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Genes and environment: effects on the development of second malignancies in retinoblastoma survivors

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Abstract

Although it is a rare cancer, retinoblastoma has served as an important model in our understanding of genetic cancer syndromes. All patients with a germinal *rb1* mutation possess a risk of the development of second malignancies. Approximately 40-50% of all retinoblastoma cases are considered germinal cases and recent work has indicated that nearly all retinoblastoma patients probably demonstrate a degree of mosaicism for the *rb1* mutation, and thus are at risk of secondary malignancies. The risk of the development of these cancers continues throughout the patients' lives due to the loss of a functional RB1 protein and its critical tumor suppressive function in all cells. These cancers can develop in diverse anatomic locations, including the skull and long bones, soft tissues, nasal cavity, skin, orbit, brain, breast and lung. Treatments used for retinoblastoma such as external-beam radiation and chemotherapy can have a significant impact on the risk for and pattern of development of these secondary cancers. Second malignancies are the leading cause of death in germinal retinoblastoma survivors in the USA and thus continue to be an important subject of study in this patient population. Second malignancies following the germinal form of retinoblastoma are the subject of this review.

Keywords

cancer risk; external-beam radiation; germinal hereditary; pinealoblastoma; radiation-induced neoplasm; retinoblastoma; sarcoma; second malignancy; Survivor

Epidemiology: incidence & survival

During the mid-20th Century, as treatment regimens for retinoblastoma improved, the number of survivors and their offspring began to increase. Treatment approaches for retinoblastoma in developed countries are now so effective that in the USA, Europe, and developed countries throughout the world, the leading cause of death among retinoblastoma survivors is not retinoblastoma itself, but second malignancies [1]. The incidence of second malignancies in survivors of retinoblastoma who carry the *rb1* mutation has been reviewed extensively [1- 13]. Cumulative incidence reports of second malignancies have varied, at least in part because of the wide range in design and population size of these reports. Most large studies with adequate long-term follow-up have reported incidence rates of approximately 0.5-1 % per year when a constant risk of cancer development per year of survival is calculated based on reported cumulative risk rates [1,3,7,10,14]. The study with

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the longest follow-up has reported a cumulative incidence rate for developing a new cancer at 50 years after diagnosis of 36% [14].

Epidemiologic evidence has indicated that the youngest patients who receive radiation early in life appear to be at highest risk of developing second malignancies. In a study of patients who developed retinoblastoma in the first month of life and received external-beam radiation treatment, more than 50% had developed second malignancies by the age of 24 years [12]. Furthermore, the cumulative incidence of second cancers decreased over the second half of the 20th Century since patients began receiving lower doses of radiation than they had in the past [14].

Survival from second malignancies in this population is relatively poor and does not appear to be improving over time. This may be due to the difficulty in screening patients for the wide range of malignancies they can develop and thus in the frequency of late diagnoses. Eng et al analyzed the long term follow-up study of patients from New York and Boston (USA) and found that the cumulative probability of death from second cancers was 26% at 40 years after bilateral retinoblastoma diagnosis [1]. Moll et al. reported similar findings from a large patient registry in The Netherlands with a cumulative probability of death among hereditary retinoblastoma patients of 18% at 35 years [13]. Deaths from second tumors are also more frequent among females for unclear reasons [1].

Risk factors for second cancer development

Several clinical and genetic risk factors and treatment-related exposures have been demonstrated to have an association with the development of second malignancies in retinoblastoma survivors (TABLE 1).

Germinal *rb1* mutation

Large cohort studies of retinoblastoma survivors have indicated that all patients with a history of retinoblastoma who develop second malignancies related to their retinoblastoma carry a germinal *rb1* mutation. *Rb1* is a tumor suppressor gene expressed in all adult tissues. The RB protein plays a vital role in controlling cell proliferation as evidenced by its frequently mutated state in human tumors of all types. Patients who carry the *rb1* mutation are at risk for long-term malignant transformation in nonocular tissues throughout their lives as each cell type initiates expression of the RB protein at a different time during development.

Approximately 85% of patients with a germinal *rb1* mutation (generally estimated to represent 40-50% of all retinoblastoma patients) will develop bilateral retinoblastoma, but approximately 15% will only develop unilateral disease and these patients are also at high risk for second cancers [1]. Mutation analysis studies over the past decade have indicated that mosaicism for the *rb1* mutation is more common than previously believed [15]. Most likely, there is a spectrum of risk for the development of second malignancies that nearly all retinoblastoma patients fall into. Where on this risk spectrum patients fall may determine not only whether they develop second malignancies, but also what type and in what anatomic location. By clinical observation, patients with unilateral retinoblastoma who should be

considered high risk for carrying the germinal mutation include: patients with a family history of retinoblastoma (including a family history of retinocytoma); patients diagnosed under the age of 6 months; and patients who present with multifocal disease. An excess of mortality from second cancers has also been found among unilateral retinoblastoma patients compared with the general population, implicating the germinal mutation in a small percentage of these patients as the cause [1,13].

Recently, several authors have argued that genomic instability, microsatellite instability, defects of the DNA mismatch repair system, and alterations in DNA methylation and acetylation/ deacetylation may be primarily responsible for the genesis of retinoblastoma, rather than mutations in the *rb1* gene [16, 17]. These authors argue that the epidemiologic patterns of unilateral versus bilateral and familial versus nonfamilial disease do not support the long-held concept that retinoblastoma is a genetic disease, but rather an epigenetic phenomenon. No clear explanation for how this theory would account for the development of secondary malignancies exists.

External-beam radiation

Impact on incidence

Numerous epidemiologic cohort studies of the use of diagnostic and therapeutic radiation in children with both benign conditions and cancer have established a risk for the late development of various forms of radiation-induced cancer including thyroid, breast, brain, skin and leukemia [11]. It has long been established that exposure to even low doses (up to 3 Gy) of ionizing radiation (such as from x-rays or atomic bomb exposure) can have a carcinogenic effect, although the details of the dose-effect relationship are controversial [18, 19].

The increased risk of second malignancies conferred by radiation in this patient population is well established in epidemiologic studies (FIGURE 1) [3,6,20]. Long-term follow-up indicates that the risk of a second malignancy in patients with the germinal mutation who underwent radiation is 3.1-fold higher than the risk of the germinal mutation alone [14]. Since survivors of germinal retinoblastoma have a strong predisposition to cell cycle dysregulation, they are at increased risk of second malignancies even in tissues that have received low doses of radiation (<0.4 Gy) [14]. For example, long-term follow-up indicates that there is an excess risk for cancer of the uterus, colon and bladder in germinal retinoblastoma survivors. Many of the uterine cancers in this population are leiomyosarcomas and are diagnosed in young patients (30-40 years of age) [14]. These are rare smooth muscle tumors that usually occur in older patients.

Impact of dosing

The linear dose-dependent relationship between the amount of radiation administered to patients and the development of second malignancies has been long established based on epidemiologic data [21]. Patients treated with higher doses and older methods of radiation delivery that lead to increased superficial skin and bone exposure are at higher risk for subsequent tumor development in the radiation field [5,14]. The odds ratio for risk of soft tissue sarcoma development after radiation with 0-4.9 Gy is 1.0, with 5-9.9 Gy is 1.6, with

10-29.9 Gy is 4.6, with 30-59.9 Gy is 6.4 and with greater than 60 Gy is 11.7 [6]. Currently, the dose prescribed to the retinal target volume ranges on average from 42-46 Gy.

Impact on tumor subtype & location

As tissues of the head and neck surrounding the radiation field receive the most radiation, these areas are at highest risk of demonstrating the mutagenic effects of high-dose incidental radiation exposure. Furthermore, these tissues are typically undergoing rapid normal growth and cell division during the time of radiation. As a result, there is a trend toward an increase in head and neck tumors and brain tumors in patients who have received radiation therapy compared with those who have not undergone radiation treatment [14,22]. These cancers and their associated standardized incidence ratio (SIR; calculated compared to the Connecticut Tumor Registry for the general population) are listed in FIGURE 2 [14]. Malignancies that are extraordinarily rare in the general population, such as cancer of the nasal cavities, pineoblastoma and bone tumors, have SIRs that are particularly high in this population.

A British study published several years ago offers unique insight into the types of malignancies that develop in patients not treated with external-beam radiation because this study group serves as a control group to examine the effect of a germinal *rb1* mutation without the effects of radiation therapy [10]. Patients in the study, who were all over 25 years of age at follow-up, had a much higher risk of developing epithelial cancers (notably lung, bladder and probably breast) than of developing sarcomas and other early-onset cancers compared with patients in the USA who received radiation. Given that alterations of the *RBI* protein are common in epithelial cancer specimens in the general population, the increased risk of epithelial cancers in this population is not surprising. Presumably the loss or inactivation of one copy of *rb1* bypasses one of the critical somatic events in carcinogenesis [10]. Patients treated with radiation seem to die of earlier onset tumors of other types (such as osteogenic sarcomas) before they, as a population, can effectively demonstrate the increased risk for epithelial cancers.

Impact on timing of second cancer development

In addition to impacting the types of malignancies that patients develop, radiation exposure appears to result in an earlier age of onset of second malignancies in retinoblastoma survivors [22,23]. Chauveinc et al. in France performed an extensive review of all published cases in order to examine the effect of radiation on the differences in the age at diagnosis of osteosarcomas. The authors reported that osteosarcomas arising within the field of radiation occurred at a mean of 1.2 years earlier than those cancers arising outside the radiation field. In addition, the latency period between radiation therapy and the onset of osteosarcoma was a mean of 1.3 years shorter for cancers arising inside the radiation field than those arising outside the radiation field. There appeared to be a bimodal distribution of osteosarcomas arising within the radiation field with a first mode at 5-7 years and a second mode at 12-14 years. The authors concluded that a radiation-induced mutation of the second *rb1* allele may account for the osteosarcomas occurring after a short latency period, whereas the later-onset sarcomas may be the result of multiple other downstream mutations.

Impact of age at radiation treatment

Epidemiologic evidence indicates that very young patients appear to be at highest risk for the development of radiation related second cancers. Our group published a retrospective study of 816 patients in the USA with bilateral retinoblastoma in 1998 demonstrating that patients who underwent external-beam radiation during the first year of life were approximately twice as likely to develop second cancers than those radiated after the age of 1 year [2]. Patients who underwent external-beam radiation after 1 year of age appeared not to be at a higher risk for developing second malignancies than patients who had a germinal mutation of the retinoblastoma gene and were never irradiated.

A subsequent retrospective study of a similar large population of both unilateral and bilateral hereditary retinoblastoma patients performed in The Netherlands confirmed an increased risk of development of second malignancies in patients treated with external-beam radiation before the age of 12 months [3]. However, the major discrepancy in the findings of this study compared with that of our group was that The Netherlands cohort developed similar numbers of second malignancies inside and outside the field of radiation. This finding suggested that radiation was not the cause of the increased risk before one year of age. Sensitivity analysis demonstrated that the results depended on how second malignancies, the irradiation field and pineoblastomas were defined.

A retrospective study was recently completed at the National Cancer Institute of 322 patients with hereditary retinoblastoma, none of whom received chemotherapy and all of whom received 'modern' doses of radiation. The study found that patients irradiated before the age of 1 year had a two fold increased risk of second cancer development [24]. Patients undergoing radiation after age 1 year had no increased risk of second cancer development compared with patients treated with surgery only (FIGURE 3). Furthermore, a recent unpublished analysis of our data from the National Cancer Institute indicates that among irradiated patients with the germinal mutation, those who were diagnosed and treated at less than 12 months of age were not only at greater risk for developing a second cancer, but also 2.2-times more likely to die of these cancers compared with those diagnosed and irradiated at an older age [CHU-LING YU. PERS. COMM.]

Chemotherapy

Recently, ophthalmic oncologists have begun exploring the possibility that the administration of chemotherapy may also contribute to the development of second cancers in this population. This theory has been explored based on two developments; first, the recent observation of several cases of leukemia in retinoblastoma survivors characterized by a typical chromosomal rearrangement characteristic of chemotherapeutic exposure, and second, a well-established phenomenon of hematologic malignancies in other patient populations induced by the chemotherapeutic agents currently being used to treat retinoblastoma patients. High doses of platinum-based drugs are well-documented to raise the risk of secondary leukemias in survivors of both childhood and adult cancers [25-27]. Etoposide-associated secondary acute myelogenous leukemia (AML) has been reported in survivors of a wide variety of childhood cancers including acute lymphoblastic leukemia, non-Hodgkin's lymphoma and Wilm's tumor [28-31]. The dose-response

relationship for these secondary cancers is unclear, but may be related to the schedule of drug delivery [32]. Secondary AML has been reported after small 'safe' doses of etoposide [33].

A recently published global survey of all previously reported and nonreported cases of AML in retinoblastoma survivors indicated that there have been 15 cases, 13 of whom occurred in children and 12 of whom received chemotherapy [34]. The patients in the review received varying multi-agent chemotherapy regimens. In total, eight of the patients received epipodophyllotoxins and five received doxorubicin or idarubicin. Nine of the patients had an M2 or M5 French-American-British (FAB) subtype, which are most often associated with chemotherapy-induced malignancies. Ten of the patients died of AML. Another case of secondary AML was also recently reported in a patient who received a regimen that included oral etoposide [35].

The overall incidence of secondary AML in this population appears low given the large cohort of retinoblastoma patients who have received etoposide worldwide over the last 10 years. The number of patients at risk is probably increasing since the continued upsurge in the use of chemotherapy for intraocular disease since the mid-1990s. The latency period of epipodophyllotoxin-induced malignancies in other patient populations is typically relatively short (6 years or less) and, therefore, a short follow-up interval does not explain the low incidence. Of note, the incidence of secondary AML in the oldest cohort of patients followed continuously in the USA (since 1914 in New York, USA), who were historically treated with surgery and radiation, is nearly zero. This observation suggests that although the current incidence of secondary AML in the chemotherapy era is low, it has increased as a result of this treatment approach. Only time and international collaboration will resolve this clinical question.

Growth hormone

Exogenous growth hormone (GH), a treatment often administered to pediatric oncology patients, may have a potentially oncogenic effect that could theoretically stimulate second cancer development. Childhood cancer survivors as a group have been demonstrated to have an elevated risk of second malignancies compared with patients who did not receive GH [36]. Our group reported the case of a patient with a history of bilateral retinoblastoma who received GH due to panhypopituitarism and developed an osteogenic sarcoma of the extremity [37]. The evidence supporting this theory is controversial. No large studies have yet been completed examining the risk of second cancer development in retinoblastoma survivors who received GH. Laboratory evidence in animals, however, supports a mutagenic function of GH on osteoblastic cells of rats [38].

Locations & subtypes

Second cancers

The most common second malignancies seen in the USA in retinoblastoma survivors are tumors of the skull and long bones, soft tissue sarcomas, tumors of the nasal cavity, cutaneous melanoma, bony tumors of the orbit, brain tumors including trilateral

retinoblastoma, breast cancer, and oral cavity cancers [6, 14]. As noted earlier, a large British cohort of adult retinoblastoma survivors, most of whom did not receive radiation, indicates that epithelial cancers are more common in older, non irradiated patients [10].

It is notable that other inherited cancer syndromes seem to follow a similar pattern in that the anatomic/histologic distribution of malignancy development is not entirely obvious. Li-Fraumeni syndrome, a rare but instructive condition in which patients inherit only one functional p53 allele, also results in the predisposition to multiple cancers over patients' lifetimes. The function of the TP53 gene is similar to the *rb1* gene, which regulates normal growth of specific organs. Similar to retinoblastoma patients with a germinal *rb1* mutation, Li-Fraumeni patients are at risk for the development of a very specific set of cancers (e.g., osteosarcomas, soft tissue sarcomas, certain breast cancers, certain brain tumors, adrenocortical carcinoma and leukemia) [39]. Interestingly, these tumors are not simply those which commonly have somatic mutations of p53. Likewise, somatic mutations of *rb1* have been identified in many forms of cancer, many of which do not occur excessively in patients with a germinal *rb1* mutation.

Particular cancers of interest

Sarcomas

After bony tumors, soft tissue sarcomas are the most commonly observed second malignancies in this population. These tumors are an interesting example of the tissue cell specificity of the germinal *rb1* mutation as they are rare in the general population. In particular, the histopathologic types observed in these patients are rare in the general population.

A long-term follow-up study examining the development of sarcomas in this population was recently completed [40]. The most frequent histologic subtypes were leiomyosarcoma, fibrosarcoma, malignant fibrous histiocytoma and rhabdomyosarcoma. The cumulative risk for any soft tissue sarcoma 50 years after external-beam radiation for retinoblastoma was 13%.

Lung cancer

Several reports from large cohorts of retinoblastoma survivors have indicated that germline *rb1* mutations confer an increased risk of lung cancer [41-43]. Despite this evidence, a recent report of a telephone survey of a large cohort of retinoblastoma survivors has indicated that 16.8% of hereditary retinoblastoma survivors currently smoke cigarettes and another 17.5% are former smokers [44]. Hereditary retinoblastoma survivors smoke significantly less frequently than nonhereditary retinoblastoma survivors and less than the US population in general, but smoke at comparable rates to other childhood cancers survivors [44]. All childhood cancer survivors have been identified as a vulnerable group with unique medical needs that are not being well met; smoking prevention and cessation counseling in the context of their disease is one of these needs [44].

Trilateral retinoblastoma

Trilateral retinoblastoma is a term that refers to bilateral retinoblastoma associated with an intracranial primitive neuroectodermal tumor in the pineal or suprasellar region of the brain. We and others have observed several rare cases in which these tumors present prior to the presentation of the ocular disease and for this reason, there has been some controversy as to whether to these tumors should be considered second malignancies or a variant of the primary tumor [3]. There are several clinical characteristics that seem anecdotally to be more common in patients with these tumors, representing markers for particularly devastating *rb1* mutations that may be more likely to confer a risk for the development of the second malignancy. These clinical risk factors include bilateral disease, a family history of the disease and diagnosis within the first 6 months of life. It is also common for these patients to have received external-beam radiation at a young age, implying that even the low dose (3-4 Gy) of radiation that reaches the pineal gland during radiation treatment to the eyes may contribute to the development of these cancers. Trilateral retinoblastoma represents the most common cause of death among retinoblastoma survivors aged 5-10 years (10%) [45]. Historically, the survival of these patients has been poor, with the largest review of patients with these tumors reporting that the longest survival in such a patient was 96 months with a median survival in all patients of 6 months (46). Souweidane et al. have reported extended survival in patients treated with new surgical approaches, chemotherapy and in some cases radiation (47). In these cases, the key novel intervention appears to be the institution of 'second-look surgery; in which the patient undergoes an initial subtotal tumor resection followed by a second procedure to complete the resection after chemotherapy and radiation have been completed (FIGURE 4).

There is controversy among clinical centers as to whether the incidence of trilateral retinoblastoma has decreased in recent years. Shields et al. retrospectively compared 142 patients treated with chemoreduction and 72 patients treated with nonchemoreduction methods from 1995 to 1999 [48]. One out of 18 (5.5%) patients at risk for developing trilateral retinoblastoma in the group that did not undergo chemotherapy subsequently developed a pinealoblastoma, a percentage consistent with a previously published series (45). None of the 99 patients at risk in the chemotherapy group developed trilateral retinoblastoma. The authors concluded that chemotherapy may play a role in preventing pineo blastoma. Moll et al. have hypothesized that the absence of external-beam radiation treatment rather than the addition of chemotherapy may explain the decreased incidence of pinealoblastoma development in the treated group of this study (49).

Benign second tumors

Li et al reported in 1997 that in a survey of 898 retinoblastoma survivors, there was an excess of lipomas among patients with hereditary disease (4). In addition, a greater than expected percentage of patients with hereditary retinoblastoma who had lipomas developed second malignancies, compared with patients with hereditary retinoblastoma without lipomas. The authors concluded that certain *rb1* germline mutations might increase the risk of both lipomas and second malignancies and that lipomas can serve as a marker for an elevated second cancer risk. A later analysis of the same cohort suggested that the presence

of lipomas was not associated with the diagnosis of a malignant sarcoma [40]. In response to these data, Genuardi et al reported a mutation analysis of a two-generation pedigree with hereditary retinoblastoma and lipomatosis and found that there was no evidence for linkage of a polymorphic focus on chromosome 13q14. The authors hypothesized that an intragenic or extragenic modifying factor was instead responsible for the lipomatosis [50].

Third, fourth & fifth cancer development

Our group has reported on the incidence and subtypes of third, fourth, and fifth malignancies in germinal retinoblastoma patients who survive a second malignancy [5]. These patients have an incidence rate of new malignancies of approximately 2% per year from the time of second tumor diagnosis, twice or more than the rate of second cancer development in this population. The average latency period between subsequent tumor diagnoses becomes progressively shorter with each additional cancer that develops. For unclear reasons, females have a higher overall mortality rate from second tumors than males, so males have a higher incidence rate of third tumors [1,5]. The distribution of tumor sites in the patients who have had second malignancies suggests a predictable pattern of third, fourth and fifth tumor development. Of patients with skin cancers as their second tumors, skin cancers also represent most of the third, fourth and fifth tumors that develop in this group. Of patients treated for a second tumor in the skull in whom a third tumor develops, most are diagnosed with a soft tissue sarcoma in the head as the third tumor.

Prevention

Although the prevention of all second malignancies in this population is probably not possible, there are several practical strategies that should be stressed with patients. Retinoblastoma survivors should be aggressively counseled to refrain from smoking (see lung cancer section). There is also sufficient evidence to support the avoidance of unnecessary exposure to ionizing radiation including x-rays, CT scans and UV radiation. Regarding UV radiation exposure, the increased incidence in this population of cutaneous melanoma is sufficient to support this recommendation although the exact dose-response relationship of UV light exposure to cancer risk in this population is unknown. An increased lifetime cancer mortality risk attributable to radiation exposure from CT scans in children has been estimated [5, 1,52]. Given that children are more sensitive to the carcinogenic effects of radiation and given that retinoblastoma patients with the germinal *rb1* mutation are at particularly elevated risk of developing dose-dependent radiation-induced cancers, these patients should avoid unnecessary radiation from any source. Some radiologists are currently recommending the complete avoidance of studies that involve exposure to ionizing radiation in all patient populations at risk for second cancers (e.g., Li-Fraumeni patients) [53].

Screening

Limited study has been performed on screening programs and no universal protocol has been adopted for the routine screening of germinal retinoblastoma survivors for second cancers. Future imaging protocols will probably utilize MRI as the primary imaging modality given the increased risk for second malignancies with repeated exposure to

ionizing radiation. Only one prospective study has been completed on this subject. In a study of 83 patients with heritable retinoblastoma, who underwent a CT scan of the brain and orbits at the time of diagnosis, followed by an MRI of the head every 6 months, Duncan et al reported no improvement in the outcome of patients who developed a pinealoblastoma [54].

Expert commentary

Thanks to our improved understanding of the relationship between external-beam radiation and the risk of second malignancy development in the germinal retinoblastoma survivor population, the incidence of second malignancies appears to be decreasing over time. However, many challenges remain in the detection, management and prevention of the second malignancies that do occur. It is critical that the families of these patients are counseled extensively about the signs and symptoms of second malignancies at the time of retinoexternal blastoma diagnosis in order to improve the chance that a second cancer is detected in its early stages [55]. Regarding early detection, a standardized screening protocol must be developed and ophthalmic oncologists must ensure that patients adhere to the protocol. It is also important that the screening protocol continue to occur as the patients transition from being cared for by pediatric subspecialists to adult oncologists or internists.

The challenges inherent in this transition have been well-documented in the pediatric oncology literature [56], and the ophthalmologist is in a unique position to help. Multicenter clinical trials examining potential screening protocols should be undertaken with these challenges in mind.

Regarding the management of second malignancies once they do occur, little progress has been made. One area of promising clinical research is the improved survival seen in patients treated with pineoblastoma (see Trilateral retinoblastoma section). This unique integrative multimodality approach to therapy should be examined on a multi-institutional scale.

Finally, more research is needed on the effect of chemotherapy on the development of second malignancies in this population.

There is some suggestive evidence that certain chemotherapeutic agents (e.g., etoposide) may be contributing to an increase in secondary leukemia, but further collaborative effort is necessary. A thorough study would require a survey of all patients receiving these drugs with long-term follow-up from several centers in order to determine an incidence rate. Many of the cases reported thus far seem clustered in South America for unclear reasons, which must also be explored [34].

Five-year view

Major advances in the care for retinoblastoma survivors in the near future will include adjustments in treatment protocols for patients with the germinal mutation in order to decrease their risk for second cancer development. Specifically, it is necessary to minimize the use of etoposide and other chemotherapeutic agents that can contribute to second malignancies; adopt protocols in order to delay the use of external-beam radiation in advanced cases until after patients reach 1 year of age; ensure that patients do not receive

CT scans or other forms of ionizing radiation. Many centers around the world have already begun adopting these modern approaches, but their adaptation must be standardized.

Studies addressing risk factors, screening protocols and prevention of second malignancies would be less costly and more likely to yield useful results if genetic testing for *rb1* mutations was more economical and available to patients and clinicians. At the present time, a single genetic test is unlikely to detect all germline RB gene mutations in patients with retinoblastoma owing to the variety of types and locations of mutations that occur. Most mutations detected in peripheral blood DNA from patients with hereditary retinoblastoma are single-base substitutions and small-length mutations and, in some patients, no mutation is identified [57].

Genetic techniques that are used to detect germline mutations in the *rb1* gene have been steadily improving over the past decade, but remain expensive and time-consuming in many centers. Nevertheless, techniques such as single-strand conformation polymorphism analysis (SSCP), heteroduplex analysis, multiplex fragment length analysis, alteration of restriction fragment length polymorphisms and direct DNA sequencing, are improving and becoming more available to families [58-60]. FISH has provided additional information about the molecular structure of the retinoblastoma locus, including the identification of translocations and inversions undetectable using other methods [61,62]. Newer techniques, such as protein truncation testing (PTT), are able to detect a broad range of common *rb1* mutation types, including small frame shift mutations, splice site alterations and nonsense mutations, reducing costs and wait-time for families [63].

Some centers have devised economical ways to routinely offer genetic screening to all retinoblastoma patients. These programs utilize multiplex ligation-dependent probe amplification (MLPA) to detect large deletions or duplications, micro satellite analysis to detect loss of heterozygosity (LOH), and denaturing high-performance liquid chromatography (D-HPLC) analysis to detect point mutations and small insertions or deletions, and quantitative multiplex PCR of short fluorescent fragments (QMPSF) [64,65]. Some authors have reported that molecular analysis will help to reduce healthcare costs and have thus advocated for mutation analysis as a standard part of the management of patients [57,66].

We expect more clinical centers to adapt standardized molecular analysis protocols over the next 5 years, enabling patients and their family members to undergo the analyses in a faster and more economical manner. Furthermore, patients germline *rb1* mutations that have been identified can be entered in clinical trials, examining screening protocols and prevention efforts as well as the effects of various treatments, such as chemotherapy, on the development of second malignancies.

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Key Issues

- Most large studies with adequate long-term follow-up have reported yearly incidence rates of second cancers in germinal retinoblastoma survivors of 0.5-1 % per year.
- Long-term follow-up indicates that the risk of a second malignancy in patients with the germinal mutation who underwent radiation is 3.1-fold higher than the risk of the germinal mutation alone.
- Patients who undergo external-beam radiation before 1 year of age are at a higher risk for developing second malignancies and dying from these cancers than patients who are radiated after 1 year of age.
- Recent global collaborative data suggest that retinoblastoma survivors may be at risk for etoposide-induced secondary acute myelogenous leukemia (AML), but more studies are needed.
- Recent modern surgical approaches combined with chemotherapy and radiation have resulted in some cases of extended survival in the previously fatal second malignancy pineoblastoma.
- Despite evidence that germinal retinoblastoma survivors are at elevated risk for lung cancer, 17% of hereditary retinoblastoma survivors currently smoke cigarettes and another 18% are former smokers.
- A standardized effective screening protocol for second malignancies integrating imaging studies, laboratory work, and clinical visits for retinoblastoma patients must be established.

Figure 1. Risk of second malignancy development in radiated versus nonradiated patients
(A) Locations and types of second malignancies developing *over* time in patients who never received external-beam radiation. **(B)** Locations and types of second malignancies developing *over* time in patients who did receive external beam radiation. Data from large studies with adequate follow-up [1,3,7,10]. Note how dramatically radiation treatment increases the risk for second malignancies. Data from large studies with adequate follow-up [1,3,7,10].

Figure 2. Standardized incidence ratio of second malignancies in hereditary retinoblastoma survivors treated with and without radiation

The cancer sites with the highest SIRs reflect cancers that are more rare in the normal population and particularly common in this cohort. The standardized incidence ratio for cancers of the nasal cavities in patients who underwent radiation is 1364 (above the graph scale). Data from Kleinerman *et al.* [14].

Figure 3. Risk of a second malignancy among germinal retinoblastoma survivors by age at retinoblastoma diagnosis

Data from Kleinerman *et al.* [24].

Figure 4. Sagittal MRI scan of a patient with a history of bilateral retinoblastoma demonstrating large pineal region tumor

The patient was treated with an endoscopic third ventriculostomy for obstructive hydrocephalus, tumor biopsy and subtotal tumor removal, followed by chemotherapy and radiation treatment and 'second look' surgery for total tumor removal. The patient is now disease-free at 42 months of follow-up. With permission from Souweidane.

Table 1

Association with risk of second cancer development in germinal retinoblastoma survivors.

Factor	Strength of association with incidence of second cancers	Ref.
Presence of germinal mutation inl gene	Definite causation (necessary risk factor)	[1]
Dose of external-beam radiation	Dose-dependent causation	[6]
External-beam radiation given at < 1 year of age	Controversial, but now shown to be associated in several studies	[2,3,24]
Presence of lipomas	Definite association	[4]
Smoking	Definite association	[43]
Chemotherapy	Possible association	[34]
Sun exposure	Possible association	[68]
Growth hormone	Possible association	[36]

Adapted with permission from Scheffler *et al*, [67],