript{1} and updated throughout the years.

Initially, retinoblastoma was treated in several hospitals around the country. The majority of the patients in this cohort were treated at the Royal Netherlands’ Eye Hospital of the University of Utrecht and University Medical Center St. Radboud Nijmegen. However, since 1991, all retinoblastoma patients
are treated in a single specialized hospital: VU University Medical Center, Amsterdam.

All patients in this registry who were diagnosed with retinoblastoma between 1862 and 2005 were included in the study. Unfortunately, the registry is possibly not complete for the period 1862–1945 because during that time, patients might have died without being diagnosed with retinoblastoma. Also, some patients may not have been traced because the medical records were not available. Patients who could not be traced (unknown birth date) and patients who apparently had retinomas (tumors with spontaneous growth arrest) were ultimately excluded from analysis. During the study, it appeared that 1 patient, previously diagnosed with retinoma, had in fact a retinoblastoma in 1 eye and a retinoma in the other eye. This person was excluded from the study because she was born before 1945.

We mailed a general health and risk factor questionnaire to all retinoblastoma patients and performed DNA analysis in hereditary retinoblastoma patients who were alive at the time of the study. To approach the retinoblastoma patients, we obtained permission from the hospitals where the patients had been treated for retinoblastoma. All practical and ethical aspects of the study protocol were extensively reviewed and finally approved by the medical ethics committees of all the individual hospitals involved. Non-hereditary retinoblastoma patients who had not yet been tested for RB1 mutations were not specifically asked to undergo DNA analysis but were provided with information about DNA analysis.

To update the vital status and to trace the recent addresses of the cohort members, we used various approaches (telephone directories, hospital records, Central Bureau of Genealogy data, and municipal population registries). Since 1994, municipal population registries in the Netherlands have been using a central computer system (GBA) that provides access to nationwide data on all living individuals. These registries are under no circumstances publicly accessible, but access for epidemiological research is permitted under strict
privacy conditions (see **C. Figure 1** for follow-up procedure). In total, the Dutch retinoblastoma cohort contains 1068 patients (1862–2005). The vital statuses of 4 patients were not accessible, because the date of birth was unknown, and 15 patients had emigrated. The vital statuses of the remaining 1049 patients were checked, and despite all possible tracing approaches used, the vital statuses of 16 patients could not be assessed. Of the remaining 1033 patients in the Dutch retinoblastoma registry, 360 were dead and 673 were still alive.

Finally, complete follow-up data in addition to the vital status were available for 1028 (96%) patients (619 with non-hereditary and 409 with hereditary retinoblastoma) from the Dutch retinoblastoma registry. Medical follow-up data were available for 92% of the study population, and the overall response rate to the questionnaire was 85%.

**C. Figure 1**
Procedure for follow-up of the Dutch retinoblastoma cohort.
C.3 Study limitations

When interpreting of our results, the strengths and limitations of our studies need to be considered. The strengths of our study include the very complete follow-up and the availability of detailed information on demographic
characteristics, family history of retinoblastoma, tumor laterality, and treatment administered for retinoblastoma, additional cancers, and cause of death for patients diagnosed with retinoblastoma during 1862–2005. The limitations of our study may include some potential misclassification of non-hereditary patients due to incomplete karyotyping or DNA analysis data. The technology to detect mutations in the RB1 gene has only become available in the past 2 decades, and it was not until the beginning of the 1990s, that DNA analysis was routinely performed for all newly diagnosed Dutch patients with retinoblastoma. As a result of many patients included in our historical retinoblastoma registry died before the study began, and so it was not possible to test for mutations in the RB1 gene. Among the patients confirmed to have hereditary retinoblastoma, 48% were found to harbor an RB1 mutation. In total, 23% of all patients diagnosed with non-hereditary retinoblastoma underwent karyotyping or DNA analysis tests, but no mutations were found among them.

Patients with hereditary retinoblastoma may have been more inclined to participate in a study investigating the late effects of retinoblastoma. We hypothesized that since patients with non-hereditary retinoblastoma do not seem to be prone to second malignancies, these patients may have been less motivated to participate in the study. Linkage of non-responders to the Netherlands Cancer Registry would reveal whether cancer rates among responding participants differed from those among non-responders. However, linkage to the Netherlands Cancer Registry is only possible with the permission of the concerned patient. In retrospective cohort studies, it is not always possible to obtain such permission because some patients may have died before the beginning of the study or they may be untraceable. Therefore, in some cases, conditional upon several requirements with regard to the research protocol, linkage of non-responders is permitted without patients’ consent. Some of these requirements are (1) non-responders must be given the opportunity to refuse linkage by approaching them at least twice via mail, and (2) the Surveillance Committee of the Netherlands Cancer Registry must give
permission to link non-responders. The Netherlands Cancer Registry became operational in 1989, and so information on patients diagnosed with retinoblastoma before 1989 is unavailable. Because most of the non-responders in our study were diagnosed long before 1989, no requests for linkage were sent to these patients, and information on their cancers is unavailable.

Additional analysis to obtain more insights into the potential for selection bias was performed. It appeared that almost all the non-responders (95%) had non-hereditary retinoblastoma. This confirms our hypothesis that patients with non-hereditary retinoblastoma were less motivated to participate in the study. We may have underestimated the risk of cancer among these patients due to the unavailability of information on second malignancies. Therefore, cancer incidence in retinoblastoma survivors who responded very late (after at least 2 reminders) was compared to cancer incidence in the early responders, assuming that non-responders would be more similar to late responders.

Surprisingly, we found that cancer incidence was lower among the late responders, which indicates that we may have slightly overestimated the risk of second malignancy in the non-hereditary retinoblastoma survivors. On the basis of these results, we believe that selection bias was a minor problem in our cohort.

Other limitations of our study are the small number of non-hereditary retinoblastoma survivors with second primary malignancies, the small sample sizes used for subgroup analysis, the small number of patients who were treated with chemotherapy only, which limited our ability to detect any association between chemotherapy and adverse effects, the small number of patients with third and subsequent malignancies, the incompleteness of IVF registries in the Netherlands, and the absence of medical files of the older cohort members.

C. References
In this study, assessment of disease outcome was based on information from several sources. First, the questionnaires that were mailed to the patients contained questions on marital status, height, and weight, education, employment, and questions on health-related topics, including congenital abnormalities, current physical complaints, medication use, information on cancers diagnosed after retinoblastoma, radiation exposure, visual acuity; and smoking habits. Unfortunately, many specific items could not be accessed through a self-administered questionnaire. Therefore, the information given was verified through medical files and, whenever possible, pathology reports.

Second, the results of this nationwide study were compared to the general Dutch population rates. Cancer incidences in the Dutch retinoblastoma population were compared with cancer incidences rates in the Dutch general population. We used sex-, age-, and calendar year-specific cancer rates up to 1990 from the Eindhoven Cancer Registry and reference rates since 1990 from the Netherlands Cancer Registry. Because the Netherlands Cancer Registry was established in 1989, cancer incidence data for the whole country were not available for the total study period. However, the reference rates for 1989–2000
from the Eindhoven Cancer Registry are very similar to those from the Netherlands Cancer Registry for the same period, which implies that the use of these regional incidence rates as reference data for earlier periods is valid.

Third, cause-specific mortality among the study population was compared with that in the Dutch general Dutch population by using reference rates from Statistics Netherlands. Since 1869, all deaths in the Netherlands are registered by Statistics Netherlands. However, data on cause-specific mortality are available only since 1961. Due to extensive research involving recoding of old classifications of the International Classification for Diseases, cause-specific mortality rates for melanoma and breast cancer were made available since 1901 and provided by Prof. Dr. Johan P. Mackenbach, Department of Public Health, Erasmus Medical Center, Rotterdam, Netherlands.

Furthermore, to assess the risk of subsequent primary malignancies among retinoblastoma survivors, we calculated SIR—an RR measure that indicates a given risk in the study population on the basis of the sex, age, and calendar-year specific incidence rates in the general population. The hazard ratio (HR) is also an RR measure that compares risks between groups within a cohort, e.g., patients treated with radiation therapy compared to those treated with other modalities. RR measures are difficult to interpret because they are strongly influenced by the background risk. For example, an SIR of 314 for bone cancer, which has a very low background risk, indicates that the risk is strongly increased compared to that in the general population, nevertheless, the absolute risk of developing bone cancer in the study group is still low because of the low background risk. Therefore, the absolute excess risk (AER) best reflects the true burden of a disease in a specific study group. For example, an AER of 2.33 per 1000 person-years for bone cancer indicates that 2.33 additional events of bone cancer per 1000 person-years occurred in that specific study group. However, the AER cannot be used to estimate the risk for individual patients. To estimate the absolute risk of second malignancies for individual patients, cumulative incidence rates were calculated. Patients were
followed up from the date of the diagnosis of retinoblastoma until the end of follow-up, e.g., date of diagnosis of a second malignancy, emigration, the date on which the patient was last known to be alive, the date of death, or the closing date of the study (June 30, 2007). A cumulative incidence of 28% at 40 years after the diagnosis of hereditary retinoblastoma means that a patient with hereditary retinoblastoma has a 28% risk of developing a second malignancy during a 40-year period following the retinoblastoma diagnosis, after adjusting for competing causes of death.

Finally, nationwide numbers on ongoing pregnancies (defined as an intrauterine pregnancy of >10 weeks duration, occurring after embryo replacement and confirmed using ultrasound) after IVF and ICSI from are available NVOG and LIR since 1996. Although all 13 certified IVF centers in the Netherlands annually report their results to the NVOG (1996–2002) and LIR (2003–2007), the number of live births after IVF was not available. Therefore, we assumed that each ongoing pregnancy resulted in a live born child, and that the number of ongoing pregnancies in 1995 was the same as that in 1996. Because some assumptions were made to estimate the overall percentage of live births after IVF and the risk of retinoblastoma among children conceived by IVF, we may have slightly under- or overestimated this risk.

The results of this cohort study can be extrapolated to other countries where retinoblastoma incidence, cancer incidence, and patient survival rates are comparable. However, our results cannot be generalized to countries in which the established risk factors for adverse effects (e.g., treatment) differ from those in the Netherlands. In large cohort studies in the United States\cite{6,7}, the number of retinoblastoma patients treated with external beam radiation therapy was greater than that in our study cohort. Consequently, in the United States, more patients are at an increased risk of developing subsequent malignancies and therefore have higher malignancy and mortality risks.

Caution and awareness should be exercised when comparing the results of different studies. The Dutch retinoblastoma cohort is a long-term and
complete population-based cohort, whereas most other studies are hospital based. The results of these studies may be biased, e.g., the estimated risk may be higher in studies including more patients with hereditary than non-hereditary retinoblastoma. Also, the definition of a subsequent primary malignancy may differ across studies. Subsequent malignancies in our study were defined as malignancies that differed in histological appearance. Consequently, pinealoblastomas were excluded because they are histologically identical to retinoblastoma. Also, basal cell carcinomas of the skin were excluded, because they do not metastasize, are rarely lethal, and are not taken into account in the Netherlands Cancer Registry.

Furthermore, the definition of radiation-induced cancers may be different in other studies. In our study, tumors were considered to be radiation induced if they originated in the field of irradiation and involved the lids, periocular sinuses, temporal bones, or skin overlying the temporal bone region.

D. References

E. Implications for Clinical Practice

Our study results indicate that lifelong follow-up is required for patients with retinoblastoma, especially, hereditary retinoblastoma. Patients with hereditary retinoblastoma are at increased risk for not only soft tissue sarcoma, bone cancers, and melanoma, but also epithelial cancers, such as bladder, breast, and lung cancer. Close surveillance of retinoblastoma patients by a multidisciplinary team, consisting of ophthalmologists, nurses who specialize in the care of retinoblastoma patients, radiologists, pathologists, oncologists, pediatric oncologists, clinical geneticists, and psychologists is recommended.

Besides the increased risk of second primary malignancies, our results indicate that the occurrence of a second primary malignancy increases the risk of a subsequent malignancy by 7-fold. Physicians should be aware of this increased risk, and close medical surveillance is required for all retinoblastoma patients, and especially for those who have developed a second malignancy.

Furthermore, radiation treatments should be avoided in retinoblastoma patients. Patients with hereditary retinoblastoma treated with radiotherapy have a greater risk of developing subsequent malignancies (located within as well as outside the field of radiation) and worse survival compared to patients with hereditary retinoblastoma treated with other modalities.

DNA analysis should be part of the diagnostic work-up, so that patients can be informed about the possible consequences of the hereditary nature of the disease. All patients with bilateral retinoblastoma are carriers of a mutation in the \textit{RB1} gene. However, some patients with unilateral retinoblastoma may also carry a mutation in the \textit{RB1} gene. Patients with a mutation in the \textit{RB1} gene have elevated risks of developing subsequent malignancies, but the underlying mechanism is unknown. We attempted to determine the relationship between specific \textit{RB1} germline mutations and the risk of second primary malignancies.
Although no statistically significant genotype-phenotype correlations emerged between hereditary retinoblastoma patients with a documented \textit{RB1} mutation and the development of a second malignancy, we observed a trend towards a greater risk for second primary malignancy among hereditary retinoblastoma patients with a nonsense or frameshift mutation compared to patients with other \textit{RB1} mutations. Larger studies are required to further investigate our findings. Confirmation of our results may lead to the identification of groups at high risk for second malignancies.

Finally, we found a statistically significantly increased risk of retinoblastoma in children conceived by IVF in the period 1995-2007. Caution and awareness while avoiding unnecessary worries on are mandatory at this stage of our research.

\section*{F. Future Research}

Long-term follow-up studies have revealed that patients with hereditary retinoblastoma are at increased risk of developing subsequent malignancies, even after more than 40 years of follow-up. Therefore, further research is needed to determine whether these risks remain elevated and whether these risks increase with the risks for other cancer types as the cohort ages. Even SIRs that are constant over time could indicate a drastically increasing AER because of the increasing incidence of cancer with age.

It would be interesting to investigate whether screening is effective in advancing the diagnosis of subsequent primary malignancies and whether it would ultimately reduce mortality from second malignancies.
Our findings of a trend towards higher risk for second malignancy among patients with hereditary retinoblastoma and a nonsense or frameshift mutation compared to other RB1 mutations should be evaluated in a larger international collaborative study. For example, data from other cohorts with complete follow-up and information on treatment, the presence of RB1 mutations, tumor laterality, and occurrence of subsequent malignancies could be used to further examine this issue, provided that the follow-up period is long enough.

The long-term effects of chemotherapeutic agents, like vincristine, etoposide, and carboplatin, warrant further research. However, follow-up of these patients is currently not long enough to enable the evaluation of the long-term adverse effects of these agents.

Our finding of a higher incidence of retinoblastoma among children conceived by IVF merits greater examination, and to explore a possible causal mechanism. This should be investigated in a larger cohort of children conceived by IVF for whom detailed information about IVF procedures, ovarian-stimulating drugs, and causes of subfertility are available.

Finally, we would like to emphasize the importance of linking patients in the Dutch retinoblastoma registry with population registries (like the GBA), the Netherlands Cancer Registry, and Statistics Netherlands for future follow-up. Unfortunately, the cause of death of some patients was unknown, and our request for individual linkage was rejected. However, coded data were made available from Statistics Netherlands, which enabled mortality analysis. Unfortunately, because only coded data were available, individual causes of death were not known, it is impossible to pool these data in an international collaborative study.

We would like to emphasize that the availability of original medical records is essential for studies on long-term adverse effects. According to the current privacy law WGBO, medical files should be maintained for up to 15 years. For the evaluation of the late effects of many medical treatments it would
be better if it were the mandatory storage duration should be much longer than
15 years and preferably more than 100 years.