MANUSCRIPT OF THE THESIS

On the road to optimizing long-term survival

Subsequent neoplasms in childhood cancer survivors across five decades

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12th July, 2024

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Chapter 1

Introduction and outline of the thesis

Introduction

Every year about 600 children under the age of 18 year are diagnosed with cancer in the Netherlands[1]. Fortunately, survival rates have improved over the past decades due to advances in treatments. The current overall survival in high-income countries is approximately 80%[1-3], although there is still quite some variation between different childhood cancer types[1]. However, only focusing on survival overlooks the complete impact of cancer and its treatments, because childhood cancer survivors (CCS) are experiencing excess morbidity and mortality compared to the general population[4]. Therefore, especially with a growing population of CCS, evaluation of long-term health is becoming increasingly important[5-9].

It is estimated that around 60 to 75% of the long-term survivors experience at least one chronic medical issue, with around 30% facing even two or more[10]. Additionally, 30-40% encounter severe, life-threatening, or disabling adverse effects[10]. Patients undergoing more intensive treatments such as intensive chemotherapy, radiotherapy and stem cell treatment are especially at high risk of experiencing adverse late effects[9,11]. Late effects include a variety of health outcomes including psychosocial, neurocognitive, cardiovascular, endocrine and subsequent neoplasms[11]. The latter attributes to a high percentage of late mortality[4].

It is important to enhance our understanding of which survivors have an increased risk of developing subsequent neoplasms. This knowledge is important for refining follow-up care guidelines for survivors. Additionally, a better understanding of the risk for developing subsequent neoplasms is important to inform future treatment protocols for newly diagnosed children with cancer. The aim is to cure every child with cancer with optimal quality of live.

Subsequent malignant neoplasms

Subsequent malignant neoplasms (SMNs) are defined as new primary malignancies after an initial cancer diagnosis, excluding a recurrence or metastasis of the initial cancer. SMNs are a leading cause of excess late mortality in CCS[4,12]. Furthermore, survivors who develop an SMN

are more prone to experiencing adverse general and mental health outcomes[13] and have an elevated rate of hospitalizations compared to survivors without an SMN [14]. There are several factors that can contribute to SMN risk. Prior cancer treatment is an important risk factor for SMNs (see also paragraph "treatment factors"), but also genetic predisposition might play an role[15] as well as general cancer risk factors, such as age, sex, lifestyle. Furthermore, the risk also depends on the primary cancer type[15]. In general, results have shown that 25 years after the diagnosis of childhood cancer 3.9% developed an SMN, which was 5 times higher compared to the expected incidence in the general population[8]. Even beyond 25 years, the risk of SMNs is still increased[5,7]. The most frequently observed SMNs are non-melanoma skin cancer[5], breast cancer[5,8], thyroid[5,8], soft tissue sarcoma[8] and CNS malignancies[5,8].

Subsequent non-malignant neoplasms

CCS may also develop subsequent non-malignant neoplasms (SNMNs) and although most SNMNs are usually not life threatening, information on their risks and risk factors can still be very valuable. SNMNs may share etiological factors and clinical manifestation with SMNs that could affect quality of life or their life expectancy[16]. Moreover, SNMNs may be cancer precursors offering potential opportunities for early detection of precancerous growth[17]. A previous study has shown that survivors have a 2 times higher developing a solid benign tumors in comparison with siblings of survivors[18]. Moreover, benign meningiomas can cause serious neurological morbidity and to mortality[16]

Treatment-related risk factors for subsequent neoplasms

Research conducted among CCS have revealed a range of associations between various treatments and the development of specific subsequent neoplasms[15]. For example, clear dose-effect relationships have been observed for radiotherapy, correlating with increased risks of breast cancer [19,20], thyroid cancer[21], colorectal cancer[22], sarcomas[23-25], central nervous system tumors[26] and basal cell carcinoma[27]. With respect to chemotherapy groups, epipodophyllotoxins and alkylating agents have been found to increase the risk of acute myeloid leukemia[28] and alkylating agents have been associated with the development of many different types of solid tumors, such as bone tumors[25,29] and colorectal cancer[22].Also specific

chemotherapeutic agents have been associated with certain subsequent neoplasm types. For example, doxorubicin was found to be associated an increased risk of female breast cancer[30], and cyclophosphamide with an increased risk of bladder cancer[31] and sarcoma[8].

Temporal trends of subsequent neoplasms

Treatment protocols have been changed over time with the aim to improve both survival and long-term health outcomes. It is likely that patterns of subsequent neoplasm risks have changed over time due to alterations in childhood cancer treatment protocols, including more complex chemotherapy and less intense radiotherapy exposures (smaller fields, lower doses). Different reports showed mixed results of the temporal trends of subsequent neoplasm risk[32,33]. In the Childhood Cancer Survivors Study (CCSS) cohort from The United States and Canada a lower risk was found for survivors diagnosed in 1990s compared to survivors diagnosed in the 1970s[33]. In the DCCSS-LATER study, there were no significant differences in SMN risk between the different diagnosis periods[8]. The association between the evolution in delivered therapies with specific outcomes, including subsequent neoplasms have not yet been investigated in Europe and ongoing follow-up of survivors from the latest treatment decade is needed to determine changes in risk over time.

Risk of subsequent neoplasms in specific childhood cancer groups

The risk of subsequent neoplasms can vary among different childhood cancer groups[5]. These variation may arise from differences in treatment protocols as well as differences in genetic predisposition to neoplasm development. Narrowing the focus to a particular childhood cancer group can enhance the effectiveness of outcomes and facilitate the formulation of targeted recommendations for treatment and follow-up care plans. Moreover, in certain childhood cancer subgroups major changes of treatment modalities took place within the past five decades, allowing for a more specific analysis on possible changes in (treatment-related) risks of the development subsequent neoplasms within those subgroups. For instance, since the 1990 treatment stratification is done risk-group for neuroblastoma patients[34]. This is accompanied by an intensified treatment for the high-risk patients including treatment with Iodine-metaiodobenzylguanidine (^{131}IMIBG). Another significant adjustment is seen in

treatment for acute lymphoblastic leukemia patients, where cranial radiotherapy was substituted by intrathecal high-dose methotrexate in December 1984[35,36].

Neuroblastoma

Neuroblastoma is the most common extracranial solid tumor of childhood[37] with 30-40 new cases diagnosed in the Netherlands each year[1]. It is a diverse tumor type with variation in location, histopathology, biology and overall outcome[38]. Thanks to advances in treatment, the survival rates have improved over the past decades with a current five-year survival rate of 95% for low- and intermediate-risk patients and 50% for high-risk patients[39].

An important aspect of the current treatment for neuroblastoma survivors involves ^{131}I-MIBG treatment. However, ^{131}I-MIBG is indicated to have the potential to damage the thyroid gland[40], and may also be implicated in the development of thyroid carcinoma[41]. While several studies observed an elevated risk of SMNs in neuroblastoma survivors[5,7,42-47], there is only little evidence regarding potential risk factors and the risk of SNMNs.

Acute lymphoblastic leukemia

Acute lymphoblastic leukemia (ALL) is the most common type of childhood cancer[48-50] with a five-year survival rate that is currently exceeding 90%[51-53]. However, compared to the general population, childhood ALL survivors have a 2.6 to 13.5 times higher risk to develop subsequent malignant neoplasms (SMNs)[54-57]. In addition to SMNs, some types of subsequent non-malignant neoplasms (SNMNs) can also cause serious morbidity, e.g. subsequent meningiomas [54,58].

The risk of developing a subsequent neoplasm was found to be higher in patients who were treated with radiotherapy[59], especially cranial radiotherapy [55,60,61]. Furthermore, patients who received hematopoietic stem cell transplantation (HSCT) also showed an increased risk of subsequent neoplasms as compared to non-transplanted leukemia survivors[62-65], which is

often suggested to be due to total body irradiation (TBI)[63,66-68]. However, the separate impact of HSCT and TBI are not fully clear.

The Dutch Childhood Cancer Survivor Study-LATER cohort

Understanding the risks of and the risk factors for subsequent neoplasms among CCS is crucial to better predict which survivors are at higher risk. Achieving this requires high-quality data on both childhood cancer diagnosis and treatment factors and subsequent neoplasm outcomes, long-term follow-up, and large samples sizes. In this thesis, we utilized data from the Dutch Childhood Cancer Survivor Study (DCCSS)-LATER cohort.

The DCCSS-LATER cohort consists of 5-year childhood cancer survivors, diagnosed before the age of 18, in one of the seven original pediatric oncologic/hematopoietic stem cell centers in the Netherlands[69]. The childhood cancer diagnoses that are included in the DCCSS-LATER cohort are malignancies covered by the International Classification of Childhood Cancer, third edition (ICD-O-3) and some additional neoplasms. This includes low-grade brain tumors and systemic multifocal or polyostotic Langerhans cell histiocytosis, as these patients were often treated in pediatric oncology wards in the Netherlands following protocols that include some of the same medications and/or radiotherapy regimens used for treating malignant conditions. The original cohort includes patients diagnosed between 1963-2001, with a total of 6,165 childhood cancer survivors. Recently, the cohort has been expanded and also includes patients diagnosed between 2002-2014, increasing to a total of over 12,000 survivors. Data on childhood cancer diagnosis and treatment for primary tumor and all recurrences were abstracted and entered into the LATER registry by trained local data managers. The available treatment data includes information on chemotherapy agents and given cumulative doses, on radiotherapy type, fields and prescribed doses, but also on other treatments like hematopoietic allogenic stem cell transplantation (HSCT).

The original DCCSS-LATER cohort (1963-2001) already resulted in two major studies: The DCCSS-LATER 1 and DCCSS-LATER 2 study. In the LATER 1 study, survivors received

questionnaires to collect data on health outcomes and lifestyle[69]. Furthermore, linkages with nationwide registries such as the Netherlands Cancer Registry (NCR)[70] and the Dutch Nationwide Pathology Databank (Palga)[71] were done to collect data on subsequent malignant and non-malignant neoplasms[8]. In the LATER 2 study, additional clinical and physical outcomes data for research were collected during a clinic visit and bio-materials such as blood or saliva sample were stored[72].

Outline of the thesis

It is essential to gain new knowledge into the risk and risk factors for subsequent neoplasms among childhood cancer survivors to better predict which survivors are at highest risk. This knowledge can inform surveillance recommendations for childhood cancer survivors and contribute to the development of treatment protocols for newly diagnosed childhood cancer patients. In this thesis, our objectives are to (1) examine temporal changes in subsequent neoplasms among childhood cancer survivors diagnosed between 1963 and 2014 and to relate these to changes in the treatment intensity **(Chapter 2)** and (2) to determine the incidence of and risk factors for subsequent neoplasms within specific childhood cancer groups **(Chapter 3, 4 & 5).**

In **Chapter 2,** we analyze the risk of subsequent neoplasms in the total Dutch Childhood Cancer Survivor Study (DCCSS)-LATER cohort, comprising over more than 10,000 childhood cancer survivors diagnosed between 1963 and 2014 and with a follow-up of over 40 years. We evaluate temporal trends of subsequent malignant neoplasms by calculating incidence rates and compare it to expected rates based on age-, sex-, and calendar period–specific cancer incidence rates in the Dutch population for different treatment eras. Furthermore, we relate these changes to changes in treatment over the past few decades.

In Chapter 3 , we systematically review and summarize the existing scientific literature on the risk of developing subsequent neoplasms in neuroblastoma survivors and associated risk factors. This knowledge is important to get insight into the survivors who are at highest risk and to address gaps in knowledge on this topic.

In **Chapter 4** we analyze the long-term risk of subsequent neoplasms among 563 5-year neuroblastoma survivors within the DCCSS-LATER cohort. In this study, we specifically focus on gaps that became apparent in our systematic review. We describe the risks of subsequent malignant and non-malignant neoplasms and compare this to the general population. Additionally, we specifically analyze treatment-related risk factors, including ^{131}I-MIBG treatment.

Chapter 5 addresses the long-term risk of subsequent neoplasms in 5-year survivors of childhood acute lymphoblastic leukemia in the DCCSS-LATER cohort. We examine the risk of and risk factors for developing subsequent malignant and non-malignant neoplasms with a median follow-up duration of over 30 years. We estimate cumulative incidences, and also stratify by treatment area. In addition, we evaluate risks attributed to different treatment variables in multivariable analyses.

Chapter 6 is a summary of our most important findings and interpretation of those findings in the context of existing literature. Furthermore, strengths and limitations are described and we discuss the clinical implications of our findings. Moreover, as we contemplate the future, we identify the gaps in our knowledge and outline the subsequent steps to move forward.

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Chapter 2

Temporal trends of Subsequent Malignant Neoplasms in Childhood Cancer Survivors and the Impact of Treatment Changes: A DCCSS-LATER 3 Study

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Manuscript in preparation

Abstract

Background The growing population of childhood cancer survivors faces an elevated risk of developing subsequent malignant neoplasms (SMNs), contributing to excess mortality. Over the past decades treatment has been modified to decrease this risk. It is essential to evaluate whether these changes have effectively reduced the risk of SMNs over time.

Methods We assessed the risk of SMNs, temporal trends, and the impact of treatment among 11,548 survivors from the Dutch Childhood Cancer Survivor Study (DCCSS)-LATER cohort (1963-2014). We calculated standardized incidence ratios (SIRs) and cumulative incidences. Multivariable Cox proportional hazard regression was used to estimate SMN risks, and mediation analysis to evaluate effects of treatment changes over time.

Results After a median follow-up of 21.2 years, 550 survivors developed an SMN (SIR: 3.5, 95%CI:3.2-3.8), with a 25-year cumulative incidence of 3.6% (95%CI:3.2-4.0) for any SMN. Overall, the risk for SMN declined over time (p-trend 0.04). Mediation analysis indicated that the decline in SMN risk was primarily associated with a decrease use of radiotherapy. Chemotherapy seemed to have the opposite effect, mainly due to the use of anthracyclines and/or epipodophyllotoxins. Survivors treated with anthracyclines (HR:1.3, 95%CI: 1.0-1.6), epipodophyllotoxins (HR:1.3, 95%CI: 1.0-1.7) and radiotherapy (HR:2.3, 95%CI: 1.9-2.8) had a significant increased risk of SMN.

Conclusion Reducing radiotherapy has lowered the SMN risk over time, but increased use of chemotherapy, especially anthracyclines and epipodophyllotoxins, counteracted this decline. Furthermore, survivors treated with radiotherapy, anthracyclines or epipodophyllotoxins have an elevated risk of developing any SMN. This highlights the need to reassess chemotherapy protocols for childhood cancer patients and to identify survivors at risk.

Introduction

Advances in treatments resulted in improved survival rates for childhood cancer, making it increasingly important to evaluate long-term health outcomes. Among childhood cancer survivors (CCS), the development of subsequent malignant neoplasms (SMNs) stands out as one of the most adverse long-term health outcome, significantly contributing to excess late mortality in CCS[1,2].

Several treatment factors have been established as risk factors for developing SMNs[3-5]. For radiotherapy, clear dose-effect relationships have been observed for various solid tumors[6-13]. For chemotherapy, epipodophyllotoxins, anthracyclines, and alkylating agents have been found to increase the risk of acute myeloid leukemia[14,15]. Anthracyclines and alkylating agents have also been associated with various solid tumors[9,12,16,17]. Also, specific chemotherapeutic agents have been associated with certain subsequent neoplasm. Recently, we showed that doxorubicin is associated with a dose-dependent increase in the risk of breast cancer[4].

Treatment protocols for childhood cancer have evolved over time, incorporating more complex chemotherapy regimens and reducing the use of radiotherapy. Currently, a report from the Childhood Cancer Survivor Study (CCSS) in the United States and Canada is the only study showing a lower SMN risk for survivors over time. This lower risk was associated with a reduction in therapeutic radiation dose yet no role of changes in chemotherapy were evident[18]. Understanding the role of chemotherapy and the risk of SMN over time is important because, while the use of radiotherapy has been minimized, chemotherapy have been intensified for various cancer types[19] to maintain cure rates. Therefore, we aimed to evaluate the temporal trends in the risk of developing SMNs among five-year CCS, and relate these to shifts in radiotherapy and chemotherapy treatments over the past five decades. In addition, we examined treatment-related risk factors for developing any SMNs.

Methods

Patients

The Dutch Childhood Cancer survivor study (DCCSS)-LATER cohort is a well-characterized cohort of five-year CCS diagnosed before the age of 18 at one of the pediatric oncology/stem cell centers in the Netherlands between January 1, 1963 and December 31, 2014[3,20]. Data collection from the original cohort (1963-2001)[3] and the expansion cohort (2002-2014)[21] has been previously reported. A total of 11,548 CCS were included in this study. The flow diagram is shown in **Figure 1**.

Data collection

The DCCSS-LATER registry includes information about demographics, diagnosis, and childhood cancer treatment for primary tumor and recurrences. Details on the informed consent procedure are provided in **Appendix A**. For 936 survivors with anonymized data, only basic yes/no treatment data were available. For the remaining 10,612 survivors, detailed treatment data were available, including the type and doses of chemotherapy and radiotherapy. Cumulative doses were calculated for different groups of chemotherapy. For anthracyclines we used the doxorubicin isotoxic equivalent (DIE) to sum doses of agents[22]. For alkylating agents, dose was summed according to the cyclophosphamide equivalent dose (CED)[23]. The cumulative doses of epipodophyllotoxins and platinum agents were calculated by summing all the individual doses of these agents without using a conversion factor. For radiotherapy, we calculated the maximum cumulative radiation dose in Gray for each body region by summing the dose for primary cancer and all recurrences (including boost dose) when the same location within the body region was irradiated. We determined the maximum dose to smallest field. In case of two or more non-overlapping fields in one body region, the dose to the field with the highest dose was assigned. Total body irradiation dose was added to the dose of each body region.

Data on SMNs were obtained by linkage with nationwide registries: the Netherlands Cancer Registry (NCR)[24] and the Dutch Nationwide Pathology Databank (Palga)[25]. The NCR, which records all cancer cases in the Netherlands since 1989 (except basal cell carcinoma), served as primary source for SMNs. Data from NCR was complete up to January 31st, 2022. For the

period before 1989, we used the partially available NCR data, supplemented by data from Palga and medical information from the DCCSS-LATER registry. Discrepancies between SMN sources were resolves by pathology report reviews. SMNs were included when they malignant neoplasms, excluding basal cell carcinomas, occurred five years or more after childhood cancer diagnosis and with evidence excluding a recurrence or metastasis from the childhood cancer.

Statistical analysis

Time at risk started five years after childhood cancer diagnosis and ended on the date of diagnosis of the first SMN of interest, date of death, date of last known vital status (emigration or loss to follow up), or end of study at January 31st, 2022, whichever came first. If a survivor presented with multiple SMNs we only included the first SMN of interest in the analysis.

Period of childhood cancer diagnosis was divided into 4 categories: <1980, 1980-1989, 1990-1999, and 2000 or onwards. For each diagnosis period, we estimated cumulative incidence considering death as competing risk and differences between diagnosis periods were compared using Gray's tests[26].

We also calculated standardized incidence ratios (SIRs) and absolute excess risk (AER) per 10,000 person-years. The SIR was calculated by dividing the observed number by the expected number based on age-, sex-, and calendar year-specific general population rates from the NCR. The AER was calculated as the excess number of SMNs per 10,000 person years. Furthermore, we performed Cox proportional hazard regression analyses to compare the hazard of developing SMNs between the diagnosis periods, adjusting for treatment variables. All models were adjusted for sex and age at diagnosis. We used mediation analysis[27,28] to examine whether changes in SMN risk over time were mediated by treatment modification. Attenuation and elimination of statistical significance of the coefficient of the period of diagnosis were used to establish the mediation role of the treatment variables. We also compared risks by diagnosis period for different childhood cancer groups. The proportional

hazard assumption was tested in all models and was not violated. All analyses were performed using SPSS v 29.0 or R studio v 4.2.

Results

Cohort characteristics

Among the 11,548 eligible CCS, the median age at primary childhood cancer diagnosis was 6.0 years (range:0-18.0). Overall, the most common primary diagnoses were leukemia (34.5%), lymphoma (17.5%) and tumors of the central nervous system (CNS) (14.1%) (**Table 1; Appendix B).** In total, 1,205 (10.4%) survivors were diagnosed before 1980; 2,169 (18.8%) during 1980-1989; 2,874 (24.9%) during 1990-1999, and 5,300 (45.9%) in or after 2000. The percentage of five-year survivors diagnosed with hematological cancers and CNS tumors was higher after 1980 compared to before 1980 **(Table 1).**

The use of any chemotherapy increased from 72% before 1980 to 83% in the period 1980-1990, after which is was fairly stable. This trend was observed across all chemotherapeutic groups, with the largest increase for anthracyclines, from 22.3% before 1980 to 54.7% in 2000 and after. The use of epipodophyllotoxins also substantially increased, from 5.5% before 1980 to 30.2% in 2000 and onwards. The proportion of five-year survivors treated with alkylating agents also increased over time (38.5% before 1980 to 59.4% in 2000 and onwards) (**Table 1)**. In contrast, the use of radiotherapy decreased considerably across the four diagnosis periods, from 75% before 1980 to 25% in 2000 and onwards. The maximum radiation dose (Gray) to any body region increased over time, with a median of 26(IQR:25-42) before 1980 and a median of 45 (IQR: 21-54) in 2000 and onwards.

After a total of 209,882 person-years, 550 survivors developed at least one SMN, of whom 75 developed multiple SMNs. In total, we observed 481 solid and 75 hematologic SMNs. The most frequently observed SMNs were breast cancer (n=101), digestive system cancers (n=67), urogenital cancers (n=54), and thyroid carcinomas (n=54) **(Table1; Appendix C)**. Types of

SMNs stratified by childhood cancer type and radiotherapy status are shown in **Appendix D and E.**

Univariate analysis subsequent malignant neoplasms

The median latency between childhood cancer diagnosis and the occurrence of an SMN was 23.5 years (range: 5-53.5). Overall SMN risk was significantly increased compared to the age-, sex-, and calendar-year matched general population with an SIR of 3.5 (95%CI: 3.2 – 3.8) and an AER of 18.8 per 10,000 person-years **(Appendix C).** When stratifying by age at diagnosis and attained age, survivors diagnosed at ages ≤10 years vs. older showed lower SIRs throughout diagnosis periods for an attained age between 15-25 years and 25-35 years (**Appendix F)**. For specific childhood cancer types, a decreasing SIR for any SMN throughout diagnosis periods was only seen for renal tumors **(Appendix G).**

The 25-year cumulative incidences of any SMN was 3.6% (95% CI 3.2-4.0), respectively. Cumulative incidences at 25 years were higher for those who received radiotherapy (yes: 5.4, 95%CI:4.7-6.3 vs. no: 2.4, 95%CI:2.0-4.2) and stem cell transplantation (yes: 9.6, 95%CI:7.2-12.8 vs no: 3.2, 95%CI: 2.8-3.6), compared to survivors not receiving those treatments (**Appendix C)**. There was no significant difference in cumulative incidence between the four diagnosis periods **(Appendix C; Supplementary Figure 1A).** Among irradiated survivors only, we observed a significantly higher cumulative incidence of SMN for survivors diagnosed in the 1980s ($p<0.001$) and 1990s ($p<0.001$) compared to those diagnosed before 1980 **(Supplementary Figure 1B).** Among non-irradiated survivors only, we observed a significant decrease in SMN cumulative incidence for survivors diagnosed in the 1980s ($p=0.006$) and 1990s ($p=0.01$) compared to those diagnosed before 1980 **(Supplementary Figure 1C).**

For survivors of hematological childhood cancers ($p=0.02$) and CNS tumor survivors ($p=0.08$), those diagnosed in the 1980s showed trends of higher cumulative incidences compared to those diagnosed before 1980, although this was not statistically significant for CNS tumor survivors. No clear trends in cumulative incidence of SMN throughout diagnosis periods were

seen for survivors of solid childhood cancers **(Supplementary Figure 2).** When analyzing specific type of childhood cancers, we observed an increased cumulative incidence throughout diagnosis periods for soft tissue and other extraosseous sarcomas, although not statistically significant **(Appendix G).**

Multivariate and mediation analysis of temporal trends

Temporal changes were evaluated using Cox proportional hazard regression models among 10,640 CCS with available detailed treatment information. After adjusting for sex and age (base model) at diagnosis, we observed a decreased risk of SMNs throughout diagnosis periods (p-trend=0.04), with HRs of 0.9, (95%CI:0.7-1.2) for 1980-1989, 0.8 (95%CI:0.6-1.1) for 1990-1999 and 0.7, (95%CI: 0.5–1.0) for 2000 and onwards, compared to survivors diagnosed before 1980 (**Table 2; Figure 2)**. When adding all treatment variables to the base model, the HRs of the period of diagnosis categories were attenuated and differences between periods were not statistically significant anymore **(Table 2).** When adding chemotherapy groups yes/no to the model, the decrease throughout diagnosis periods was stronger, with a p-trend of 0.001. A similar effect of stronger decrease throughout diagnosis periods was observed when anthracyclines and epipodophyllotoxins yes/no were added to the base model, with a p-trend of 0.003. Adding other chemotherapy groups, other than anthracycline and epipodophyllotoxins, to the base model did not materially change the effect estimates of the diagnosis periods (**Appendix H).** In contrast, when adding radiotherapy yes/no to the model, the differences in risk throughout diagnosis periods disappeared, with HRs of 1.2 (95%CI: 0.9-1.5) for 1980-1999, 1.2 (95%CI:0.9-1.7) for 1990-1999 and 1.1 (95%CI:0.7-1.5) for 2000 and onward, compared to period 1980> (p-trend=0.5) (**Table 2; Figure 2).** Inclusion of chemotherapy doses, radiotherapy doses or body regions did not further influence estimates (**Appendix H)**. These analyses showed that the decrease in use of radiotherapy is the main contributor to the decline of SMN risk that we observed across the four diagnosis periods. Analysis stratified for childhood cancer groups showed decreased risk for SMN throughout periods for solid childhood cancers **(Appendix I).**

Similar analysis were done among the subgroups of irradiated and non-irradiated survivors. Among irradiated survivors, an increase in SMN risk was seen across diagnosis periods, with a HR of 1.5 (95%CI:1.1-2.0) for 1980-1989, 2.0 (95%CI: 1.4-2.8) for 1990-1999, and 1.4 (95%ci: 0.9-2.3) for 2000 and onwards compared to diagnosis before 1980 (p-trend 0.005). Mediation analysis revealed that chemotherapy doses were the main contributors to this increased SMN risk among the irradiated survivors **(Table 3; Figure 3)**. Among non-irradiated survivors only, a decrease of SMN risk was seen throughout the diagnosis periods. Adjusting for chemotherapy agents and doses, stem cell transplantation did not substantially alter those risks **(Appendix J)**.

Multivariate model of treatment-related risk factors

Survivors treated with anthracyclines (HR:1.3, 95%CI: 1.0-1.6) and epipodophyllotoxins (HR:1.3, 95%CI: 1.0-1.7) had significantly higher risks of developing SMNs compared to the survivors who did not receive those treatments **(Table 2).** Furthermore, survivors who were treated with radiotherapy had a significantly higher risk compared to survivors treated without radiotherapy (HR:2.3, 95%CI: 1.9-2.8).

Discussion

This study, in a well-established cohort of Dutch five-year CCS, shows that the overall risk of developing SMNs decreased over time, which was associated with a reduced use of radiotherapy. An innovative finding of this study is that changes in chemotherapy administration counteracted the decline of SMN risk over time, which can be mainly attributed to an increase in use of epipodophyllotoxins and anthracyclines. Furthermore, survivors treated with radiotherapy, anthracyclines, or epipodophyllotoxins were found to have a significantly elevated risk of developing any SMN compared to the patients treated without those treatments.

In this study, we observed that the decreased use of radiotherapy was the main contributor to the observed decline in the SMN risk over time. The use of radiotherapy decreased from

75% before 1980 to just over 25% in 2000 and beyond. This finding aligns with results from the childhood cancer survivors study (CCSS) from North America, which reported a lower SMN risk for survivors diagnosed in 1990, compared to survivors diagnosed in 1970s[18]. This CCSS study primarily attributed this reduction to changes in radiation dose, while in our first European study on temporal trends we found an association with the use of radiotherapy, but changes in doses or body regions did not significantly further impact this decline. Furthermore, it is important to recognize that the enhanced quality of radiotherapy, with increased efforts to spare healthy tissue, might have also contributed to a reduction in risk. However, despite the reduced use of radiotherapy our results indicate that radiotherapy continues to be an important risk factor for developing any SMN.

Regarding our novel finding on the effect of chemotherapy, we observed that the temporal decline of SMN risk due to decreased use of radiotherapy, was counteracted by the increased use of chemotherapy. This was mainly attributed to an increased use of anthracyclines and epipodophyllotoxins in the more recent diagnosis periods. This is in contrast with the abovementioned CCSS study which observed no associations were identified between chemotherapy changes and SMN risk over time[18]. Interestingly, anthracyclines and epipodophyllotoxins were both significantly associated with a higher risk of developing SMNs in our multivariable analyses. Both groups of chemotherapy have previously been found to increase the risk of acute myeloid leukemia[14,15], though epipodophyllotoxins are not a well-known risk factor for other types of SMNs. Anthracyclines are also associated with risk of subsequent breast cancer[4] and sarcomas[3]. Breast cancers were among the most frequently observed SMN in our study. While alkylating agents have been associated with development various solid tumors such as bone tumors[12,16] and colorectal cancer[9], we did not find a significant association with the risk of developing any SMN. This increased risk due to chemotherapy is supported by observations among the subgroup of irradiated survivors. Among the irradiated survivors SMN risk increased over time, which was mainly explained by the higher chemotherapy doses in recent periods.

Concerning the temporal changes it is also important to realize that the population of childhood cancers survivors has likely changed over time. Due to increased and varying survival rates across childhood cancers, the composition of the survival cohort has changed. In our analysis stratified by childhood cancer group, all groups showed a decreasing temporal trend in SMN risk, however this trend was only significant for solid childhood cancers possibly due to a lower power in subgroups.

Major strengths of our study are the large cohort size with over 11,000 childhood cancer survivors, the availability of comprehensive individual-level treatment data, and the extended follow-up time of more than five decades from 1963 through January 2022 and. A previous study within our DCCSS-LATER cohort revealed no significant differences in SMN risk between different diagnosis periods[3], possibly due to the shorter follow-up time or smaller sample size. However, our current study also included the expanded DCCSS-LATER cohort (2002-2014) with extended follow-up until 2022, thereby enhancing statistical power and providing more robust findings. In addition, linkage with Netherlands Cancer Registry (NCR) provided complete follow-up data, including objective data on SMNs. When interpreting the results, we also need to consider some limitations. First, earlier treatment periods have a longer follow-up time compared to more recent decades, inevitably leading to more SMNs that typically occur at long latency periods and/or at higher ages. Ongoing follow-up of the survivors from the latest period is needed to compare SMN risk over a longer period of time. Second, due to lack of genetic data, we were unable to account for genetic predispositions in our analysis.

This study underscores that efforts to reduce radiotherapy usage have been effective in lowering the long-term risk of SMNs. However, the increased use of certain chemotherapy agents increased the risk of SMNs and mitigated this decline of risk over time. This highlights the need to reevaluate chemotherapy protocols to better balance treatment efficacy and long-term health outcomes for childhood cancer patients and the need to identify CCS at risk. Furthermore, this study provides a valuable reference for future research examining the impact of the increasing use of targeted therapies in recent decades on SMN risk.

In conclusion, the reduced use of radiotherapy resulted in a decline over time in the risk of developing SMNs risk among CCS, whereas the increased use of chemotherapy, in particular anthracyclines and epipodophyllotoxins, counteracted this decline. Furthermore, our results show that CCS treated with radiotherapy, anthracyclines and/or epipodophyllotoxins have an increased risk of developing any SMN.

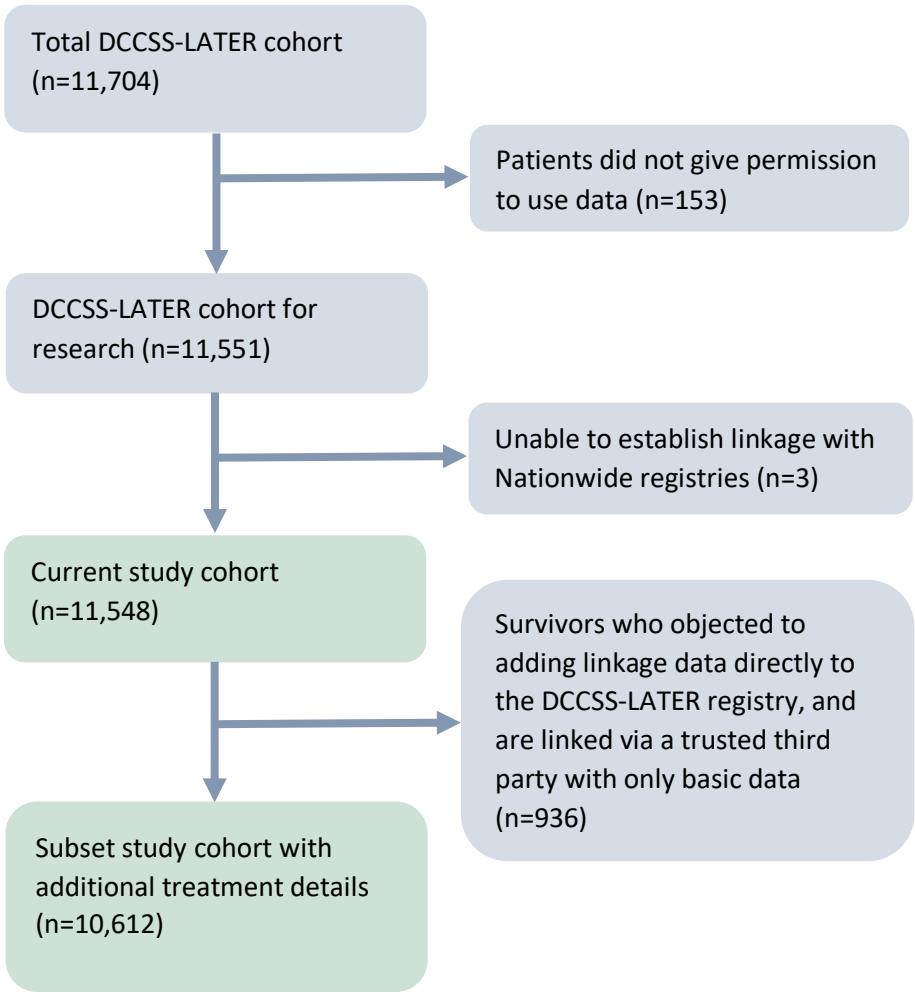


Figure 1. Flow chart of Dutch Childhood Cancer Survivor Study (DCCSS)-Later cohort

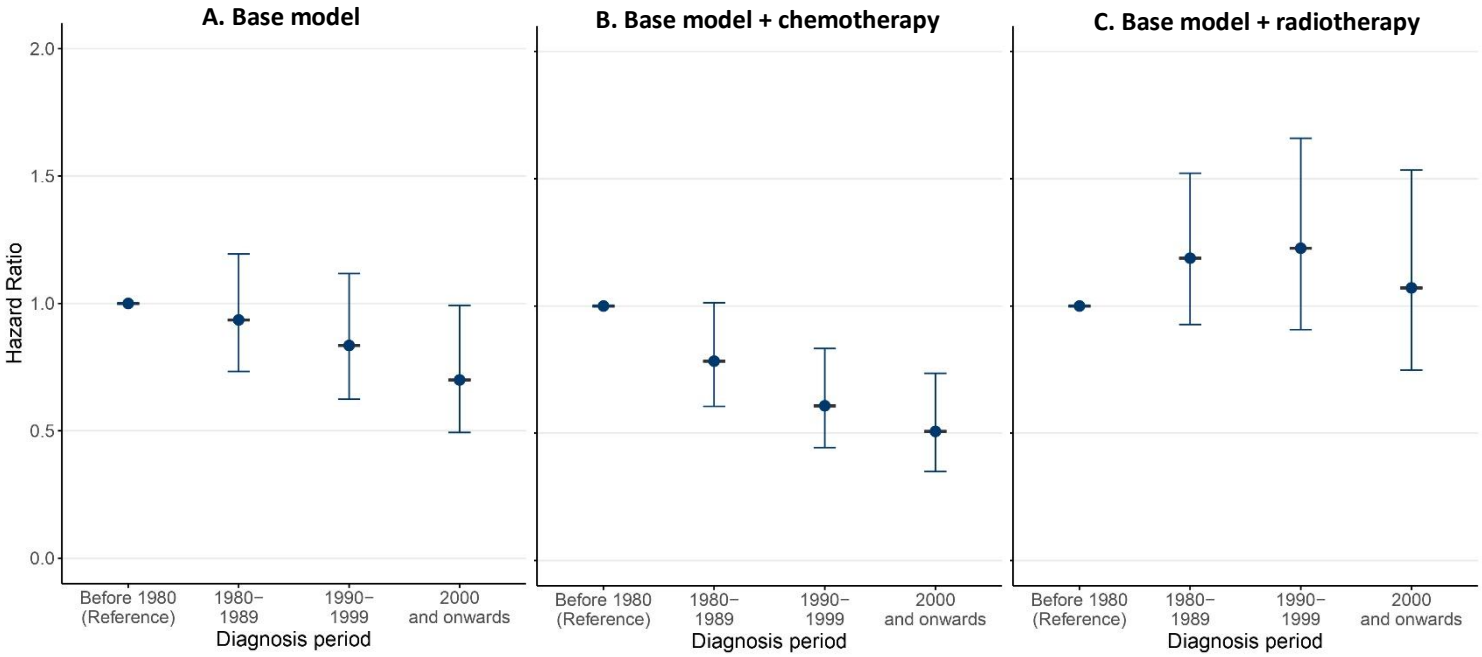


Figure 2. Hazard Ratios and their 95% confidence interval for overall SMN risk from the DCCSS-LATER cohort, stratified by period of childhood cancer diagnosis, with diagnosis <1980 as reference group. Calculated by multivariate Cox proportional hazard regression, all models are adjusted for sex and age at diagnosis. **A.** Base model, without adjusting for treatment-related factors **B**. Model adjusted for chemotherapy agents yes/no including alkylating agents, anthracyclines, epipodophyllotoxins, platinum agents, and vinca alkaloids **C.** Model adjusted for radiotherapy yes/no.

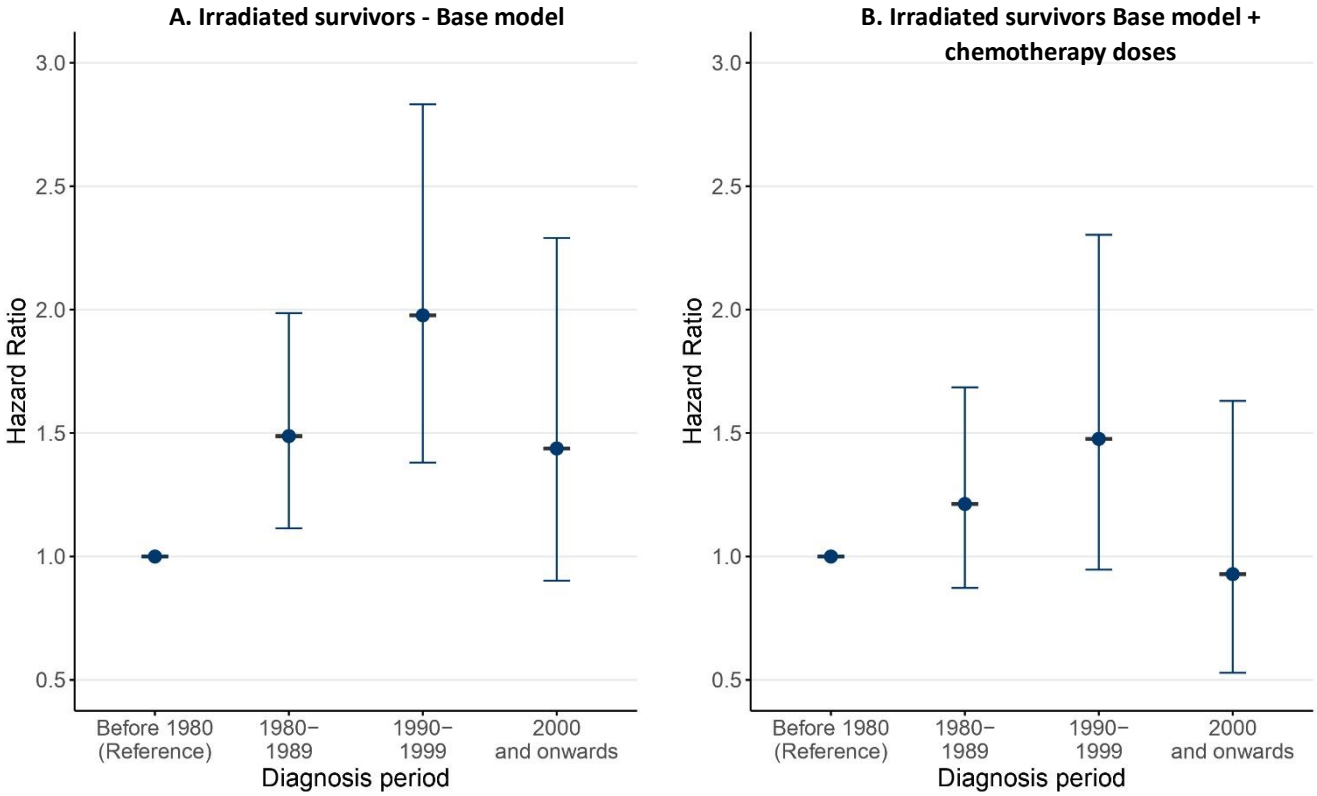


Figure 3. Hazard Ratios and their 95% confidence interval of the irradiated survivors of the DCCSS-LATER cohort, stratified by period of childhood cancer diagnosis, with diagnosis <1980 as reference group. Calculated by multivariate Cox proportional hazard regression, both models are adjusted for sex and age at diagnosis. **A.** Irradiated survivors, base model without adjusting for treatment-related variables **B.** Irradiated survivors, model adjusted for adjusting for chemotherapy doses.

Table 1. Clinical and treatment characteristics of the 11.548 childhood cancer survivors from the DCCSS-LATER cohort, overall and by diagnosis period

Characteristics	Total cohort	< 1980	1980-1989	1990-1999	≥ 2000
Total cohort	**11.548**	**1205**	**2169**	**2874**	**5300**
Median Fu time since Dx	21.5 (5.0 – 59.0)	43.9(5.1 – 59.0)	35.5 (5.0-42.1)	25.8 (5.0 – 32.1)	14.1 (5.1 – 22.1)
Subsequent malignant neoplasms					
No	11.024 (95.2%)	1036 (86.0%)	1980 (91.3%)	2752 (95.8%)	5230 (98.7%)
Yes	550 (4.8%)	169 (14.0%)	189 (8.7%)	122 (4.2%)	70 (1.3%)
Sex					
Male	6489 (56.2%)	635 (52.7%)	1210 (55.8%)	1667 (58.0%)	2975 (56.1%)
Female	5064 (43.8%)	570 (47.3%)	959 (44.2%)	1207 (42.0%)	2325 (43.9%)
Childhood cancers					
Hematological malignancies	**6000 (52.0%)**	**525 (43.6%)**	**1135 (52.3%)**	**1516 (52.7%)**	**2824 (53.3%)**
Leukemia	3984 (34.5%)	338 (28%)	729 (33.6%)	1008 (35.1%)	1909 (36.0%)
ALL	3072	271	564	735	1502
AML	397	12	57	99	229
Lymphoma	2016 (17.5%)	187 (15%)	406 (18.7%)	508 (17.7%)	915 (17.2%)
HL	742	53	142	160	387
NHL	589	65	114	156	254
Central nervous system tumors	**1623 (14.1%)**	**113 (9.4%)**	**256 (11.8%)**	**452 (15.7%)**	**802 (15.1%)**
Solid tumors	**3925 (34.0%)**	**567 (47.1%)**	**778 (35.9%)**	**906 (31.5%)**	**1674 (31.6%)**
Neuroblastoma	577 (5.0%)	81 (6.7%)	130 (6.0%)	129 (4.5%)	237 (4.5%)
Retinoblastoma	101 (0.9%)	7 (0.6%)	18 (0.8%)	13 (0.5%)	63 (1.2%)
Renal Tumors	970 (8.4%)	160 (13.3%)	218 (10.1%)	244 (8.5%)	348 (6.6%)
Hepatic tumors	129 (1.1%)	3 (0.2%)	11 (0.5%)	40 (1.4%)	75 (1.4%)
Bone tumors	682 (5.9%)	100 (8.3%)	154 (7.1%)	136 (4.7%)	292 (5.5%)
Soft tissue and other extraosseous sarcomas	800 (6.9%)	129 (10.7%)	161 (7.4%)	177 (6.2%)	333 (6.3%)
Germ cell tumors	450 (3.9%)	47 (3.9%)	60 (2.8%)	132 (4.6%)	211 (4.0%)
Other malignant epithelial neoplasms and malignant melanomas	201 (1.7%)	38 (3.2%)	24 (1.1%)	33 (1.1%)	106 (2.0%)
Other and unspecified	15 (0.1%)	2 (0.1%)	2 (0.1%)	2 (0.1%)	9 (0.2%)
Vital status					
Alive	10613 (91.9%)	897 (74.4%)	1911 (88.1%)	2670 (92.9%)	5131 (96.8%)
Deceased	940 (8.1%)	308 (25.6%)	258 (11.9%)	204 (7.1%)	169 (3.2%)
Chemotherapy					
No	1976 (17.1%)	317 (26.3%)	334 (15.4%)	512 (17.8%)	813 (15.3%)
Yes	9528 (82.5%)	873 (72.4%)	1820 (83.9%)	2354 (81.9)	4476 (84.5%)
Unknown	49 (0.4%)	15 (1.3%)	15 (0.7%)	8 (0.3%)	11 (0.2%)
Radiotherapy					
No	7511 (65.0%)	290 (24.1%)	1232 (56.8%)	2068 (72.0%)	3917 (73.9%)
Yes	3990 (34.5%)	906 (75.2%)	929 (42.8%)	795 (27.7%)	1359 (25.6%)
Unknown	52 (0.5%)	9 (0.7%)	8 (0.4%)	11 (0.3%)	24 (0.5%)
Stem cell transplantation					
No	10572 (91.5%)	1155 (95.9%)	2014 (92.9%)	2595 (90.3%)	4807 (90.7%)
Yes	853 (7.4%)	11 (0.9%)	113 (5.2%)	260 (9.0%)	465 (8.8%)
Unknown	128 (1.1%)	39 (3.2%)	42 (1.9%)	19 (0.7%)	28 (0.5%)
Treatment groups					
Surgery only	1075 (9.3%)	107 (8.9%)	160 (7.4%)	322 (11.2%)	486 (9.2%)
CT no RT	6224 (53.9%)	179 (14.9%)	1041 (48.0%)	1705 (59.3%)	3295 (62.2%)
RT no CT	703 (6.1%)	205 (17.0%)	148 (6.8%)	154 (5.4%)	196 (3.7%)
CT +RT	3237 (28.0%)	679 (56.3%)	766 (35.3%)	633 (22.0%)	1158 (21.8%)
No surgery, no CT. no RT	194 (1.7%)	4 (0.3%)	25 (1.2%)	34 (1.2%)	131 (2.5%)
Treatment (partially) unrecorded	120 (1.0%)	31 (2.6%)	29 (1.3%)	26 (0.9%)	34 (0.6%)
*Chemotherapy **					
Alkylating (a)	5901 (51.1%)	409 (38.5%)	951 (50.4%)	1459 (58.9%)	3077 (59.4%)
Anthracyclines (b)	5460 (47.3%)	237 (22.3%)	878 (46.5%)	1385 (55.9%)	2960 (54.7%)
Epipodophyllotoxins (c)	2689 (23.3%)	58 (5.5%)	319 (16.9%)	745 (30.1%)	1566 (30.2%)
Platinum (d)	1718 (14.9%)	14 (1.3%)	185 (9.8%)	454 (18.3%)	1064 (20.5%)
Vinca alkaloids (e)	7448 (64.5%)	690 (65%)	1467 (77.7%)	1758 (70.9%)	3533 (68.2%)
*Cumulative doses for chemotherapy groups (mg/m^2)**					
Alkylating (a) median (IQR)	**4800 (2000-9989)**	**10200 (4800-14557)**	**6000 (3435 – 11625)**	**4638 (3000-8784)**	**4000.0 (2000–9200)**
None	4644 (43.8%)	630 (59.3%)	917 (48.6%)	1010 (40.7%)	2087 (40.3%)
1 – 3999	2297 (21.6%)	50 (4.7%)	247 (13.1%)	614 (24.8%)	1386 (26.7%)
4000 – 7999	1195 (11.3%)	61 (5.7%)	232 (12.3%)	338 (13.6%)	654 (10.9%)
8000+	1752 (16.5%)	196 (18.5%)	338 (17.9%)	374 (15.1%)	844 (16.3%)
Anthracyclines (b) median (IQR)	**200 (132-300)**	**180 (60-420)**	**228 (140-360)**	**180 (132-280)**	**200 (126 -300)**
None	5078 (47.9%)	799 (75.2%)	992 (52.6%)	1083 (43.7%)	2204 (42.5%)
1 – 99	700 (6.6%)	62 (5.8%)	152 (8.1%)	51 (2.1%)	435 (8.4%)
100 - 199	1954 (18.4%)	40 (3.8%)	190 (10.1%)	731 (25.5%)	993 (19.2%)
200+	2679 (25.2%)	96 (9.0%)	504 (26.7%)	566 (22.8%)	1513 (29.2%)
Epipodophyllotoxins (c) median (IQR)	**1350 (950-2250)**	**1980 (881-3135)**	**1050 (600-1875)**	**1300 (900 – 2200)**	**1440 (1050-2400)**
None	7846 (73.9%))	977 (92.0%)	1547 (82.0%)	1726 (69.6%)	3596 (69.4%)
1 – 999	693 (6.5%)	15 (1.4%)	138 (7.3%)	225 (9.1%)	315 (6.1%)
1000 - 1999	1058 (10.0%)	10 (0.9%)	79 (4.2%)	256 (10.3%)	713 (13.8%)
2000+	795 (7.5%)	24 (2.3%)	68 (3.6%)	223 (9.0%)	480 (9.3%)
Platinum (d) median (IQR)	**1000 (450-2520)**	**360 (300-10050)**	**600 (400-900)**	**1252 (480-2405)**	**1200 (469-2701)**

None	8804	(83.0%)	1018	(95.9%)	1680	(89.0%)	2012	(81.2%)	4094	(79.0%)
1 – 799	749	(7.1%)	6	(.6%)	114	(6.0%)	171	(6.9%)	458	(8.8%)
800 - 1599	219	(2.1%)	0	(0%)	21	(1.1%)	73	(2.9%)	125	(2.4%)
1600	64	(6.1%)	3	(0.3%)	29	(1.6%)	180	(7.3%)	435	(8.4%)
Vinca alkaloids (e) median (IQR)	**24 (12 – 59)**		**47 (25-76)**		**31 (16 – 64)**		**15 (12-41)**		**23 (10 – 58)**	
Radiotherapy body compartments *(f)										
TBI (g)	333 (3.1%)		6 (0.6%)		69 (3.7%)		131 (5.3%)		127 (2.4%)	
Head (h)	2195 (20.7%)		267 (44.0%)		567 (30.0%)		440 (17.7%)		721 (13.9%)	
Neck (i)	1311 (12.3%)		158 (14.9%)		296 (15.7%)		341 (13.8%)		516 (9.9%)	
Chest (g)	960 (9%)		139 (13.1%)		187 (9.9%)		241 (9.7%)		393 (7.6%)	
Abdominal (j)	917 (8.6%)		187 (17.6%)		175 (9.3%)		225 (9.1%)		330 (6.4%)	
Pelvis (k)	627 (5.9%)		91 (8.6%)		115 (6.1%)		180 (7.3%)		241 (4.6%)	
Upper extremities (l)	408 (3.8%)		31 (2.9%)		78 (4.1%)		133 (5.4%)		166 (3.2%)	
Lower extremities (m)	494 (4.7%)		70 (6.6%)		91 (4.8%)		152 (6.1%)		181 (3.5)	
Spinal (n)	977 (9.2%)		94 (8.9%)		231 (12.2%)		267 (10.8%)		385 (7.4%)	
Testis (o)	368 (3.5%)		17 (1.6%)		79 (4.2%)		135 (5.4%)		137 (2.6%)	
MIBG	89 (0.8%)		0		4 (0.2%)		30 (1.2%)		55 (1.1%)	
Max radiation dose to any body region (Gy)*										
None	6923 (65.2%)		256 (24.1%)		1070 (56.7%)		1767 (71.3%)		3830 (73.9%)	
0.1 – 10	204 (1.9%)		30 (2.8%)		58 (3.1%)		68 (2.7%)		48 (0.9%)	
10.1 – 20	587 (5.5%)		87 (8.2%)		144 (7.6%)		118 (4.8%)		238 (4.6%)	
20.1 – 0	818 (7.7%)		333 (31.4%)		235 (12.5%)		72 (2.9%)		178 (3.4%)	
30.1 – 40	390 (3.7%)		112 (10.5%)		91 (4.8%)		72 (2.9%)		115 (2.2%)	
40.1 – 50	388 (3.7%)		100 (9.4%)		92 (4.9%)		93 (3.8%)		103 (2.0%)	
>50	1042 (9.8%)		105 (9.9%)		159 (8.4%)		220 (8.9%)		558 (10.8%)	
Unknown	259 (2.5%)		39 (3.7%)		38 (2.0%)		69 (2.8%)		114 (2.2%)	
Median (IQR)	**35 (21-54)**		**26 (25 – 42)**		**26 (20 – 50)**		**40 (15 – 54)**		**45 (21 – 54)**	

ALL: Acute lymphoblastic leukemia, AML: Acute myeloid leukemia, HL: Hodgkin lymphoma, NHL: Non-Hodgkin lymphoma, Rt: radiotherapy. CT: chemotherapy, RT: radiotherapy, TBI: Total body irradiation, MIBG: , IQR: Interquartile range, GY: gray * Based on sub cohort with detailed treatment information (n= 10.617). Five patients were diagnosis with simultaneously a SMN and SNMN a). 72 (0.7%) unknown b). 74 (0.7%) unknown c). 78 (0.7%)unknown d) 91 (0.9%) unknown e) 61 (0.6%)unknown f) compartments are including TBI g)104 unknown h) 79 unknown i) 201 unknown j) 108 unknown k) 347 unknown l) 123 unknown m) 108 unknown n)96 unknown o) 110 unknown

Table 2. Multivariable Cox regression models and mediation analysis with and without treatment variables, of any SMNs

		SMN		
Variable	**Total survivors**	**SMNs**	**HR**	**95% CI**
Base model – Not adjusted for any treatments*				
Period of childhood diagnosis				p-trend = 0.04
<1980	1062	156	1 (ref)	
1980 – 1989	1887	163	0.9	0.7 – 1.2
1990 – 1999	2479	105	0.8	0.6 – 1.1
≥ 2000	5189	70	**0.7**	**0.5 – 1.0**
Model adjusted for all treatments*[1]				
Period of childhood diagnosis				p-trend = 0.2
<1980	1062	156	1 (ref)	
1980 – 1989	1887	163	1.0	0.8 – 1.3
1990 – 1999	2479	105	0.9	0.7 – 1.3
≥ 2000	5189	70	0.8	0.5 – 1.1
Alkylating agent [5]				
No	4644	211	1 (ref)	
Yes	5896	280	1.2	0.9 – 1.6
Anthracyclines [6]				
No	5078	248	1 (ref)	
Yes	5460	241	**1.3**	**1.0 – 1.6**
Epipodophyllotoxins [7]				
No	7846	370	1 (ref)	
Yes	2688	120	**1.3**	**1.0 – 1.7**
Platinum agents [8]				
No	8804	425	1 (ref)	
Yes	1717	64	1.2	0.9 - 1.6
Vinca alkaloids [9]				
No	3103	144	1 (ref)	
Yes	7448	348	0.9	0.8 – 1.2
Radiotherapy				
No	6923	166	1 (ref)	
Yes	3638	325	**2.3**	**1.9 – 2.8**
Model adjusted for use of chemotherapy only*[2]				
Period of childhood diagnosis				p-trend = 0.001
<1980	1062	156	1(ref)	
1980 – 1989	1887	163	0.8	0.6 – 1.0
1990 – 1999	2479	105	**0.6**	**0.4 – 0.8**
≥ 2000	5189	70	**0.5**	**0.3 – 0.7**
Model adjusted for use of anthracyclines and/or epipodophyllotoxins*[2]				
Period of childhood diagnosis				p-trend = 0.003
<1980	1062	156	1(ref)	
1980 – 1989	1887	163	0.8	0.6 – 1.0
1990 – 1999	2479	105	**0.6**	**0.5 – 0.9**
≥ 2000	5189	70	**0.5**	**0.4 – 0.8**
Model adjusted for use of radiotherapy only*[4]				
Period of childhood diagnosis				p-trend = 0.5
<1980	1062	156	1 (ref)	
1980 – 1989	1887	163	1.2	0.9 – 1.5
1990 – 1999	2479	105	1.2	0.9 – 1.7
≥ 2000	5189	70	1.1	0.7 – 1.5

***all models were adjusted for sex and age at diagnosis** SMNs: subsequent malignant neoplasms. The model for chemotherapy is adjusted for yes/no administration of alkylating agents anthracyclines, epipodophyllotoxins, platinum agents and vinca-alkaloids. The model for radiotherapy is adjusted for yes/no radiotherapy. The model adjuster for anthracyclines and/or epipodophyllotoxins is adjuster for yes/no administration of anthracyclines and or epipodophyllotoxins. SMNs: Subsequent malignant neoplasms [1.] 116 observations omitted due to missing data [2.] 103 observations omitted due to missing data [3.] 98 observations were omitted due to missing data. [4.] 51 observations omitted due to missing data [4.] 72 (0.7%) unknown, [5.] 74 (0.7%) unknown [6.] 78 (0.7%) unknown [7.] 91 (0.9%) unknown [8.] 61 (0.6%) unknown

Table 4. Multivariable Cox regression models and mediation analysis for irradiated survivors, with and without treatment variables for any SMNs

Variable	Total survivors	SMNs	HR	95% CI
Irradiated survivors – not adjusted for treatment variables*[1]				
Period of childhood diagnosis				p-trend = 0.005
<1980	797	119	1 (ref)	
1980 - 1989	809	109	**1.5**	**1.1– 2.0**
1990 – 1999	702	64	**2.0**	**1.4 – 2.8**
≥ 2000	1330	33	1.4	0.9 – 2.3
Irradiated survivors – adjusted for RT doses*[2]				
Period of childhood diagnosis				p-trend = 0.01
<1980	797	119	1 (ref)	
1980 - 1989	809	109	**1.4**	**1.1– 1.9**
1990 – 1999	702	64	**1.9**	**1.3 – 2.7**
≥ 2000	1330	33	1.4	0.8 – 2.3
Irradiated survivors – adjusted for RT body regions*[1]				
Period of childhood diagnosis				p-trend = 0.03
<1980	797	119	1 (ref)	
1980 - 1989	809	109	**1.5**	**1.1– 2.0**
1990 – 1999	702	64	**1.8**	**1.3 – 2.6**
≥ 2000	1330	33	1.3	0.8 – 2.1
Irradiated survivors – adjusted for TBI*[1]				
Period of childhood diagnosis				p-trend=0.03
<1980	797	119	1 (ref)	
1980 - 1989	809	109	**1.4**	**1.1– 1.9**
1990 – 1999	702	64	**1.8**	**1.2 – 2.6**
≥ 2000	1330	33	1.3	0.8 – 2.1
Irradiated survivors – adjusted for SCT*[3]				
Period of childhood diagnosis				p-trend = 0.1
<1980	797	119	1 (ref)	
1980 - 1989	809	109	**1.3**	**1.0– 1.8**
1990 – 1999	702	64	**1.6**	**1.1 – 2.3**
≥ 2000	1330	33	1.2	0.7 – 1.9
Irradiated survivors – adjusted for use of chemotherapy agents* [4]				
Period of childhood diagnosis				p-trend = 0.4
<1980	797	119	1 (ref)	
1980 - 1989	809	109	**1.3**	**1.0– 1.7**
1990 – 1999	702	64	**1.5**	**1.0 – 2.3**
≥ 2000	1330	33	1.0	0.6 – 1.7
Irradiated survivors – adjusted for chemotherapy doses* [5]				
Period of childhood diagnosis				P-trend = 0.6
<1980	797	119	1 (ref)	
1980 - 1989	809	109	1.2	0.9– 1.7
1990 – 1999	702	64	1.5	0.9– 2.3
≥ 2000	1330	33	0.9	0.5 – 1.6

***all models were adjusted for sex and age at diagnosis** SMNs: subsequent malignant neoplasms, TBI: Total body irradiation, SCT: Stem cell transplantation. The model for chemotherapy is adjusted for yes/no administration of alkylating agents anthracyclines, epipodophyllotoxins, platinum agents and vinca-alkaloids. The model for radiotherapy is adjusted for yes/no radiotherapy. SMNs: Subsequent malignant neoplasms [1] 3 observations omitted due to missing data [2.] 212 observation omitted due to missing data [3.] 68 observations omitted due to missing data. [4.] 57 observation omitted due to missing data [5.] 575 observations omitted due to missing

Supplementary materials chapter 2

Appendix A. Informed consent procedure

Informed consent was obtained for most survivors who had been invited for active participation DCCSS-LATER research projects. For survivors who had been invited for active participation in DCCSS-LATER research projects, but did not respond after repeated requests via a standardized protocol, and for survivors who had not yet been invited for active participation in any DCCSS-LATER research projects, specific consent was not needed in accordance with Dutch legislation. For 936 survivors who objected to adding linkage data directly to the DCCSS-LATER registry, we anonymized a minimal dataset via a trusted third party. Survivors who declined use of their health care data for research purposes were excluded from the eligible study cohort.'

Appendix B. Types of childhood cancer per decade in percentages, stratified by irradiated survivors and non-irradiated survivors

Irradiated survivors (%)				
Childhood cancer	**<1980**	**1980-1989**	**1990-1999**	**2000≥**
Leukemia's, myeloproliferative disease and myelodysplastic disease	34.5	38.9	22.1	11.9
Lymphomas and reticuloendothelial neoplasms	15.2	18.7	14.8	14.8
Central nervous system and miscellaneous intracranial and intraspinal neoplasms	10.4	18.4	26.4	26.3
Neuroblastoma and other peripheral nervous cell tumors	5.2	2.1	5.3	6.8
Retinoblastoma	0.4	0.8	0.3	1.5
Renal tumors	13.5	7.5	8.8	7.5
Hepatic tumors	0	0	0	0
Bone tumors	8.5	4.8	5.7	7.4
Soft tissue and other extraosseous sarcomas	7.6	6.0	9.8	15.4
Germ cell tumors, trophoblastic tumors and neoplasms of gonads	2.2	1.5	5.2	4.6
Other malignant epithelial neoplasms and malignant neoplasms	2.2	1.3	1.8	3.8
Other and unspecified malignant neoplasms	0.2	0	0	0.1
Non-irradiated survivors (%)				
Childhood cancer	**<1980**	**1980-1989**	**1990-1999**	**2000≥**
Leukemia's, myeloproliferative disease and myelodysplastic disease	7.2	29.6	40.0	44.5
Lymphomas and reticuloendothelial neoplasms	16.9	18.8	18.7	18.1
Central nervous system and miscellaneous intracranial and intraspinal neoplasms	5.9	6.7	11.6	11.2
Neuroblastoma and other peripheral nervous cell tumors	11.7	8.9	4.2	3.7
Retinoblastoma	1.0	0.9	0.5	1.1
Renal tumors	13.1	12.0	8.4	6.3
Hepatic tumors	1.0	0.9	1.9	1.9
Bone tumors	7.6	8.8	4.4	4.8
Soft tissue and other extraosseous sarcomas	20.0	8.5	4.8	3.1
Germ cell tumors, trophoblastic tumors and neoplasms of gonads	9.3	3.7	4.4	3.8
Other malignant epithelial neoplasms and malignant neoplasms	**6.2**	**1.1**	**0.9**	1.4
Other and unspecified malignant neoplasms	**0**	0.1	0.1	0.2

Appendix C. SIRs and AERs by patient and treatment characteristics and by type of subsequent malignant neoplasm

					Cumulative incidence %			
	Observed	SIR	95% CI	AER	15 year	95% CI	25 year	95% CI
Overall cohort	**550**	**3.5**	**3.2 – 3.8**	**18.8**	**1.5**	**1.3-1.8**	**3.6**	**3.2-4.0**
By patient and treatment characteristics								
Period of Diagnose								
<1980	169	2.7	2.3 – 3.1	25.8	1.4	0.9 - 2.3	3.7	2.8 – 5.0
1980-1989	189	3.7	3.2 – 4.3	22.4	1.8	1.3 – 2.4	3.8	3.1 – 4.7
1990-1999	122	4.2	3.5 – 5.0	16.0	1.5	1.1 – 2.0	3.6	2.0 – 4.4
2000>	71	5.2	4.0– 6.5	11.8	1.5	1.1 – 1.9	3.1[1]	1.6 – 5.8
Gender								
Male	250	3.4	3.0 – 3.9	15.1	1.2	1.0 – 1.5	2.8	2.4 – 3.4
Female	301	3.6	3.2 - 4.1	23.7	1.9	1.6 – 2.4	4.6	3.9 – 5.3
Time since childhood cancer diagnosis								
5-15	157	6.9	5.8 – 8.1	13.4				
15-25	141	3.9	3.3 – 4.6	16.6				
25-35	149	3.3	2.8 – 3.9	31.6				
35+	104	2.0	1.6 – 2.4	36.6				
Attained age								
5-15	71	11.0	8.6 – 13.9	13.2				
15-25	108	4.5	3.7 – 5.5	10.9				
25-35	143	3.6	3.0 – 4.2	20.2				
35+	229	2.7	2.4 – 3.0	44.1				
Radiotherapy[2]								
No	186	2.5	2.1 – 2.9	8.8	1.0	0.8 – 1.3	2.4	2.0 – 2.8
Yes	363	4.5	4.0 – 5.0	34.1	2.4	2.0 – 3.0	5.4	4.7 – 6.3
Chemotherapy[3]								
No	104	2.6	2.1 – 3.1	16.2	1.5	1.0 – 2.1	3.1	2.4 – 4.2
Yes	446	3.9	3.9 – 4.3	19.4	1.5	1.3 – 1.8	3.7	3.3 – 4.2
Stem cell transplantation[4]								
No	479	3.2	2.9 – 3.5	16.8	1.4	1.1 – 1.6	3.2	2.8 – 3.6
Yes	66	12.4	9.6 – 15.8	55.0	3.4	2.3 – 5.1	9.6	7.2 – 12.8
By type of SMN*								
Solid tumors	**481**	**3.5**	**3.2 - 3.9**	**16.4**	**1.2**	**1.0 – 1.4**	**3.1**	**2.7 – 3.5**
Head and neck	39	8.8	6.3 – 12.1	1.6	0.1	0.05 – 0.2	0.3	0.2 – 0.4
Digestive organs	67	3.7	2.9 –4.7	2.9	0.07	0.03 – 0.2	0.2	0.1 – 0.4
Pulmonary	24	3.1	2.0 – 4.6	0.8	0	0	0.09	0.04 – 0.2
Bone	32	12.2	8.3 – 17.2	1.4	0.2	0.1 – 0.3	0.3	0.2 – 0.4
Soft tissue	43	14.7	10.5 – 19.8	1.8	0.1	0.09 – 0.2	0.3	0.2 – 0.5
Female breast	101	3.2	2.6 – 3.9	3.3	0.1	0.07 – 0.2	0.6	0.5 – 0.8
Urogenital system[5]	54	1.6	1.2 – 2.2	1.0	0.1	0.09 – 0.2	0.3	0.2 – 0.4
Female genital organs	17	1.7	1.0-2.7	0.3	0.05	0.02 – 0.1	0.08	0.03 – 0.2
Male genital organs	19	1.0	0.6-1.6	0.02	0.07	0.03 – 0.2	0.2	0.09 – 0.3
Testis	8	0.5	0.2-1.0	-0.3	0.03	0.008 – 0.08	0.08	0.04 – 0.2
Central nervous system	46	7.3	5.3 – 9.7	1.9	0.2	0.1 – 0.3	0.4	0.2 – 0.5
Brain	34	5.7	4.0-8.0	1.3	0.2	0.09 – 0.2	0.3	0.2 – 0.4
Meninges	7	119.9	48.2 – 247.0	0.3	0.01	0.001-0.07	0.02	0.005 – 0.1
Thyroid	54	12.2	9.1 –15.9	2.3	0.1	0.09 – 0.2	0.5	0.4 – 0.7
Melanoma	34	1.5	1.1 – 2.2	0.6	0.07	0.03 – 0.1	0.2	0.1 – 0.3
Nonmelanoma skin (BCC excluded)	18	3.6	2.2 – 5.8	0.6	0.03	0.009 – 0.09	0.09	0.04 – 0.2
Hematological	**75**	**3.4**	**2.6 – 4.2**	**2.5**	**0.4**	**0.3 – 0.5**	**0.5**	**0.4 – 0.7**
Leukemias	28	3.9	2.6 – 5.7	1.0	0.2	0.1 – 0.3	0.2	0.2 – 0.4
Myeloid	16	5.6	3.2 – 9.1	0.6	0.1	0.07 – 0.2	0.1	0.08 – 0.2
Lymphoblastic	10	2.7	1.3 – 5.0	0.3	0.07	0.04 – 0.1	0.08	0.04 – 0.1
Lymphomas	31	2.1	1.4 – 3.2	0.8	0.1	0.05- 0.2	0.2	0.1 – 0.3
Non-Hodgkin lymphoma	20	2.7	1.7 – 4.2	0.6	0.05	0.02 – 0.1	0.1	0.06 – 0.2
Hodgkin-lymphoma	11	1.5	0.8 – 2.8	0.2	0.05	0.02 – 0.1	0.09	0.05 – 0.2
Other hematologic	2	2.2	0.3 – 8.1	0.05	0	0	0	0

SMN: Subsequent malignant neoplasm, SIR: standardized Incidence Ratio, AER: Absolute Excess Risk per 10,000 person-years, CI: Confidence interval *This is excluding 15 subsequent malignant neoplasms with and unknown morphology and/or topography. [1] This is 21 year cumulative incidence [2] 2 SMN with unknown radiotherapy status, [3] 1 SMN with unknown CT status, [4] 6 SMN with unknown SCT status, [5] Including adrenal gland

Appendix D. Percentages of types of subsequent malignant neoplasm per decade, stratified by childhood cancer groups

Hematological childhood cancers (%)				
Type of SMN	<1980 (n=525)	1980-1989 (n=1135)	1990-1999 (n=1516)	2000≥ (n=2824)
Solid tumors	**9.3**	**7.8**	**3.5**	**0.8**
Head and Neck	1.0	0.3	0.1	0.1
Digestive system	1.3	1.4	0.6	0.1
Pulmonary	1.3	0.5	0.1	0.0
Bone	0.6	0.1	0.1	0.1
Soft tissue	0.4	0.3	0.3	0.1
Mamma	1.3	1.9	0.9	0.1
Urogenital system	1.3	0.5	0.1	0.1
Female reproductive	0.2	0.4	0.0	0.0
Male reproductive	0.4	0.1	0.1	0.1
Central nervous system	2.5	0.5	0.1	0.1
Meningiomas	0.8	0.1	0.0	0.0
Thyroid	0.2	1.6	0.7	0.2
Melanoma	1.1	0.8	0.3	0.0
Nonmelanoma skin[1]	0.0	0.4	0.2	0.0
Hematological tumors	**1.0**	**1.4**	**0.5**	**0.2**
Leukemia	0.2	0.7	0.1	0.1
Lymphoma	0.4	0.7	0.4	0.1
Central nervous system childhood cancers (%)				
Type of SMN	<1980 (n=113)	1980-1989 (n=256)	1990-1999 (n=451)	2000≥ (n=802)
Solid tumors	**5.3**	**7.8**	**3.3**	**0.9**
Head and Neck	0.0	0.8	0.4	0.0
Digestive system	0.9	0.4	0.2	0.2
Pulmonary	0.0	0.8	0.2	0.0
Bone	0.0	0.4	0.2	0.0
Soft tissue	0.0	0.8	0.7	0.0
Mamma	0.0	1.2	0.0	0.0
Urogenital system	0.0	1.6	0.4	0.2
Female reproductive	0.0	0.4	0.0	0.1
Male reproductive	0.0	1.2	0.4	0.0
Central nervous system	3.5	0.8	0.7	0.5
Meningiomas	0.0	0.4	0.0	0.0
Thyroid	0.0	0.8	0.4	0.1
Melanoma	0.9	0.4	0.0	0.0
Nonmelanoma skin[1]	0.0	0.4	0.0	0.0
Hematological tumors	**0.9**	**0.4**	**0.2**	**0.1**
Leukemia	0.0	0.4	0.2	0.1
Lymphoma	0.9	0.0	0.0	0.0
Solid childhood cancers (%)				
Type of SMN	<1980 (n=567)	1980-1989 (n=778)	1990-1999 (n=906)	2000≥ (n=1674)
Solid tumors	**14.8**	**7.2**	**4.2**	**1.3**
Head and Neck	0.9	1.2	0.6	0.1
Digestive system	3.0	0.8	0.4	0.1
Pulmonary	0.7	0.1	0.1	0.0
Bone	1.4	0.4	0.7	0.3
Soft tissue	1.4	0.9	0.9	0.1
Mamma	4.4	2.3	0.4	0.2
Urogenital system	2.3	0.9	0.4	0.2
Female reproductive	0.7	0.5	0.0	0.2
Male reproductive	0.4	0.1	0.2	0.1
Central nervous system	0.2	0.3	0.2	0.1
Meningiomas	0.0	0.0	0.1	0.0
Thyroid	0.7	0.5	0.3	0.1
Melanoma	0.9	0.5	0.2	0.1
Nonmelanoma skin[1]	0.7	0.3	0.1	0.1
Hematological tumors	**1.4**	**0.5**	**0.6**	**0.3**
Leukemia	0.2	0.4	0.2	0.2
Lymphoma	1.2	0.1	0.3	0.1

SMN: subsequent malignant neoplasm [1.] Basal cell carcinoma's excluded

Appendix E. Percentages of types of subsequent malignant neoplasm per diagnosis period, stratified by irradiated survivors and non-irradiated survivors

Irradiated survivors (%)				
Type of SMN	<1980	1980-1989	1990-1999	2000≥
Solid tumors	**11.9**	**12.2**	**8.6**	**2.1**
Head and Neck	0.9	1.2	1.0	0.1
Digestive system	2.3	1.8	0.8	0.3
Pulmonary	1.1	0.9	0.5	0
Bone	1.1	0.4	0.8	0.3
Soft tissue	0.9	0.9	1.3	0.2
Mamma	2.4	3.2	1.6	0.4
Urogenital system	1.8	0.6	0.5	0.1
Female reproductive	0.3	0.4	0	0
Male reproductive	0.4	0.2	0.4	0.1
Central nervous system	1.7	1.0	0.6	0.2
Meningiomas	0.4	0.2	0.1	0
Thyroid	0.4	1.9	1.3	0.5
Melanoma	0.9	0.8	0.5	0.1
Nonmelanoma skin[1]	0.3	0.6	0.3	0
Hematological tumors	**0.9**	**1.1**	**0.4**	**0.1**
leukemia	0.2	0.5	0.1	0.1
lymphoma	0.4	0.5	0.3	0
Non-irradiated survivors (%)				
Type of SMN	<1980	1980-1989	1990-1999	2000≥
Solid tumors	**10.7**	**4.1**	**1.8**	**0.6**
Head and Neck	0.7	0.2	0.0	0.1
Digestive system	1.4	0.5	0.4	0
Pulmonary	0.3	0.1	0	0
Bone	0.3	0.1	0.1	0.1
Soft tissue	0.7	0.3	0.3	0
Mamma	3.4	1.1	0.2	0.1
Urogenital system	1.4	0.9	0.2	0.2
Female reproductive	0.7	0.5	0.1	0
Male reproductive	0	0.2	0.1	0.1
Central nervous system	1.0	0.1	0.1	0.1
Meningiomas	0	0	0	0
thyroid	0.3	0.5	0.3	0.1
Melanoma	1.4	0.6	0.1	0
Nonmelanoma skin[1]	0.3	0.1	0.1	0.1
Hematological tumors	**2.1**	**0.9**	**0.5**	**0.3**
leukemia	0	0.6	0.2	0.2
lymphoma	2.1	0.3	0.3	0.1

SMN: subsequent malignant neoplasm [1.] Basal cell carcinoma's excluded

Appendix F. Standardized incidence ratios for SMNs, by attained age and decade of primary cancer diagnosis, stratified by age of diagnosis

Diagnosis up to age 10 (n=8031)								
Characteristics	< 1980 (n=879)		1980-1989 (n=1585)		1990-1999 (n=2068)		2000≥ (n=3499)	
	SMNs	SIR (95% CI)	SMNs	SIR (95% CI)	SMNs	SIR (95% CI)	SMNs	SIR (95% CI)
Attained age								
5-15	7	9.6 (3.9 – 19.7)	19	14.4 (8.7 – 22.4)	18	10.3 (6.1 – 16.3)	26	9.9 (6.5 – 14.5)
15-25	14	7.0 (3.8 – 11.7)	22	4.9 (3.1 – 7.4)	23	3.6 (2.8 – 5.5)	8	2.3 (1.0 – 4.5)
25-35	22	4.1 (2.6 – 6.2)	29	2.4 (1.7 – 3.5)	24	2.4 (2.2 – 5.0)	1	2.8 (0.07 – 15.8)
35+	64	2.5 (1.9 – 3.2)	39	3.2 (2.3 – 4.4)	2	3.4 (0.4 – 12.3)	NA	NA
Diagnosis above the age of 10 (n=3517)								
	< 1980 (n=326)		1980-1989 (n=584)		1990-1999 (n=806)		2000≥ (n=1801)	
	SMNs	SIR (95% CI)	SMNs	SIR (95% CI)	SMNs	SIR (95% CI)	SMNs	SIR (95% CI)
Attained age								
5-15	0	0	0	0	0	0	0	0
15-25	4	7.0 (1.9 – 17.9)	10	8.2 (3.9 – 15.0)	9	4.5 (2.1 – 9.5)	18	4.9 (2.9 – 7.7)
25-35	6	3.3 (1.2 – 7.2)	17	4.4 (2.6 – 7.1)	27	4.4 (2.9 – 6.4)	17	5.0 (2.9 – 7.8)
35+	52	2.0 (1.5 – 2.6)	53	3.3 (2.5 – 4.4)	19	3.8 (2.3 – 5.9)	0	0

SMNs: Subsequent malignant neoplasms, SIR: Standardized incidence ratio, CI: Confidence interval

Appendix G. Standardized incidence ratios, absolute excess risks, and cumulative incidence of SMNs for each childhood cancer diagnosis category, total and stratified by decade of diagnosis.

	Total cohort						Before 1980						1980-1989					
	Total	SMNs	SIR (95% CI)	AER	15y Cumulative incidence (95% CI)	25y Cumulative incidence (95% CI)	Total	SMNs	SIR (95% CI)	AER	15y Cumulative incidence (95% CI)	25y Cumulative incidence (95% CI)	Total	SMNs	SIR (95% CI)	AER	15y Cumulative incidence (95% CI)	25y Cumulative incidence (95% CI)
Leukemia's, myeloproliferative disease and myelodysplastic disease	3984	142	3.3 (2.8 - 3.8)	14.1	1.1 (0.8 - 1.5)	2.7 (2.2 - 3.5)	338	28	2.1 (1.4 - 3.1)	13.9	1.5 (0.6 - 3.5)	2.4 (1.2 - 4.7)	729	65	4.0 3.1 - 5.0)	23.3	1.5 (0.8 - 2.7)	3.0 (2.0 - 4.6)
Lymphomas and reticuloendothelial neoplasms	2016	114	3.8 (3.1 - 4.6)	22.3	1.4 (0.8 - 1.9)	4.4 (3.4 - 5.7)	187	30	3.1 (2.1 - 4.5)	30.2	1.1 (0.3 - 4.2)	3.8 (1.8 - 7.7)	406	41	3.8 (2.8 - 5.2)	25.7	2.0 (1.0 - 3.9)	4.2 (2.6 - 6.7)
Central nervous system and miscellaneous intracranial and intraspinal neoplasms	1624	60	3.3 (2.5 - 4.2)	16.1	1.5 (1.0 - 2.2)	3.3 (2.4 - 4.6)	113	9	1.5 (0.7 - 2.8)	8.6	0	1.8 (0.4 - 6.9)	256	21	3.8 (2.4 - 5.8)	23.5	1.6 (0.6 - 4.1)	3.9 (2.1 - 7.2)
Neuroblastoma and other peripheral nervous cell tumors	577	23	3.5 (2.2 - 5.3)	14.1	1.6 (0.8 - 3.2)	2.1 (1.1 - 4.0)	81	10	3.3 (1.6 - 6.0)	2.3	1.3 (0.2 - 8.5)	3.8 (1.2 - 11.2)	130	4	1.8 (0.5 - 4.3)	4.3	0	0
Retinoblastoma	101	8	12.4 (5.3 - 24.4)	49.0	4.7 (1.8 - 12.4)	9.8 (4.3 - 22.0)	7	2	10.8 (1.3 - 39.0)	8.9	1.4 (2.1 - 6.7)	1.4 (2.1 - 6.7)	18	4	13.9 (3.8 - 35.4)	73.9	11.1 (2.9 - 37.6)	11.1 (2.9 - 37.6)
Renal tumors	970	50	3.7 (2.8 - 4.9)	17.1	0.7 (0.4 - 1.6)	1.6 (1.0 - 2.8)	160	33	4.9 (3.4 - 6.9)	4.3	1.9 (0.6 - 5.7)	3.1 (1.3 - 7.3)	218	14	3.5 (1.9 - 5.8)	15.2	0.5 (0.06 - 3.2)	2.3 (1.0 - 5.4)
Hepatic tumors	129	2	2.8 (0.3 - 10.2)	6.4	NA	2.5 (0.6 - 9.4) (f)	3	0	NA	NA	NA	NA	11	0	NA	NA	NA	NA
Bone tumors	682	60	3.6 (2.7 - 4.6)	34.8	3.6 (2.4 - 5.4)	6.8 (4.9 - 9.4)	100	19	2.1 (1.3 - 3.3)	31.2	2.0 (0.5 - 7.8)	6.0 (2.7 - 12.9)	154	21	4.2 (2.6 - 6.4)	37.5	4.0 (1.8 - 8.5)	7.8 (4.5 - 13.3)
Soft tissue and other extraosseous sarcomas	800	53	3.7 (2.8 - 4.8)	24.9	2.4 (1.5 - 3.8)	5.7 (4.1 - 8.0)	129	21	2.6 (1.6 - 3.9)	29.0	0	5.4 (2.6 - 11.0)	161	11	3.1 (1.5 - 5.5)	16.3	2.5 (0.9 - 6.5)	5.6 (3.0 - 10.5)
Germ cell tumors, trophoblastic tumors and neoplasms of gonads	450	19	2.6 (1.6 - 4.1)	14.5	2.3 (1.2 - 4.3)	2.6 (1.4 - 4.9)	47	9	2.6 (1.2 - 4.9)	30.4	2.1 (0.3 - 14.2)	2.1 (0.3 - 14.2)	60	2	1.1 (0.1 - 4.0)	1.2	1.7 (0.2 - 11.2)	1.7 (0.2 - 11.2)
Other malignant epithelial neoplasms and malignant neoplasms	201	17	3.5 (2.1 - 5.7)	34.3	2.8 (1.2 - 6.6)	8.1 (4.3 - 15.2)	38	8	2.5 (1.1 - 5.0)	38.6	5.3 (1.4 - 19.7)	8.0 (2.7 - 22.9)	24	4	4.6 (1.2 - 11.7)	45.2	0	8.3 (2.2 - 29.4)
Other and unspecified malignant neoplasms	15	2	15.5 (1.9 - 56.1)	83.6	6.7 (1.0 - 8.7)	NA	2	0	NA	NA	NA	NA	2	2	504.5 (61.1 - 1822.7)	1254.5	50 (8.9 - 99)	NA

	1990-1999						2000 and after					
	Total	SMNs	SIR (95% CI)	AER	15y Cumulative incidence (95% CI)	25y Cumulative incidence (95% CI)	Total	SMNs	SIR (95% CI)	AER (95% CI)	15y Cumulative incidence (95% CI)	25y Cumulative incidence (95% CI)
Leukemia's, myeloproliferative disease and myelodysplastic disease	1008	36	3.9 (2.7 - 5.4)	13.1	1.2 (0.7 - 2.1)	3.4 (2.5 - 4.8)	1909	13	2.8 (1.5 - 4.8)	4.7	0.7 (0.4 - 1.3)	NA
Lymphomas and reticuloendothelial neoplasms	508	26	3.9 (2.6 - 5.7)	18.1	0.4 (0.1 - 1.6)	4.0 (2.6 - 6.3)	915	17	5.5 (3.2 - 8.8)	16.6	1.6 (0.9 - 3.0)	NA
Central nervous system and miscellaneous intracranial and intraspinal neoplasms	452	17	3.6 (2.1 - 5.8)	13.9	1.6 (0.7 - 3.2)	3.2 (2.0 - 5.3)	803	13	6.5 (3.4 - 11.1)	15.5	1.5 (0.8 - 2.8)	NA
Neuroblastoma and other peripheral nervous cell tumors	129	5	6.9 (2.2 - 16.1)	16.7	2.3 (0.8 - 7.0)	2.3 (0.8 - 7.0)	237	4	11.9 (3.2 - 30.4)	17.1	2.8 (1.0 - 7.7)	NA
Retinoblastoma	13	1	9.6 (0.2 - 53.5)	30.3	0	7.7 (1.1 - 43.3)	63	1	14.6 (0.3 - 81.4)	18.7	1.6 (0.2 - 10.7) (a)	NA
Renal tumors	244	3	1.6 (0.3 - 4.6)	2.1	1.2 (0.4 0- 3.8)	NA	348	0	NA	NA	0.3 (0.05 - 2.3) (b)	NA
Hepatic tumors	40	2	7.5 (0.9 - 27.2)	21.9	NA	5.0 (1.3 - 18.5)	75	0	NA	NA	NA	NA
Bone tumors	136	11	6.1 (3.0 - 10.9)	36.1	3.7 (1.6 - 8.7)	6.1 (3.1 - 11.9)	292	9	9.4 (4.3 0 17.8)	33.6	4.0 (2.1 - 7.6)	NA
Soft tissue and other extraosseous sarcomas	177	15	8.7 (4.9 -14.3)	37.5	4.0 (1.9 - 8.1)	6.4 (3.6 - 11.3)	333	6	7.1 (2.6 - 15.5)	17.1	2.4 (1.1 - 5.4) ©	NA
Germ cell tumors, trophoblastic tumors and neoplasms of gonads	132	3	2.1 (0.4 - 6.2)	5.9	1.5 (0.4 - 5.9)	NA	211	5	8.2 (2.7 - 19.3)	24.2	3.5 (1.3 - 8.7)	NA
Other malignant epithelial neoplasms and malignant neoplasms	33	3	6.9 (1.4 - 20.2)	38.8	3.0 (0.4 - 19.6)	9.4 (2.1 - 26.4) (d)	106	2	5.4 (0.7 - 19.6)	17.4	2.5 (0.6 - 9.7) (e)	NA
Other and unspecified malignant neoplasms	2	0	NA	NA	0	0	9	0	NA	Na	0	0

SMNs: Subsequent malignant neoplasms, SIR: Standardized incidence ratio, AER: Absolute Excess Risk, CI: Confidence interval, NA: Not applicable

Appendix H. Multivariable Cox regression model and mediation analysis with treatment variables of any SMN

		SMN		
Variable	Total survivors	SMNs	HR	95% CI
Basic model – Not adjusted for any treatments*				
Period of childhood diagnosis				
<1980	1062	156	1 (ref)	
1980 – 1989	1887	163	0.9	0.7 – 1.2
1990 – 1999	2479	105	0.8	0.6 – 1.1
2000 ≥	5189	70	**0.7**	**0.5 – 0.99**
Model adjusted radiotherapy dose*[1]				
Period of childhood diagnosis				
<1980	1062	156	1 (ref)	
1980 – 1989	1887	163	1.1	0.8 – 1.4
1990 – 1999	2479	105	1.1	0.8 – 1.5
2000 ≥	5189	70	2.0	0.7 – 1.4
Model adjusted for radiotherapy fields*[2]				
Period of childhood diagnosis				
<1980	1062	156	1(ref)	
1980 – 1989	1887	163	1.0	0.8 – 1.3
1990 – 1999	2479	105	0.9	0.7 – 1.2
2000 ≥	5189	70	0.8	0.5– 1.1
Model adjusted for use of TBI*[3]				
Period of childhood diagnosis				
<1980	1062	156	1(ref)	
1980 – 1989	1887	163	0.9	0.7 – 1.1
1990 – 1999	2479	105	0.8	0.6 – 1.0
2000 ≥	5189	70	0.7	0.5– 1.0
Model adjusted for chemotherapy doses*[4]				
Period of childhood diagnosis				
<1980	1062	156	1(ref)	
1980 – 1989	1887	163	0.8	0.6 – 1.0
1990 – 1999	2479	105	0.7	0.5 – 1.0
2000 ≥	5189	70	**0.5**	**0.3– 0.9**
Model adjusted for use of alkylating agents*[5]				
Period of childhood diagnosis				
<1980	1062	156	1(ref)	
1980 – 1989	1887	163	0.9	0.7 – 1.1
1990 – 1999	2479	105	0.8	0.6 – 1.0
2000 ≥	5189	70	**0.7**	**0.5– 0.9**
Model adjusted for use of anthracyclines*[6]				
Period of childhood diagnosis				
<1980	1062	156	1(ref)	
1980 – 1989	1887	163	0.8	0.7 – 1.1
1990 – 1999	2479	105	**0.7**	**0.5 – 0.97**
2000 ≥	5189	70	**0.6**	**0.4– 0.9**
Model adjusted for use of epipodophyllotoxins*[7]				
Period of childhood diagnosis				
<1980	1062	156	1(ref)	
1980 – 1989	1887	163	0.9	0.7 – 1.0
1990 – 1999	2479	105	**0.7**	**0.5 – 0.9**
2000 ≥	5189	70	**0.6**	**0.4– 0.8**
Model adjusted for use of platinum agents*[8]				
Period of childhood diagnosis				
<1980	1062	156	1(ref)	
1980 – 1989	1887	163	0.9	0.7 – 1.2
1990 – 1999	2479	105	0.8	0.6 – 1.1
2000 ≥	5189	70	**0.6**	**0.4– 0.9**
Model adjusted for use of vinca-alkaloids*[9]				
Period of childhood diagnosis				
<1980	1062	156	1(ref)	
1980 – 1989	1887	163	0.9	0.7 – 1.2
1990 – 1999	2479	105	0.8	0.6 – 1.1
2000 ≥	5189	70	**0.7**	**0.5– 0.99**

***all models were adjusted for sex and age at diagnosis** SMNs: Subsequent malignant neoplasms, TBI: Total body irradiation**.** The models with 'the use of' are adjuster for yes/no administration of the treatment variable of interest, [1.] 271 observations omitted due to missing data [2.] 11 observations omitted due to missing data [3.] 62 observations omitted due to missing data [4.] 905 observations omitted due to missing data [5.] 83 observations omitted due to missing data [6.] 85 observations omitted due to missing data [7.] 89 observations omitted due to missing data [8.] 102 observations omitted due to missing data [9.] 72 observations omitted due to missing data

Appendix I. Multivariable Cox regression model for differences in risk between different diagnosis periods stratified by childhood cancer

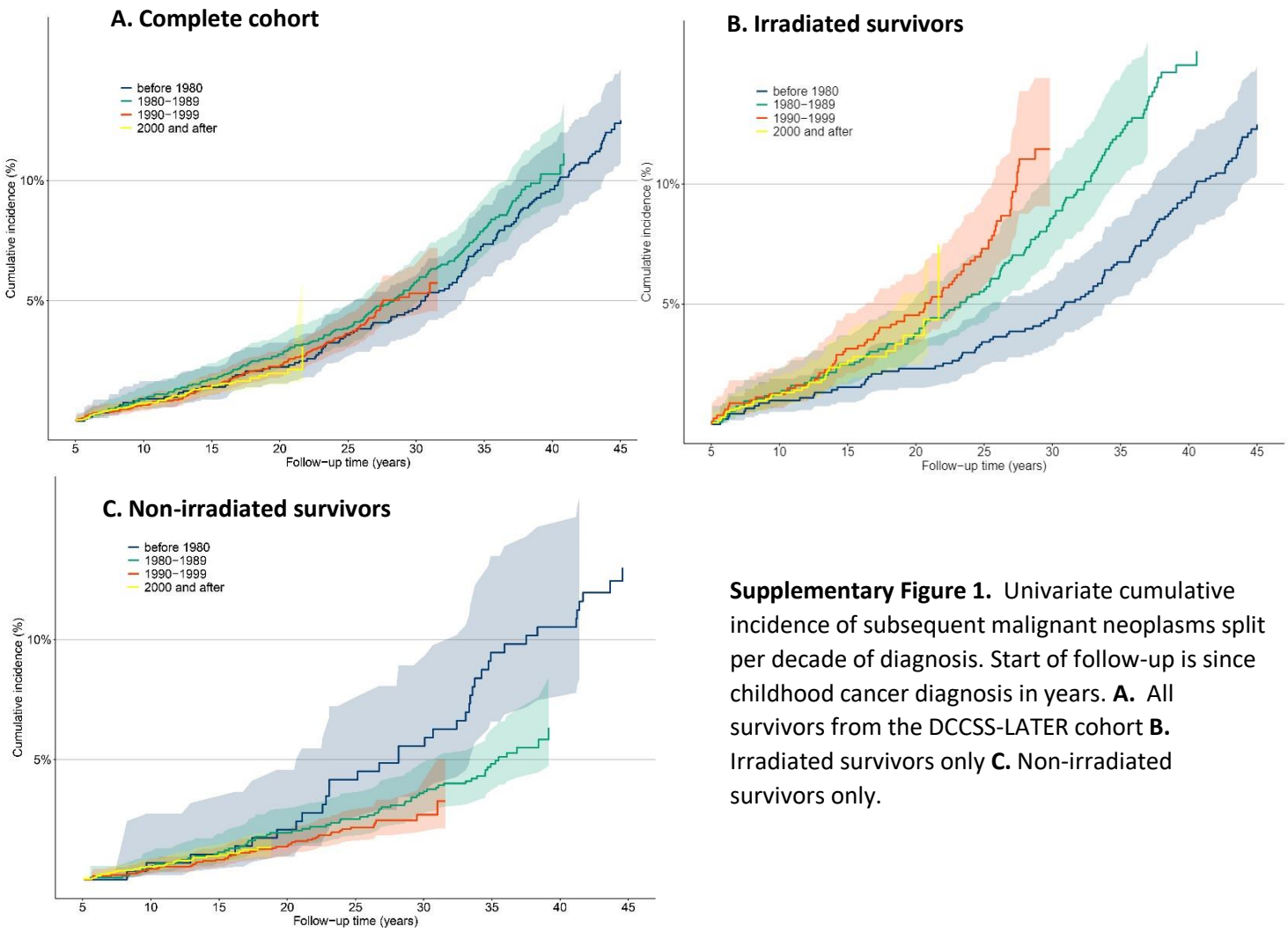
Variable	Total survivors	SMNs	HR	95% CI
Hematological childhood cancers*				
Period of childhood diagnosis				
<1980	461	55	1 (ref)	
1980 – 1989	989	93	1.1	0.8– 1.7
1990 – 1999	1317	54	0.9	0.6 – 1.4
2000 >	2755	30	0.8	0.4 – 1.3
Central nervous system childhood cancers*				
Period of childhood diagnosis				
<1980	105	8	1 (ref)	
1980 – 1989	222	18	2.2	0.7 – 6.5
1990 – 1999	390	16	1.7	0.5 – 5.2
2000 >	787	12	1.5	0.4 - 5.2
Solid childhood cancers*				
Period of childhood diagnosis				
<1980	496	93	1 (ref)	
1980 – 1989	676	52	**0.7**	**0.4 - 1.0**
1990 – 1999	772	35	0.7	0.5 – 1.1
2000 ≥	1642	27	0.6	0.4 – 1.0

***all models were adjusted for sex and age at diagnosis** SMNs: Subsequent malignant neoplasms, HR: Hazard ratio, CI: Confidence interval, CNS: Central nervous system

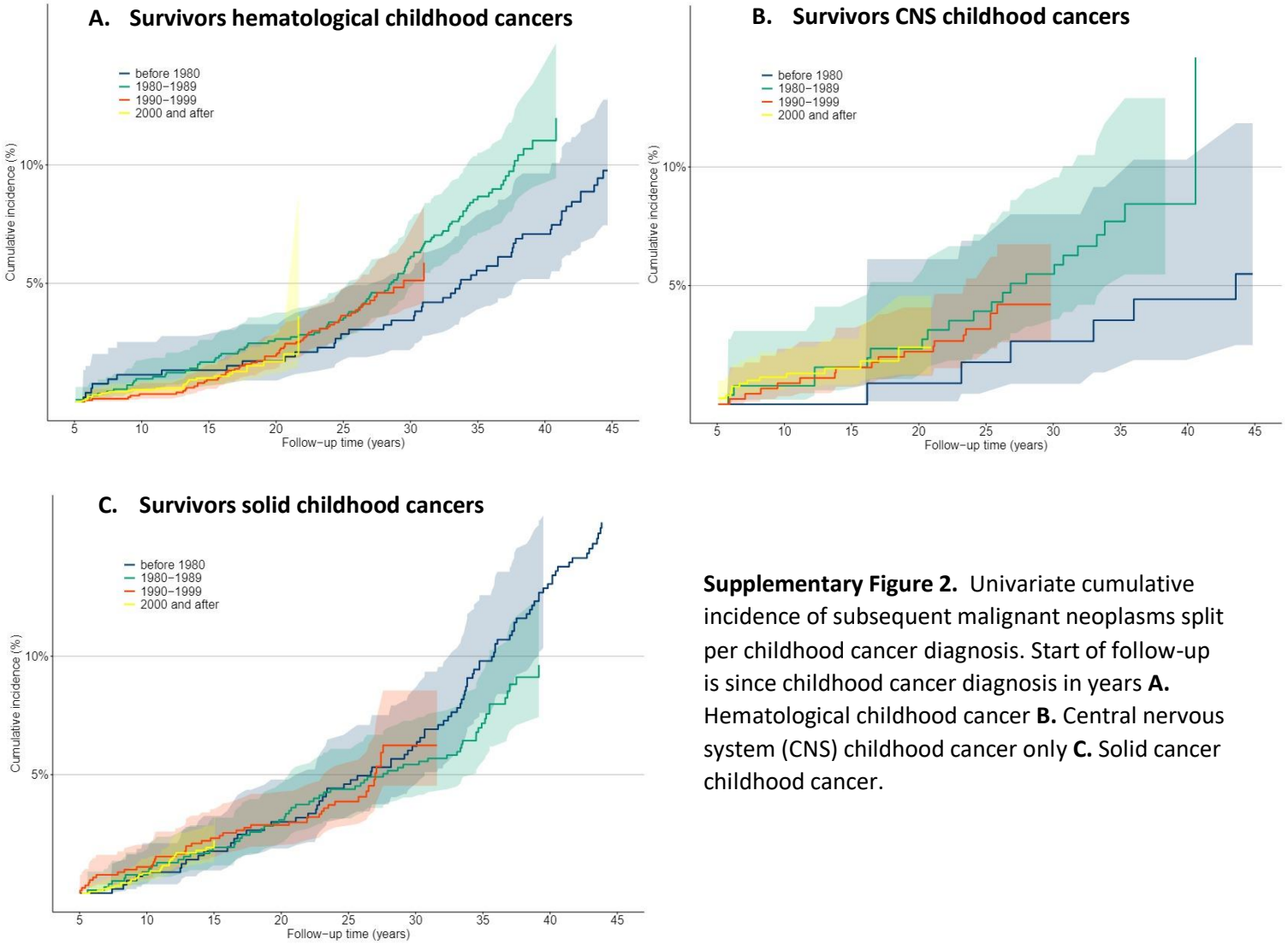
Appendix J. Multivariable Cox regression models and mediation analysis for irradiated survivors, with and without treatment variables for any SMNs

Variable	Total survivors	SMNs	HR	95% CI
Non-irradiated survivors – not adjusted for treatment variables*[1]				
Period of childhood diagnosis				
<1980	256	37	1 (ref)	
1980 - 1989	1070	153	**0.5**	**0.3– 0.8**
1990 – 1999	1767	40	**0.4**	**0.2 – 0.6**
2000 ≥	3830	36	**0.4**	**0.2 – 0.7**
Non-irradiated survivors – adjusted for use of chemotherapy agents* [2]				
Period of childhood diagnosis				
<1980	256	37	1 (ref)	
1980 - 1989	1070	153	**0.4**	**0.3– 0.7**
1990 – 1999	1767	40	**0.3**	**0.2 – 0.5**
2000 ≥	3830	36	**0.3**	**0.2 – 0.6**
Non-irradiated survivors – adjusted for chemotherapy doses* [3]				
Period of childhood diagnosis				
<1980	256	37	1 (ref)	
1980 - 1989	1070	153	**0.5**	**0.3– 0.8**
1990 – 1999	1767	40	**0.4**	**0.2 – 0.7**
2000 ≥	3830	36	**0.4**	**0.2 – 0.8**
Non-irradiated survivors – adjusted for SCT* [4]				
Period of childhood diagnosis				
<1980	256	37	1 (ref)	
1980 - 1989	1070	153	**0.5**	**0.3– 0.8**
1990 – 1999	1767	40	**0.4**	**0.2 – 0.6**
2000 ≥	3830	36	**0.4**	**0.2 – 0.7**

***all models were adjusted for sex and age at diagnosis** SMNs: subsequent malignant neoplasms, TBI: Total body irradiation, SCT: Stem cell transplantation [1.] 8 observations omitted due to missing data [2.] 19 observations omitted due to missing data [3.] 322 observations omitted due to missing data [4.] 26 observations omitted due to missing data.



Supplementary Figure 1. Univariate cumulative incidence of subsequent malignant neoplasms split per decade of diagnosis. Start of follow-up is since childhood cancer diagnosis in years. **A.** All survivors from the DCCSS-LATER cohort **B.** Irradiated survivors only **C.** Non-irradiated survivors only.



Supplementary Figure 2. Univariate cumulative incidence of subsequent malignant neoplasms split per childhood cancer diagnosis. Start of follow-up is since childhood cancer diagnosis in years **A.** Hematological childhood cancer **B.** Central nervous system (CNS) childhood cancer only **C.** Solid cancer childhood cancer.

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Chapter 3

Neuroblastoma survivors at risk for developing subsequent neoplasms: a systematic review

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Cancer Treatment Review. 2022 Mar; 104:102355.

Abstract

Neuroblastoma survivors have an increased risk of unfavorable long-term health outcomes, of which developing subsequent neoplasms is one of the most serious. We aimed to provide an overview of the current knowledge on the risk of subsequent neoplasms in neuroblastoma survivors. We conducted a systematic literature search in Medline/Pubmed (01-01-1945 - 13-01-2022) to identify studies that reported on ≥100 neuroblastoma survivors and assessed subsequent neoplasms as an outcome. We identified 410 potentially eligible articles, of which we eventually included 13 reports. All articles described retrospective cohorts with sizes varying from 145 to 5,987 neuroblastoma survivors. Within these cohorts 0.7% – 17.2% of the survivors developed a subsequent neoplasm. A wide variety of types of subsequent malignant and non-malignant neoplasms were observed, of which thyroid carcinoma and acute myeloid leukemia were most frequently reported. The risk of developing a subsequent neoplasm was 2.8 to 10.4 times higher in neuroblastoma survivors than in the general population. Although no statistically significant risk factors for subsequent neoplasms were observed in multivariable analyses, high-risk group survivors, women and those treated with radiotherapy seemed to have a higher risk. In conclusion, the studies in this systematic review consistently show that neuroblastoma survivors are at elevated risk of developing subsequent neoplasms. Future research should further explore risk factors for subsequent neoplasms in neuroblastoma survivors, so future treatment protocols and follow-up care can be improved.

Introduction

Neuroblastoma is the most common extracranial pediatric solid tumor [1], with a median age at diagnosis of 18 months [2, 3]. This tumor originates from the peripheral sympathetic nervous system and is known for its diversity, exhibiting great variation in location, histopathology, biology and overall outcome [4]. Due to this clinical diversity, current treatment protocols can be quite different between neuroblastoma patients, depending on the expected risk and overall prognosis. Risk classification is based on both clinical and biological factors [5, 6]. Factors that are often used are the patients' age, tumor stage, *MCYN* amplification and additional tumor genetic findings [5, 6]. However, the exact factors differ across research groups [5, 6]. Overall, most high-risk patients are treated with more intensive multi-modality approaches including induction chemotherapy, surgery, stem cell transplantation, radiotherapy and immunotherapy, while low-risk patients are treated with less intensive chemotherapy, and sometimes only with surgery or watchful waiting [4].

Survival rates have improved over the past decades [4, 7]. Currently, five-year survival ranges from 50% for high-risk patients to 95% for low-risk patients [2]. Because more neuroblastoma patients are surviving, it is becoming increasingly important to evaluate long-term health outcomes. Neuroblastoma survivors and other childhood cancer survivors have an increased risk of unfavorable long-term health outcomes, of which subsequent neoplasms are considered to be one of the most serious [8-13].

Although several studies have reported on subsequent neoplasms in neuroblastoma survivors, a systematic overview of the current knowledge on risk of and risk factors for subsequent neoplasms in neuroblastoma survivors is lacking. This knowledge is necessary to identify the survivors who are at higher risk. This might help to improve future treatment protocol designs, follow-up care, and improve the long-term quality of life and overall health of neuroblastoma survivors. Therefore, we systematically reviewed the existing scientific literature on the risk of developing subsequent neoplasms in neuroblastoma survivors and the possible associated risk factors.

Methods

We have used the PRISMA guideline as guidance for the reporting of our review [14].

Literature search

We conducted a systematic literature search in Medline/PubMed from 1945 to January 13th 2022. We combined search terms for neuroblastoma and subsequent neoplasms (Appendix A). We also manually screened the reference lists of the included studies and relevant review papers and consulted experts within this field to identify additional studies that were not obtained through the Medline/PubMed search. We did not impose language restrictions.

We used the following inclusion criteria for the selection of studies: (1) the study population consisted of ≥100 neuroblastoma patients; (2) ≥75% of the neuroblastoma patients were followed for at least two years after primary cancer diagnosis (if the article did not provide information about the number of participants with a follow-up of at least two years, we included the article when the median follow-up of the cohort was >5 years); (3) data on subsequent neoplasms in neuroblastoma survivors were presented (subsequent neoplasms were defined as new neoplasms, not including recurrence of the primary childhood tumor and could include both benign and malignant tumors); (4) the study reported results of an original research investigation; (5) full text publication was available (i.e. not only a conference abstract). We excluded case reports and case series (i.e. a description of non-consecutive participants).

Titles and abstracts were screened by two independent reviewers. If the study seemed to meet the inclusion criteria, we selected it for full-text screening. Full-text articles were also screened by two independent reviewers. Discrepancies between reviewers were resolved by re-examining the article and discussing until consensus was reached. When multiple reports describing the same cohort were identified, we selected the report with the longest follow-up time or the report that focused the most on subsequent tumors. If the other, non-selected, report(s) presented results on specific subsequent neoplasms that were not presented in the selected report, we also included these results.

Data extraction

Data extraction was done by two independent reviewers. We extracted data on study characteristics, participant characteristics, treatment characteristics, outcome measures, (i.e. the numbers of subsequent tumors and their type, and, if reported, risk estimates of subsequent tumors compared to the general populations (standardized incidence and absolute excess risks), cumulative incidence, and risk factors from multivariable analyses). Discrepancies were resolved by discussing to reach a consensus.

Risk of bias assessment

The risk of bias in included studies was assessed by two independent reviewers. The risk of bias was scored for selection bias, attrition bias and detection bias. If a study assessed risk factors in a multivariable analysis, we also scored confounding bias (Appendix B). Discrepancies were resolved by discussing to reach a consensus or consultation of a third reviewer.

Results

Results of the search

Our search identified 410 unique articles. We selected 100 articles for full-text screening. Of those, we eventually included 15 articles [9, 10, 15-27]. In addition, we included two more articles after consulting experts in this field [28, 29]. Screening references lists did not yield any additional articles. Finally, we excluded four articles [9, 10, 24, 25] because they published data on the same cohorts as other included articles. In total, we included 13 articles in this systematic review [15-23, 26-29]. Figure 1 shows a flow diagram of the selection of studies.

Study characteristics

The main study characteristics of the included studies are listed in Table 1. Of the 13 studies, seven included only neuroblastoma survivors, while six evaluated multiple childhood cancer types, but reported separate results on neuroblastoma survivors. The inclusion period of all studies was between 1936 and 2015. All articles described retrospective cohort studies. Seven articles reported a cohort size between 145 to 1,000 neuroblastoma survivors [15, 17, 19, 23, 26, 27, 29] and five articles reported a cohort size of more than 1,000 neuroblastoma patients [16, 18, 20-22]. For one study, the exact cohort size was unknown because they only reported

the number of person years [28]. Six studies included 5-year neuroblastoma survivors, one study included 2-year survivors, and one study included 2-month survivors, while the other studies did not have a minimum survival period. The median follow-up of neuroblastoma survivors, if reported, ranged from 5.3 years to 24.3 years [15-23, 26-29]. Due to the heterogeneity of included studies pooling of results was not feasible.

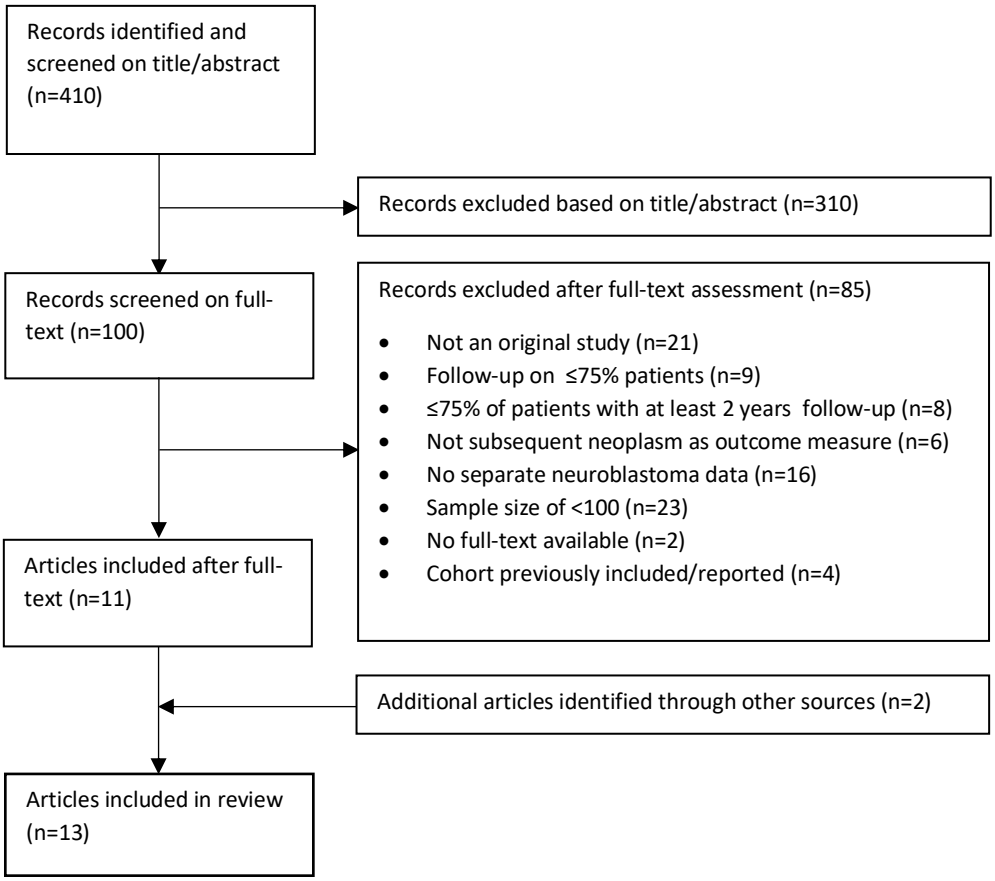


Figure 1. Selection of the articles identified through literature search

Most studies focused on subsequent malignant neoplasms (SMNs) only. However, three studies also included benign neoplasms in addition to SMNs: Friedman et al. included nonmalignant meningiomas [15], Reulen et al. included nonmalignant bladder neoplasms [28], and Haghiri et al. included all benign neoplasms [26]. One study examined only thyroid carcinoma as a subsequent neoplasm [17].

Number of subsequent neoplasms

The numbers of reported subsequent neoplasms ranged from 5 to 46 [15-23, 26-29] as shown in Table 2. The percentage of subsequent neoplasms varied between 0.7% and 17.2% and the highest percentage was found in the study by Haghiri in a high-risk neuroblastoma cohort after a median follow-up of 15.2 years after primary diagnosis [range: 5.0-35] [26]. This was the only study that also included all benign tumors. However, even when only considering malignant tumors, the percentage (7.6%) was higher than other studies. For one study it was not possible to calculate a percentage [28]. The most frequently described subsequent neoplasms were thyroid carcinoma [15, 16, 19, 20, 22, 23, 26-28, 30], acute myeloid leukemia (AML) [15, 16, 18, 20-23, 26, 27].

Standardized incidence ratio, absolute excess risk and cumulative incidence of subsequent neoplasms in general

Most studies (n=7; 53%) reported the standardized incidence ratio (SIR) of any SMN after neuroblastoma, calculated as the ratio of the observed number of SMNs and the expected number of SMNs based on age-, sex-, and calendar year-specific general population. SIRs ranged from 2.8 to 10.4 [15, 16, 18, 20-23, 28] (Table 3). Six studies (46%) also estimated the absolute excess risk (AER), calculated as the absolute excess numbers of SMNs after neuroblastoma per number of person-years of follow-up. AERs of any SMN ranged from 7.5 to 17 per 10,000 person years of follow up [15, 16, 18, 21, 23, 28]. Cumulative incidence of SMNs was calculated in seven studies (53%). The studies reported estimates on different time points. Four studies (31%) reported the 20-year cumulative incidence with values ranging between 1.4% and 7.1% [16, 18, 22, 23]. Three studies (23%) reported the 30-year cumulative incidence with values ranging between 2.9% and 8.9% [15, 16, 23].

In addition, eight studies (62%) reported SIRs for specific SMN subtypes, with the highest SIRs reported for thyroid cancer, AML and renal carcinomas. SIRs for thyroid cancer were reported in five studies and ranged between 12.4 and 350 [16, 17, 20, 22, 24]. SIR for AML among any neuroblastoma survivors was reported in one study (SIR, 15; 95% CI: 4.0-34) [16]. SIRs for renal carcinoma were reported by three studies and ranged between 27 and 128.2 [16, 20, 25]. In addition, four studies (31%) reported AERs for specific SMN subtypes [15-17, 28], with the highest AERs reported for thyroid cancer (AERs ranging between 1.3 and 14 excess cases per

10,000 person-years) and kidney and renal pelvis tumors (AERs ranging between 2.1 and 4.2 excess cases per 10,000 person-years [16, 25]).

Stratified standardized incidence ratio, absolute excess risk and cumulative incidence of subsequent neoplasms in different subgroups

Three studies (23%) reported risks of any SMN stratified for different risk groups of neuroblastoma patients. One study analyzed only high risk patients, reporting a SIR of 25.0 (95% CI: 12.4–44.5) and an AER of 47.1 (95% CI: 25.8–86.1) [26] In another study, SIRs were reported to be 17.5 (95% CI: 11.4-25.3) for the high-risk neuroblastoma patients, 4.8 (95% CI: 2.1-9.4) for intermediate-risk patients and 3.1 (95% CI: 1.4-6.2) for low-risk patients [21]. In the same study, the AER per 10,000 person-years of follow-up was 27.6 for the high-risk group, 6.0 for the intermediate-risk group, and 3.7 for the low-risk group [21]. Another study presented SIRs for AML after neuroblastoma by risk group, which were 106.8 for the high-risk patients, 127.7 for intermediate-risk patients and 23.2 for the low-risk patients [21]. Additionally, two studies presented separate cumulative incidence measures for different risk groups of neuroblastoma patients, both reporting a higher cumulative incidence for the high-risk groups compared to the low-risk groups [20, 21]. The first study reported 30-year cumulative incidences of 3.57% (95% CI: 1.87-6.12) for the low-risk group and 10.44% (95% CI: 3.98-20.52) for the high-risk group [20]. The second study reported 10-year cumulative incidences of 0.38% (95% CI: 0.22-0.94) for the low-risk group, 0.56% (95% CI: 0.34 - 1.3) for the intermediate-risk group and 1.8% (95% CI: 1.0-2.6) for the high-risk group [21]. Another study analyzed only the high-risk group and reported a 10-year cumulative probability of 2.4 (95% CI: 0–5.1) and a 20-year cumulative probability of 12 (95% CI: 3–21) [26]. One study that focused on infants with stage 4(m) nonamplified MYCN neuroblastoma, reported a 10-year cumulative incidence of 2.7%. (95% CI: 1.6–3.8%)[27]. Another study evaluated risk of any SMN by sex and found SIRs of 13 (95% CI: 7–24) for women and 5 (95% CI: 1.3–13) for men [23].

Furthermore, two studies (18%) calculated risk of any SMN by calendar period of neuroblastoma diagnosis. One study showed that SMN risk was higher for those diagnosed before 1970 compared to those diagnosed in the 1970s (1948-1959 SIR: 21.3 (95% CI 7.6–45.8); 1960-1969: SIR: 10.4 (95% CI 3.7–22.4); 1970-1979: SIR: 6.8 (95% CI 1.1–20.9), p=0.04)

[23]. The other study did not observe any differences in incidence rates between treatment eras [20]. Compared with era 1 (1973-1989), the incidence rate ratios were 0.34 (95% CI: 0.12–1.01; p=0.051) in era 2 (1990–1996) and 0.76 (95% CI: 0.35–1.62; p=0.48) in era 3 (1997–2006) [20]. Ten years from diagnosis, the cumulative incidences were 0.49% (95% CI 0.17 – 1.20%) for era 1 and 1.26% (95% CI 0.55–2.51%) for era 3, but this difference was not statistically significant (p=0.28) [20].

Risk factors for subsequent neoplasms (multivariable analyses)

Two studies (18%) evaluated risk factors for developing subsequent neoplasms in multivariate analyses [17, 23]. However, no factors were identified that were clearly statistically significantly associated with subsequent neoplasm risk. One study compared the risk between women and men and showed a relative risk of SMN of 2.9 (95% CI: 0.7–9), after adjusting for chemotherapy and radiotherapy [23]. For radiotherapy, one study compared risk in neuroblastoma patients treated with and without and showed a relative risk of SMN of 4.3 (95% CI: 0.8–78) after adjusting for diagnostic period, sex, age at diagnosis and follow-up duration [23]. Another study examined effects of radiotherapy dose on risk of thyroid cancer and found an excess absolute risk of 2.1 per 1,000,000 person years per centigray increase of exposure (p-value not reported) [17]. For chemotherapy, a relative risk of 0.4 (95% CI: 0.1–1.9) was reported for patients treated with compared to treated without chemotherapy [23].

Risk of bias in included studies

Most studies (69%) clearly reported the number of neuroblastoma patients that were included from the original cohort and at least 90% was included in the study, scoring low at risk for selection bias. The other four studies (31%) had an unclear risk of bias. The risk for attrition bias was scored as low in nine studies (69%) and high in one study (8%).Three studies (23%) scored an unclear risk of attrition bias as they did not clearly report the follow-up completeness. All studies had an unclear risk of detection bias as no information on the blinding of outcome assessors was provided. Two (15%) studies performed multivariable risk factor analyses, but it was unclear if they also adjusted for either chemotherapy or age and follow-up duration. Therefore, these studies had an unclear risk of confounding bias (Table 4).

Table 1 Characteristics of included studies.

Author (year)	Origin cohort	Study design	Inclusion period	NB cohort size	Age at NB diagnosis	Risk groups	Gender M/F	Treatment	Minimal survival after NB diagnosis	Median follow-up time (years)	Follow-up starting point	Method of ascertainment second neoplasms	Number of second neoplasms (%)
Neuroblastoma specific studies													
Rubino (2003) [23] [a]	French and Great Britain treatment centers	Retrospective cohort	1948-1986	544	Median: 11 months	NM	272 (50%) 272 (50%)	CT: 173 (31.8%) RT: 85 (15.6%) CT + RT: 214 (39.4%) Surgery: 72 (13.2%)	5 years [o]	15	After diagnosis	Clinical records by physicians or hospital physicist	12 (2.2)
Applebaum (2015)[20] [b]	Surveillance, Epidemiology and End Results (SEER) + sub database 2013 Hurricane Katrina Impacted Louisiana Cases	Retrospective cohort	1973-2006	2801	Median with SMN: 1.5 years Median no SMN: 1 year	Low risk: 1,694 (60.5%) High risk: 946 (33.8%) [L]	1477 (52.7%) 1324 (47.3%)	RT: 25.0% Other treatment details not mentioned	NM	6.2	NM	Linkage to registry	34 (1.2)
Haupt (2010)[22] [c]	Italian Neuroblastoma Registry (ING)	Retrospective cohort	1979-2005	2216	0-17 months: 1010 (45.6%) 12-59 months: 896 (40.4%) >60 months: 310 (14%)	INSS stage: 1: 372 (16.8%) 2: 301 (13.6%) 3: 424 (19.1%) 4: 929 (41.9%) 4S: 190 (8.6%)	1219 (55%) 997 (45%)	NM	None	7.0	After diagnosis	Clinical follow-up	21 (0.9)
Youlden (2020)[18]	Australian Childhood Cancer Registry (ACCR)	Retrospective cohort	1983-2015	1148	<18 months: 596 (47.0%) 18 months - 4years: 519 (40.9%) 5–14 years: 125 (9.9%) 10–14 years: 29 (2.3%)	Non-metastatic: 541 (42.6%) Metastatic: 668 (52.6%) Not stated: 60 (4.7%)	631 (55.0%) 517 (45.0%)	CT: 777 (67.7%) RT: 275 (24.0%) Surgery: 734 (63.9%)	None	NM	2 months after diagnosis	Linkage with Australian Cancer Database	13 (1.1)
Applebaum (2017)[21] [d]	The International Neuroblastoma Risk Group (INRG)	Retrospective cohort	1990-2010	5987	Median with SMN: 27.5 months Median no SMN: 18. months	Low risk: 2334 Intermediate risk: 1493 High risk: 2161 [m]	SMN: 16 (37.2%) 27 (62.8%) No SMN: 3174 (53.4%) 2770 (46.6%)	NM	NM	5.3	After diagnosis	Institutional reporting SMN according to ICD-O-10	43 (0.7)
Haghiri (2021)[26] [e]	Gustave Roussy (GR) hospital	Retrospective cohort	1980-2012	145	Median: 2.6 years (0–18.2)	High risk	77(53.1%) 68 (46.9%)	High dose CT: 145 (100%) RT: 66 (45.5%) (mean dose = 27 Gy) Surgery: 142 (97.7%) ASCR: 145 (100%) Immunotherapy: 8 (5.5%)	5 years	15.2	After diagnosis	Medical files	Total: 25 (17.2%) SMN: 11 (7.6%) Benign: 14 (9.6%)

								Retinoic acid: 47 (32.4%)					
Berthold (2021)[27]	TheNB90, NB97, andNB2004 trials of the German Pediatric Oncology Society	Retrospective cohort	1990-2015	177	0–2 months 12 (7%) 3–5 months 19 (11%) 6–8 months 29 (16%) 9–11 months 29 (16%) 12–14 months 46 (26%) 15–17 months 42 (24%)	stage 4(M) MYCN nonamplified	88 (50%) 89 (50%)	CT: 166 (94%) RT: 25 (14%) Surgery: 146 (82%) MIBG: 12 (7%) ASCT: 39 (22) Antibody therapy: 34 (19%)	NM	9.7	NM	NM	6 (3.3%)
General childhood cancer survivor studies with neuroblastoma as subgroup													
Tucker (1991)[17] f	Late effect study group	Retrospective cohort	1936-1979 j	790	Mean: 2 years	NM	NM	Mean dose to the thyroid: 660 cGy [range: 0-3000 cGy] Other treatment details not mentioned	2 years	Mean: 5.5 j	2 years after diagnosis	Registry	7 (0.9) n
Reulen (2011)[28] g	British Childhood Cancer Survivors	Retrospective cohort	1940-1991	16 970 PY k	<15 years	NM	NM	NM	5 years	24.3 j	After diagnosis	National population-based death and cancer registration systems	29
Smith (1993) [19] d	The University of Texas MD Anderson Cancer Center	Retrospective cohort	1951-1991	202	NM	NM	NM	NM	NM	Mean: 15 j	After diagnosis	Records	5 (2.5)
Teepen (2017)[29] c	Dutch Childhood Cancer Oncology Group-Long term Effects after Childhood Cancer Cohort (DCOG LATER)	Retrospective cohort	1963-2001	324	<18 years	NM	NM	NM	5 years	20.7 j	After diagnosis	Linkages with the Netherlands Cancer and pathology Registry + Medical follow-up	9 (2.8)
Zong (2017)[16] h	Surveillance, Epidemiology and End Results (SEER) and eight provincial cancer registries in Canada	Retrospective cohort	1969-2010	472 6	<15 years	NM	NM	NM	2 months	7.4 j	After diagnosis	Linkage to registry	46 (1.0)
Friedman (2010)[15] i	Childhood Cancer Survivor Study (CCSS)	Retrospective cohort	1970-1986	955	<21 years	NM	NM	NM	5 years	22.9 j	After diagnosis	Self- or proxy report in questionnaires and/or death certificate. Followed by pathology report or other medical records	45 (4.7)

NB. Neuroblastoma; SMN: Subsequent malignant neoplasms; NM. Not mentioned; PY. Person years; CT. Chemotherapy; RT. Radiotherapy: INSS: International Neuroblastoma Staging System; ASCR: Autologous stem cell rescue; ASCT: Autologous stem cell transplantation; MIBG : iodine-metaiodobenzylguanidine

a Potentially partly overlaps with Reulen (2011), but the level of overlap is unclear.
b Overlaps partly with Zong (2017) but is still included because additional data was presented. Also potentially partly overlaps with Applebaum (2017), Tucker (1991), Smith (1993), and Friedman (2010), but the level of overlap is unclear.
c Potentially partly overlaps with Tucker (1991) but the level of overlap is unclear.
d Potentially partly overlaps with Applebaum (2015) and Zong (2017) but the level of overlap is unclear.
e Potentially partly overlaps with Rubino (2003), but level of overlap is unclear
f Potentially partly overlaps with Haupt (2010), Reulen (2011), Friedman (2010), Teepen (2017), Applebaum (2015) and Zong (2017), but the level of overlap is unclear.
g Potentially partly overlaps with Rubino (2003), but the level of overlap is unclear.
h Potentially partly overlaps with Applebaum (2015) but is still included because additional data was presented. Also possibly partly overlaps with Friedman (2010), Applebaum (2017), Smith (1993) and Tucker (1991), but the level overlap is unclear.
i Potentially partly overlaps with Smith (1993), Applebaum (2015), Tucker (1991) and Zong (2017), but the level of overlap is unclear.
j Reported for the whole cohort, not only for the neuroblastoma group.
k Number was not reported, only person years.
L Low-risk: less than one year old at diagnosis or localized disease. High-risk: older than one year old at diagnosis with distant spread of their disease.
m Risk group was assigned according to the COG classification system, based on INSS stage, age, tumor histology, ploidy, and MYCN status.
n Only included thyroid cancer as subsequent neoplasms.
o It was not clearly stated that this was after primary diagnosis in the articles but can be assumed

Table 2 Overview of types of subsequent neoplasms in the included studies.

			Hematologic neoplasms						Solid neoplasms											Unspecified neoplasms
			Leukemia			Lymphoma		Unspecified hematological neoplasms	Sarcoma			Breast	Thyroid	Kidney	Skin		CNS	Other solid	Unspecified solid neoplasms	
Author (year)	Total SNs (%)	Median interval since NB diagnosis (years)	AML	ALL	Other or unspecified leukemia	HL	NH		Bone	Soft tissue	Unspecified sarcoma				NMSC	Melanoma				
Rubino (2003)[23] [k]	12 (2.2)	19.5	1						1	1		3	5				1			
Applebaum (2015)[20]	34 (1.2)	11.6	6					4			7	1	5	7		1	1	2 [c]		
Haupt (2010)[22]	21 (0.9)	11.3	3		4	1	2			1			8					2 [g]		
Youlden (2020)[18]	13 (1.1)	4.5	6																	7
Applebaum (2017)[21]	43 (0.7)	3.4	10		9 [f]						12						6		6	
Haghiri (2021)[26] [k]	25 (27.2%)	18.3	2						1				5 [h]	1		1	2	13 [i]		
Berthold (2021)[27]	6 (3.3%)	Range 3.8-10.4	1		2								1	1				1 [j]		
Tucker (1991)[17] [b]	7 (0.9)	NM											7							
Reulen (2011)[28] [k]	29	NM			2					3		1	1		8	1	3	6 [d]	3 [a]	1
Smith (1993)[19]	5 (2.5)	NM							3				2							
Teepen (2017)[29] [k]	9 (2.8)	NM																		9
Zong (2017)[16]	46 (1.0)	NM	4		2				2	4			6	9				4 [e]		15
Friedman (2010)[15] [k]	45 (4.7)	NM	3	1		1			4			2	9		6		2		17	
Total			**36**	**1**	**19**	**2**	**2**	**4**	**11**	**9**	**19**	**7**	**49**	**18**	**14**	**3**	**13**	**29**	**26**	**32**

NB: Neuroblastoma; SN: Subsequent neoplasms; NM: Not mentioned; AML: Acute Myeloid Leukemia; ALL: Acute Lymphocytic Leukemia; HL: Hodgkin lymphoma; NH: Non-Hodgkin lymphoma; NMSC: Non-melanoma skin cancer; CNS: Central nervous system

a The three unspecified solid neoplasms were all genitourinary neoplasms, not further specified, which potentially also include neoplasms of the kidney.
b This study only included thyroid cancer as subsequent neoplasm.
c One tongue and one ovarian.

d Two oral cavity, three digestive and one respiratory.
e Two saliva gland, one non-epithelial skin and one within the Trachea, mediastinum and other respiratory organs.
f Nine cases were reported as 'ALL or lymphoma'
g Two schwannomas
h Three thyroid carcinomas and two thyroid adenomas
l Second malignancies: one cholangiocarcinoma, one neurofibrosarcoma Second benign neoplasms: Six osteochondroma, one chondroma, one osteid osteoma, one schwannoma, one hepatic adenoma, one pilomatrixoma,
j One nerve sheath tumor
k These studies only included 5 year survivors and may therefore underestimate the number of subsequent neoplasms that often occur within the first five years, , such as myelodysplastic syndromes and acute myeloid leukemia

Table 3 Risk measures for developing subsequent malignant neoplasms

Author (year)	NB cohort size	Median follow-up time (years)	Follow-up starting point	Standardized Incidence Ratio (95% CI)		Absolute Excess Risk per 10,000 person years (95% CI)		Cumulative incidence % (95% CI)	
				Any SMN	Specific SMN subtypes	Any SMN	Specific SMN subtypes	Any SMN	Specific SMN subtypes
Neuroblastoma specific studies									
Rubino (2003)[23] [j]	544	15	After diagnosis	10.4 (3.5-17.4) Women: SIR: 13 (7–24) Men: SIR: 5 (1.3–13) By treatment era: 1948-1959: 21.3 (7.6–45.8) 1960-1969: 10.4 (3.7–22.4) 1970-1979: 6.8 (1.1–20.9	Thyroid RT dose none or <5 Gy: 160 (51-365) [h] RT dose ≥5 Gy: 1532 (363-4062) [h]	12.91 (5.71–23.56) [ł]	NM	20y: 2.2 (0.3-4.1) 25y: 3.6 (0.9-6.3) 30y: 8.9 (2.5-15.5)	NM
Applebaum (2015)[20]	2801	6.2	NM	5.6 (3.9-7.9);	Thyroid: 12.4 (4.0-28.9) Renal: 128.2 (51.3-254.0)	NM	NM	30y high risk group: 10.44 (3.98-20.52) 30y low risk group: 3.57 (1.87-6.12)	NM
Haupt (2010)[22]	2216	7.0	After diagnosis	8.4 (5.1-13.2)	Thyroid 131.7 (56.9 - 295.5)	NM	NM	20y: 7.1 (4.1 - 12.1)	NM
Youlden (2020)[18]	1148		2 months after diagnosis	All SMN overall: 5.18 (3.01-8.91) All SMN (primary diagnose NBL >18 months): 13.57 (6.47-28.46)	NM	9.2 (7.6-10.2)	NM	20y: 1.4 (0.7–2.4)	NM
Applebaum (2017)[21]	5987	5.3	After diagnosis	Overall: 7.5 (5.4 - 10.0) High risk: 17.5 (11.4-25.3) Intermediate risk: 4.8 (2.1-9.4) Low risk: 3.1 (1.4-6.2)	AML: High risk: 106.8 (28.7–273.4) Intermediate risk: 127.7 (25.7–373.3) Low risk: 23.2 (0.3–128.9)	Overall: 10.8 High risk: 27.6 Intermediate risk: 6.0 Low risk: 3.7	NM	10y high risk: 1.8 (1.0-2.6) 10y intermediate risk: 0.56 (0.34 - 1.3) 10y low risk: 0.38 (0.22-0.94)	NM
Haghiri (2021)[26] [j]	145	15	After diagnosis	High risk: 25.0 (12.4–44.5)	NM	High risk: 47.1 (25.8–86.1)	NM	10y high risk: 2.4 (0–5.1) [i] 20y high risk: 12 (3–21) [i]	NM
Berthold (2021)[27]	177	9.7	NM	NM	NM	NM	NM	10y stage 4(M): 2.7 (1.6–3.8)	NM
General childhood survivor studies with neuroblastoma as subgroup									

Tucker (1991)[17]	790	Mean: 5.5 [a]	2 years after diagnosis	NM	Thyroid: 350	NM	Thyroid: 14	NM	NM
Reulen (2011)[28] [j]	16 970 PY [c]	24.3 [a]	After diagnosis	2.8 (1.8 - 4.3)	Digestive: 4.5 (1.5 to 14.0) Glioma: 3.0 (0.8 to 12.2) Breast: 0.8 (−0.8 to 2.4) Genitourinary: 1.8 (0.6 to 5.7)	7.5 (2.4-12.7)	Digestive: 1.4 (−0.6 to 3.4) Glioma: 0.8 (−0.8 to 2.4) Breast: −0.2 (−1.3 to 1.0) Genitourinary: 0.8 (−1.2 to 2.8)	NM	NM
Zong (2017)[16] [b]	4726	7.4 [a]	After diagnosis	5.3 (3.9 - 7.0)	Thyroid: 13 (4.7 - 25) Kidney and renal pelvis: 27 (12-48) Colon [e] 16 (0 - 64) Bones and joints 7.1 (0.7-20) Soft tissue including heart: 11 (2.8 - 24) Other non-epithelial skin: 22 (0-88) Salivary gland: 66 (6.2-190) AML: 15 (4.0-34) Other leukemia: 21 (2-61)	8.9 [ǂ]	Thyroid: 1.3 [ǂ] Kidney and renal pelvis: 2.1 [ǂ] Colon [e]: 0.22 [ǂ] Bones and joints: 0.41 [ǂ] Soft tissue including heart: 0.87 [ǂ] Other non-epithelial skin: 0.23 [ǂ] Salivary gland: 0.47 [ǂ] AML: 0.89 [ǂ] Other leukemia: 0.46 [ǂ]	20y: 1.6 [d] 25y: 2.1 [d] 30y: 2.9 [d]	NM
Friedman (2010)[15] [j]	955	22.9 [a]	After diagnosis	6.9 (4.9 - 9.7)	Thyroid: 27.4 (14.3 – 52.7) [f] Renal: 85.8 (38.4 - 175.2) [g]	16 [ǂ] (10-24)	Renal: 4.21 (1.79 – 8.34) [g ǂ]	30y : 5.9 (3.6 - 8.3) [b]	30y NMSC: 2.0 (0.0-4.3) 30y Meningioma: 0.0

NB: Neuroblastoma; SMN: Subsequent malignant neoplasm; NM: Not mentioned; PY: person years; AML: acute myeloid leukemia; y: year; NMSC: non-melanoma skin cancer

a Reported for the total cohort, not only for the neuroblastoma group.
b Excluding non-melanoma skin cancer.
c Number was not reported, only person years.
d Numbers were extracted from a Figure (Fig.2b) and therefore might deviate slightly from the true numbers.
e Excluding rectum, trachea, mediastinum and other respiratory organs.
f Result comes from Bhatti (2010), which was eligible based on our inclusion criteria, but was excluded because Friedman (2010) analyzed the same cohort and reported on all subsequent neoplasms and Bhatti (2010) only on thyroid cancer. However, because Friedman (2010) did not report a separate risk estimate for thyroid cancer, this result from Bhatti (2010) was added to this table.
g. Result comes from Wilson (2012), which was eligible based on our inclusion criteria, but was excluded because Friedman (2010) analyzed the same cohort and reported on all subsequent neoplasms and Bhatti (2010) only on renal cancer. However, because Friedman (2010) did not report a separate risk estimate for renal cancer, this result from Wilson (2010) was added to this table.
h Result comes from Vathaire (1999), which was eligible based on our inclusion criteria, but was excluded because Rubino (2003) analyzed the same cohort and reported on all subsequent neoplasms and Vathaire (1999) only on thyroid cancer. However, because Rubino (2003) did not report a separate risk estimate for thyroid cancer, this result from Vathaire (1999) was added to this table.
i Cumulative probability estimated by the Kaplan-Meier method
j These studies only included 5 year survivors and may therefore underestimate the number of subsequent neoplasms that often occur within the first five years, such as myelodysplastic syndromes and acute myeloid leukemia

ǂ Calculated from 100,000 person years to 10,000 person years by dividing all numbers with 10.

Discussion

This systematic review summarized for the first time the current evidence on subsequent neoplasms in neuroblastoma survivors. Neuroblastoma survivors are at increased risk of developing subsequent neoplasms as compared to the general population, even many years after their primary diagnosis. A wide variety of types of subsequent malignant and non-malignant neoplasms were observed, of which thyroid carcinoma and acute myeloid leukemia were most frequently reported. Studies suggest that high-risk neuroblastoma patients, neuroblastoma patients treated with radiotherapy, and women have a higher risk of subsequent neoplasms. However, no statistically significant risks factors have been identified in multivariable analyses.

Thyroid carcinoma was one of the most frequently reported subsequent neoplasms in neuroblastoma survivors [15, 16, 19, 20, 22, 23, 26-28] and also showed high SIRs (range: 12.4-350) [17]. The association between thyroid carcinoma and radiation exposure has been evaluated by only one study in our review, which identified a dose-dependent increased risk for estimated dose to the thyroid gland, but did not report whether this was statistically significant or not [17]. However, other studies in childhood cancer survivors and in other radiation-exposed populations have also shown dose-dependent risks of developing thyroid carcinoma after radiation exposure [24, 31, 32]. Neuroblastoma survivors have been suggested to be more susceptible than other childhood cancer survivors to develop thyroid carcinoma after radiation [24, 33], which might be due to the younger age at exposure for neuroblastoma patients or to other, unknown reasons. In addition, neuroblastoma survivors have undergone iodine-metaiodobenzylguanidine (MIBG) delivery for diagnostic (^{131}I MIBG or ^{123}I MIBG) and/or therapeutic (^{131}I MIBG) purposes, with difficulties in effective protection of the thyroid gland [34, 35]. MIBG treatment is also associated with damage of the thyroid gland [34], which can possibly contribute to development of thyroid carcinoma [35]. However, none of our included articles evaluated thyroid carcinoma in relation to MIBG related radiation exposure.

We also observed that AML was one of the most reported subsequent neoplasm in neuroblastoma survivors [15, 16, 18, 20-23, 26, 27]. The SIR for AML exceeded 100 in high-risk

and intermediate-risk patients, while no significant elevated SIR was observed among low-risk neuroblastoma patients [21]. The association between chemotherapy and treatment-related AML is well-known for many cancer types [36] and is mainly attributed to exposure to alkylating agents and topoisomerase-II inhibitors [37]. High-risk neuroblastoma patients usually receive intensive multi-modality treatments, including, sometimes dose-intensive, protocols with alkylating agents and topoisomerase-II inhibitors [4]. Reducing the number of dose-intensive induction cycles has been shown to decrease the risk of leukemia [38]. Of note, treatment-related AML often presents within five years of diagnosis [39] and would therefore not be recorded in studies that only include patients with a minimum survival time of five years after diagnosis (five studies in our review) [15, 23, 26, 28, 29], which may have resulted in an underrepresentation of AML as a subsequent neoplasm.

Renal cell carcinoma was a frequently reported subsequent neoplasm in neuroblastoma survivors [16, 20, 26, 27] with one of the highest reported SIRs (range 27-128.2) among all subsequent neoplasms [20]. Renal cell carcinoma in childhood cancer survivors has been linked to renal-directed radiotherapy of 5 Gy or greater and possibly also to platinum-based chemotherapy [25]. Specific chromosome translocations in renal carcinomas arising after chemotherapy have been reported [40]. However, renal cell carcinomas also occur in neuroblastoma patients who have not been treated with chemotherapy or abdominal radiotherapy [41]. This might suggest that an underlying genetic predisposition may play a role. Neuroblastoma survivors seem to be at higher risk of developing renal cell carcinoma compared to other childhood cancer survivors for unknown reasons [25]. Renal cell carcinoma after neuroblastoma is recognized as a distinct subtype by the World Health Organization since 2004 [42], but the tumors seem to be very heterogeneous between neuroblastoma patients with regard to morphological, immunohistochemical, and molecular features, with possibly also different etiologies [41].

Two studies reported on possible risk factors for developing subsequent neoplasms in multivariable analyses. Radiotherapy was associated with a non-significantly increased risk of any subsequent neoplasm [23] and with a dose-dependent increased risk of thyroid cancer (significance level not reported) [17]. Chemotherapy was not found to be significantly associated with risk of any subsequent neoplasm [23]. Furthermore, women were reported to

have a non-significantly higher risk of subsequent neoplasms than men [23]. Non-significant differences could be a result of low power due to a small sample size. Also, subsequent neoplasms can develop years after treatment, so the length of follow-up could have been too short to have sufficient number of cases to detect significant differences between factors.

Treatment protocols for neuroblastoma have evolved over time [43]. Differences between treatment eras were analyzed by two studies in this systematic review: one study reported no differences in risk between different treatment eras [20], while the other study reported a significant decreased risk for those treated after 1970 compared to those treated earlier [23]. Before 1990 treatment was based on age and stage of the patient. Since risk-based approaches started, high-risk patients have been treated with increasingly intensive and multi-modality approaches, whereas intermediate and mostly low-risk patients have been treated with less aggressive therapy [44-46].

Also, treatment protocols differ across countries and even hospitals. The studies included in our review likely overrepresented low- and intermediate-risk patients compared to current clinical practice due to limited survival of high-risk patients in earlier decades. Treatment reductions over time in low- and intermediate-risk patients may lead to a decreased risk of subsequent neoplasms in those subgroups, but this overall decrease might be counteracted by subsequent neoplasms developed by the increasing number of heavily treated high-risk neuroblastoma survivors. Also, due to the changes in treatment over time there are only few studies that included patients who received more modern therapies like immunotherapy, chemoimmunotherapy, ALK-inhibitors, or therapeutic MIBG.

In Figure 2, we visualized major adjustments within the standard protocol along a timeline from 1970 to 2009, including knowledge on treatment-related risks of subsequent neoplasms from other (childhood) cancer survivor studies. This Figure gives us a basic framework against which we can interpret analysis of subsequent neoplasms. Although this review did not identify significant treatment-related risk factors for subsequent neoplasms in neuroblastoma survivors in multivariable analyses, knowledge from other (childhood) cancer survivors have taught us about treatment-related risks that likely can also be translated to neuroblastoma survivors. Radiotherapy is known to increase risk of subsequent neoplasms, especially solid neoplasms [47-49]. Radiotherapy became part of high-risk neuroblastoma treatment in the

1990s (total body irradiation and ^{131}I-MIBG) and external beam radiotherapy has been introduced in the early 2000s for intermediate- and high-risk patients (Figure 2). Treatment with topoisomerase-II inhibitors, known to increase AML risk [37], has been intensified for moderate-risk and high-risk patients since the 1990s, which may increase their AML risk. Alkylating agents, also a known risk factor of AML [37] and associated with various solid cancers [50], have been part of protocols for intermediate- and high-risk neuroblastoma patients since the 1990s. Platinum compounds treatment, associated with AML [51] and possibly also associated with certain solid cancers [52, 53], has been intensified in high- and intermediate-risk patients. For low-risk patients, treatment has been reduced and since the 1990s treatment with chemotherapy is restricted to specific situations.

Low-risk neuroblastoma patients, who are often treated with surgery only recently, still showed an elevated risk of developing a subsequent neoplasms compared to the general population. This suggests that factors other than treatment may play a role in the etiology of subsequent neoplasms, e.g. genetic susceptibility. One study analyzed the potential association between germline variations and development of SMN in neuroblastoma survivors and identified multiple variants that are involved in DNA repair to be associated with risk of SMN [21]. However, associations were not significant when corrected for multiple testing, which might be due to a lack of statistical power. Other studies in our review did not report on genetic variants and risk of subsequent neoplasms. Large-scale information on germline genetic variants is typically not available in those historical cohort studies that sometimes include patients that have been treated back in the latter half of the 20th century. As sequencing techniques have become more cost affordable and widespread, it can be valuable to incorporate these data in future studies. Also, it would be of interest to evaluate interactions between treatment and genetic factors, in order to identify mutations that modify treatment-related risk of subsequent neoplasms. In an ongoing study of the Children's Oncology Group [54], germline and tumor DNA of 367 five-year high-risk neuroblastoma survivors will be sequenced and combined with clinical, treatment, and toxicity data, including SMNs, which might give new insights into genetic factors associated with SMN[55].

The results of this systematic review should be interpreted in the light of several considerations. First, comparing risk measures between the different studies is difficult due to

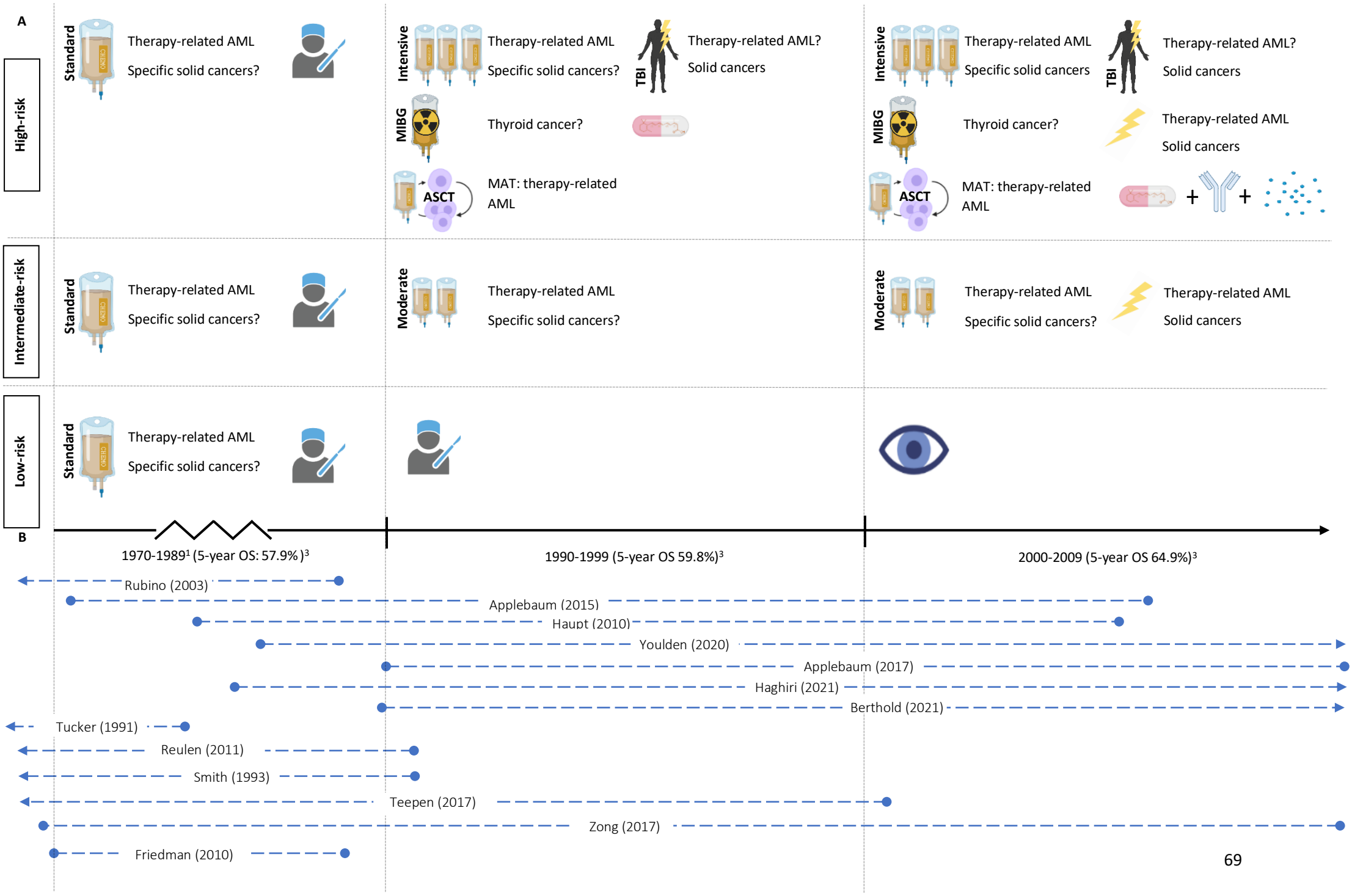
heterogeneity in study characteristics, like follow-up duration, treatment, and minimum survival period. As a result pooling of results was not feasible. Second, most studies only reported on malignant neoplasms. Various studies used linkage to cancer registries for ascertainment and those registries typically include only malignant neoplasms [17, 18, 20, 28, 29]. This focus might result in an underrepresentation of subsequent benign neoplasms, which can also be a serious late health condition as can also be seen in the study of Haghiri et al., showing 14 benign neoplasms in a cohort of 145 high risk neuroblastoma patients[26]. Third, although we did exclude articles with (almost) complete cohorts overlap with another included report (n=4), we cannot exclude that there is some overlap in presented cases between the included studies, because of partial overlap between their cohorts.

The risk of bias in the included studies was mostly low (Table 4). However, in 31% of included studies selection bias and in 23% of the included studies attrition bias could not be ruled out, which could have affected their results. In all studies, the risk of detection bias was unclear, but this is unlikely to have influenced the results, because subsequent neoplasms, especially SMNs, are serious and objective outcomes. Finally, two studies performed a multivariable risk assessment and confounding bias could not be ruled out in both studies. This systematic review used a very broad search strategy for identifying eligible studies, making it unlikely that eligible studies were missed. As we did not impose language restrictions language bias is not an issue.

In conclusion, neuroblastoma survivors are at increased risk of developing subsequent neoplasms. The findings of this systematic review can help to improve awareness of this increased risk in neuroblastoma survivors and their health care providers. Future studies should focus on potential risk factors for subsequent neoplasms, including treatment factors and genetic predisposition. More information on risk factors is important to enhance risk stratification for neuroblastoma survivors and therefore improve follow-up care. In addition, this information can give important input to the development of new treatment protocols for neuroblastoma patients because treatment efficacy and (long-term) toxicity can then be better evaluated.

Table 4 Risk of bias of included studies with support for judgement

Author (year)	Type of bias	Outcome	Support for judgement
Friedman (2009)[15]	Selection	?	For all childhood cancer survivors: 20,626 eligible --> 3,058 not located / 3,205 refused / 4 no complete medical records available --> 14,359 (69.6%) included. But no information on the inclusion percentage for neuroblastoma survivors specifically.
	Attrition	?	Data SMN on self-report. Ascertainment may not have been complete if survivors did not report their SMN.
	Detection	?	No information on the blinding of outcome assessors was provided
	Confounding	NA	No multivariate analysis on risk factors for SMN
Haupt (2010)[22]	Selection	+	Only 13 patients not included, because of inconsistent dates. All other 2,216 eligible survivors included.
	Attrition	+	SMN data for all 2,216 patients available
	Detection	?	No information on the blinding of outcome assessors was provided
	Confounding	NA	No multivariate analysis on risk factors for SMN
Applebaum (2015)[20]	Selection	+	Identification by cancer registry – Assuming completeness of SEER database
	Attrition	+	17 of patients without SMN follow-up, so follow-up information on SMN available for >90% of cohort.
	Detection	?	No information on the blinding of outcome assessors was provided
	Confounding	NA	No multivariate analysis on risk factors for SMN
Applebaum (2017)[21]	Selection	?	9,173 patients diagnosed - study was limited to 5,987 who were enrolled on risk adapted therapeutic (COG) trials. But unclear how many COG trial survivors there were in total.
	Attrition	+	SMN data available for all 5,987 patients.
	Detection	?	No information on the blinding of outcome assessors was provided
	Confounding	NA	No multivariate analysis on risk factors for SMN
Youlden (2020)[18]	Selection	+	Identification based on population-based childhood cancer registry – Assuming completeness of registry
	Attrition	+	Ascertainment of SMN based on linkage with cancer registry – Assuming completeness of registry
	Detection	?	No information on the blinding of outcome assessors was provided
	Confounding	NA	No multivariate analysis on risk factors for SMN
Rubino (2003)[23]	Selection	?	544 5-year neuroblastoma survivors were selected from a cohort that included all of the 4,400 children treated for a first primary cancer before 16 years old in eligible treatment centers. Based on this information we do not know how many of those 4,400 were 5-year neuroblastoma survivors what percentage of the 5-year neuroblastoma survivors were included in the study.
	Attrition	+	48 (9%) were lost to follow-up. Thus >90% with a follow-up
	Detection	?	No information on the blinding of outcome assessors was provided
	Confounding	?	Multivariate models not clear for either adjustments of chemotherapy or age and follow-up duration.
Smith (1993)[19]	Selection	+	All childhood cancer patients were included in de eligible period.
	Attrition	?	Unclear how SMN were ascertained and how complete this ascertainment was.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1970-1989[1] (5-year OS: 57.9%)[3]
1990-1999 (5-year OS 59.8%)[3]
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Youlden (2020)
Applebaum (2017)
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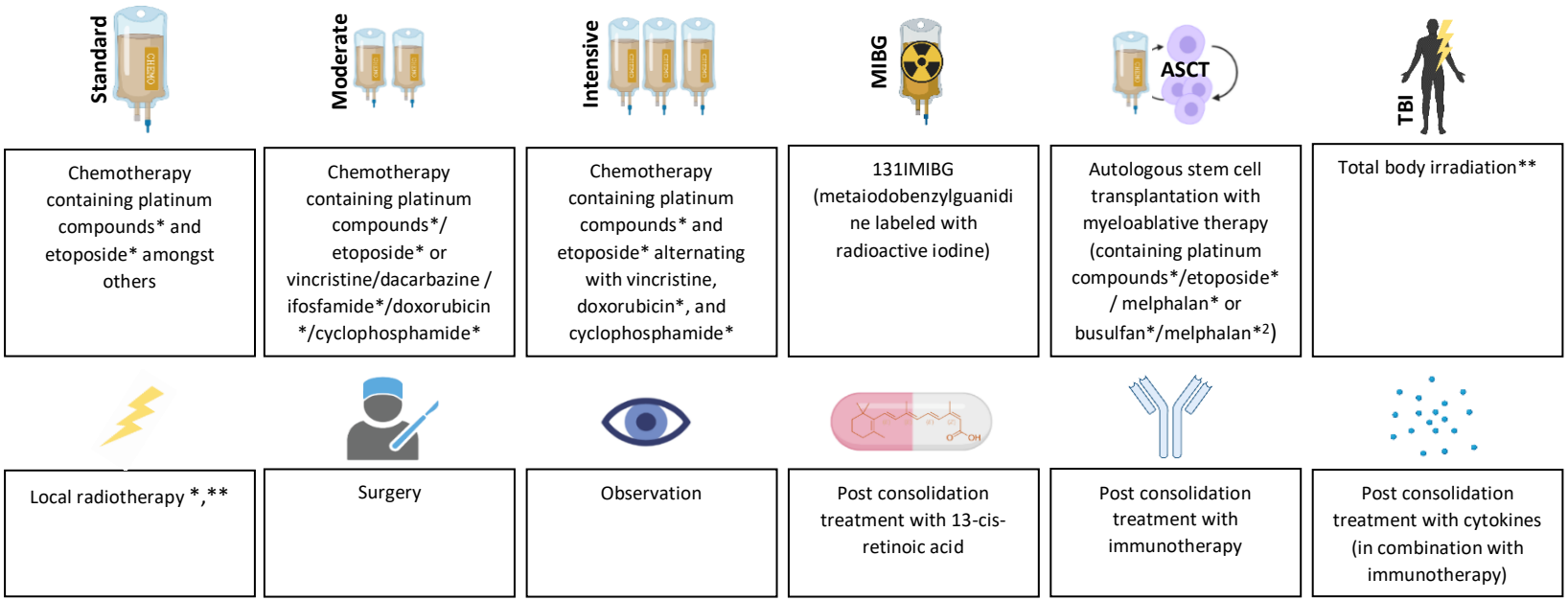

Figure 2 Overview of changes in treatment protocols for neuroblastoma patients per risk group, indicating associations between specific therapies and possible risk of subsequent neoplasms based on knowledge from other (childhood) cancer survivor studies**. A** The icons indicate major treatment adjustments within the treatment protocols for neuroblastoma patients per risk groups. Note that these treatments are options and not necessarily given all together. For each icon, possible risks for subsequent neoplasms are mentioned. Treatment for high-risk patients has been intensified with a multimodal approach including induction chemotherapy, surgery, radiotherapy, stem cell therapy, consolidation and post consolidation. Agents for induction, consolidation and post-consolidation have also been subject to change. Treatment for the intermediate-risk patients has been slightly intensified over time with moderate doses of multi-agent chemotherapy. Treatment for low-risk patients has been reduced by treating with surgery only and restricting chemotherapy to specific situations. In later periods, observation alone could even be considered as a standard therapy for infants that fulfill certain criteria. **B** Included studies are shown within the corresponding inclusion period for their cohort. **C** Legend of the icons used in figure 2A *Abbreviations*: *CT: chemotherapy; ^{131}I MIBG: metaiodobenzylguanidine labeled with radioactive iodine; TBI: total body irradiation; SMN: subsequent malignant neoplasm; MAT: Myeloablative therapy; ASCT: Autologous stem cell transplantation;* *Known to increase the risk for developing therapy-related acute myeloid leukemia; **Known to increase the risk for developing solid cancers; ? depicts possible association. Figure based on information from multiple articles [4, 6, 7, 30, 34, 38, 45, 46, 58-66]

1. Treatment was based on stage and age and not on risk group. This was introduced around 1990.
2. In the era 1990-1999, myeloablative therapy included carboplatin/etoposide/melphalan, since 2000 busulfan/melphalan became an alternative option.
3. The 5-year overall survival rate for 1970-1989 was extracted from Madanat-Harjouja (2014), for 1990-1999 and 2000-2009 the overall survival rates were averaged from studies of Madanat-Harjouja (2014) and Tas (2020).

Supplementary materials chapter 3

Appendix A Search strategy for MEDLINE (PubMed)

1. For Neuroblastoma the following MeSH headings and text words were used:

neuroblastoma OR neuroblastomas OR neuroblast* OR ganglioneuroblastoma OR ganglioneuroblastomas OR ganglioneuroblast* OR neuroepithelioma OR neuroepitheliomas OR neuroepitheliom* OR esthesioneuroblastoma OR esthesioneuroblastomas OR esthesioneuroblastom* OR schwannian

2. For Subsequent neoplasms the following MeSH headings and text words were used:

Neoplasms, Radiation-Induced [Mesh] OR "Neoplasms, Radiation-Induced" OR "Radiation-Induced Neoplasms" OR "Neoplasm, Radiation-Induced" OR "Radiation Induced Neoplasms" OR "Radiation-Induced Neoplasm" OR "Radiation Induced Cancer" OR "Cancers, Radiation-Induced" OR "Radiation Induced Cancer" OR "Radiation-Induced Cancers" OR "Cancer, Radiation-Induced" OR "Cancer, Radiation Induced" OR Neoplasm, Second Primary [Mesh] OR "Neoplasms, Second Primary" OR "Neoplasm, Second Primary" OR "Second Primary Neoplasm" OR "Metachronous Second Primary Neoplasms" OR "Neoplasms, Metachronous" OR "Second Malignancy" OR "Malignancies, Second" OR "Malignancy, Second" OR "Second Malignancies" OR "Second Neoplasm" OR "Neoplasm, Second" OR "Neoplasms, Second" OR "Second Neoplasms" OR "Second Primary Neoplasms" OR "Metachronous Neoplasms" OR "Metachronous Neoplasm" OR "Therapy Associated Neoplasm" OR "Neoplasms, Treatment-Related" OR "Neoplasms, Treatment Related" OR "Treatment-Related Neoplasm" OR "Therapy-Related Neoplasms" OR "Therapy Related Neoplasms" OR "Treatment-Associated Neoplasms" OR "Treatment Associated Neoplasms" OR "Treatment-Related Neoplasms" OR "Treatment Related Neoplasms" OR "Neoplasms, Therapy-Related" OR "Neoplasm, Therapy-Related" OR "Neoplasms, Therapy Related" OR "Therapy Related Neoplasm" OR "Therapy Associated Cancer" OR "Cancer, Therapy-Associated" OR "Therapy Associated Cancer" OR "Therapy-Related Cancer" OR "Cancer, Therapy-Related" OR "Cancers, Therapy-Related" OR "Therapy Related Cancer" OR "Therapy-Related Cancers" OR "Treatment-Related Cancer" OR "Cancer, Treatment-Related" OR "Cancers, Treatment-Related" OR "Treatment Related Cancer" OR "Treatment Related Cancers" OR "Treatment-Associated Cancer" OR "Cancer, Treatment-Associated" OR "Treatment Associated Cancer" OR "Treatment-Associated Cancers" OR "Cancer, Second Primary" OR "Cancers, Second Primary" OR "Second Primary Cancer" OR "Second Primary Cancers" OR "Second Cancer" OR "Cancer, Second" OR "Cancers, Second" OR "Second Cancers" OR "Neoplasms, Radiation effects" OR "second primary malignancy" OR "second primary malignancies" OR "second malignant neoplasm" OR "second malignant neoplasms" OR "SMN" OR "second neoplasm" OR "second neoplasms" OR "secondary breast cancer" OR "subsequent malignant neoplasm" OR "subsequent malignant neoplasms" OR "subsequent neoplasm" OR "subsequent neoplasms" OR "second malignancy" OR "new malignancy" OR "new malignancies" OR "subsequent primary malignancy" OR "subsequent primary malignancies" OR "subsequent primary neoplasm" OR "subsequent primary neoplasms" OR "subsequent primary tumor" OR "subsequent primary tumors" OR "subsequent malignancy" OR "subsequent malignancies" OR "subsequent tumor" OR "subsequent tumors" OR "secondary cancer" OR "secondary neoplasm" OR "secondary malignancy" OR "secondary tumor" OR "secondary cancers" OR "secondary neoplasms" OR "secondary malignancies" OR "secondary tumors" OR "secondary primary malignancy" OR "second tumor" OR "second tumors" OR "second primary tumor" OR "second primary tumors" OR "second malignant tumor" OR "second malignant tumors" OR "subsequent malignant tumor" OR "subsequent malignant tumors"

Final search 1 AND 2

Appendix B Cochrane Childhood Cancer Risk of bias assessment criteria for observational studies*

	Type of bias
Study group	Selection bias (representative: yes/no) The described study group consisted of more than 90% of the neuroblastoma survivors included in the original cohort of the eligible patients Or If it was a random sample with respect to important prognostic factors (i.e. Age, Follow-up time, Treatment factors (RT yes/no, Chemotherapy yes/no)
Follow-up	Attrition bias (adequate: yes/no) Drop-outs & lost to follow up - if subsequent neoplasms were assessed for more than 90% of the study group of interest
Outcome	Detection bias (blind: yes/no) If the outcome assessors were blinded to the investigated determinant
Risk assessment **	Confounding (adjustment for other factors: yes/no) If important prognostic factors (i.e. Age, Follow-up time, Treatment factors (RT yes/no, Chemotherapy yes/no)) were taken adequately into account.

Each bias item was scored as low risk, high risk or unclear risk

* based on previously described checklists according to evidence-based medicine criteria [56, 57].

** Only applicable when risk factors were assessed in a multivariable manner.

Acknowledgements

We are grateful to the late Edith Leclercq, information specialist for Cochrane Childhood Cancer, for her help with developing the search strategy.

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Chapter 4

Long-term Risk of Subsequent Neoplasms in 5-Year Survivors of Childhood Neuroblastoma: A DCCSS-LATER 3 Study

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Accepted in Journal of Clinical Oncology, 2024

Abstract

Purpose

Neuroblastoma survivors have an increased risk of developing subsequent malignant neoplasms (SMNs), but the risk of subsequent non-malignant neoplasms (SNMNs) and risk factors are largely unknown. We analyzed the long-term risks and associated risk factors for developing SMNs and SNMNs in a well-characterized cohort of five-year neuroblastoma survivors.

Patients and methods

We included 563 five-year neuroblastoma survivors from the Dutch Childhood Cancer Survivor Study (DCCSS)-LATER cohort, diagnosed during 1963-2014. Subsequent neoplasms were ascertained by linkages with the Netherlands Cancer Registry and the Dutch Nationwide Pathology Databank (Palga) and medical chart review. We calculated standardized incidence ratios (SIRs), absolute excess risk (AERs), and cumulative incidences. Multivariable competing risk regression analysis was used to evaluate risk factors.

Results

In total, 23 survivors developed an SMN and 60 an SNMN. After a median follow-up of 23.7 (range:5.0-56.3) years, the risk of SMN was elevated compared to the general population (SIR:4.0; 95%CI, 2.5-5.9; AER per 10,000 person-years: 15.1). The 30-year cumulative incidence was 3.4% (95%CI:1.9-6.0%) for SMNs and 10.4% (95%CI:7.3-14.8%) for SNMNs. Six survivors developed an SMN after metaiodobenzylguanidine (^{131}I-MIBG) treatment. Survivors treated with ^{131}I-MIBG had a higher risk of developing SMNs (subdistribution hazard ratio [SHR]:5.7, 95%CI:1.8-17.8) and SNMNs (SHR:2.6, 95%CI:1.2-5.6) compared to survivors treated without ^{131}I-MIBG; results for SMNs were attenuated in high-risk patients only (SMNs SHR:3.6, 95%CI:0.9-15.3, SNMNs SHR:1.5, 95%CI:0.7-3.6).

Conclusion

Our results demonstrate that neuroblastoma survivors have an elevated risk of developing SMNs and a high risk of SNMNs. ^{131}I-MIBG may be a treatment-related risk factor for the development of SMN and SNMN, which needs further validation. Our results emphasize the need for awareness of subsequent neoplasms and the importance of follow-up care.

Introduction

Neuroblastoma is the most common extracranial solid tumor of childhood[1], with a median age at diagnosis of 18 months[2]. In the Netherlands, 30-40 new cases of neuroblastoma are diagnosed each year[3]. It is a diverse tumor type with variation in location, histopathology, biology and overall outcome[4]. Because of treatment advances, survival rates have improved over the past decades with a current five-year survival rate of 95% for low- and intermediate-risk patients and 50% for high-risk patients[5]. Due to this growing population of survivors it is becoming more important to evaluate long-term health outcomes, including development of subsequent neoplasms[6-11]. We recently systematically summarized the evidence on risk of subsequent neoplasms in neuroblastoma survivors[12]. Previous studies reported a 2.8-10.4 times higher risk of developing a subsequent malignant neoplasms (SMN) in neuroblastoma survivors than in the general population[12-20]. However, there was little evidence on potential risk factors. Women and patients treated with radiotherapy seemed to be at higher risk in univariate analyses, but no statistically significant risk factors were observed in multivariable analyses[12]. Furthermore, only few studies included data on subsequent non-malignant neoplasms (SNMN). More knowledge on risk factors for SMNs and SNMNs can contribute to better identifying which survivors are at highest risk of developing subsequent neoplasms. This can help to improve future treatment protocols, follow-up care, and long-term quality of life. In this study we analyzed the long-term risks and associated risk factors for developing SMNs and SNMNs in a cohort of neuroblastoma survivors.

Methods

Patients

Neuroblastoma patients were selected from the Dutch Childhood Cancer survivor study (DCCSS)-LATER cohort[21]. This cohort consists of five-year childhood cancer survivors who were diagnosed before the age of 18 years in one of the paediatric oncology/stem cell centres in the Netherlands. The original DCCSS-LATER cohort included survivors diagnosed between January 1 1963, and December 31 2001[22]. In the current study we also included the expansion DCCSS-LATER cohort with survivors diagnosed up to December 31 2014. The total DCCSS-LATER cohort consists of 10,785 childhood cancer survivors of which we included 563 neuroblastoma survivors in this study (Figure 1). We included patients diagnosed with neuroblastoma or ganglioneuroblastoma (ICD-O-3 morphology code 9490/3) according to the *International Classification of Disease for Oncology, Third Edition (ICD-D-O-3)*[23] (ICD-O-3 morphology code 9500/3).

Data collection

Information about demographics, diagnosis and treatment, including treatment data on recurrences, of the childhood cancer was collected by trained data managers and entered into the DCCSS-LATER registry. The informed consent procedure is described in appendix A.

For the 57 survivors with anonymized data we only had basic treatment data available. For the other 506 neuroblastoma patients, detailed treatment information, including chemotherapy agents and doses and the radiotherapy type was available. Risk group classification is shown in appendix B.

Subsequent neoplasms were ascertained by linkages with nationwide registries: the Netherlands Cancer Registry (NCR)[24] and the Dutch Nationwide Pathology Databank (Palga)[25]. The NCR was used as the main source for subsequent malignant tumors (SMNs). The NCR records all cancer cases in the Netherlands, except for basal cell carcinoma (BCC), and has

nationwide coverage from 1989. The linkage procedure for the original DCCSS-LATER has been reported previously[22]. For the current study, we received an update with longer follow-up time. In addition, the expansion DCCSS-LATER cohort was linked with the NCR. Information from the NCR was complete up to January 31st, 2022 for the whole cohort. For the pre-1989 era we used the partially available data from NCR and data from Palga. Furthermore, we obtained SMN data from the LATER registry based on medical records. In case of discrepancies between SMN sources, we reviewed pathology reports to resolve this. Palga, which records all pathology examinations performed in the Netherlands with nationwide coverage since 1991, was used as source for histologically confirmed subsequent non-malignant neoplasms (SNMNs). SNMNs included subsequent benign, borderline malignant, in situ tumors, and BCCs. BCCs are officially malignant tumors, but were treated as SNMNs in our analyses, because their ascertainment method was similar to SNMNs and because of their indolent behaviour. Non-malignant tumors of the skin were excluded. Information from Palga was complete up to April 7th, 2022 for the original cohort and up to November 30th for the expansion cohort. Excerpts were manually reviewed to identify and classify SNMNs according to the *ICD-D-O-3*[23]. Equivocal cases were discussed with a pathologist (RdK). Subsequent neoplasms were included when they occurred five years or more after neuroblastoma diagnosis, excluding recurrences or metastases.

Statistical analysis

Follow-up started five years after neuroblastoma diagnosis (for SMNs) or five years after neuroblastoma diagnosis or January 1, 1991, whichever occurred last (for SNMNs) and ended on the date of diagnosis of the first tumor of interest, death, last known vital status (emigration, lost to follow-up) or the end of the study, whichever came first. Analyses were done separately for SMNs and SNMNs, because of the differences in entry time. If a patient presented with multiple SMNs or SNMNs we only included the first subsequent malignant or non-malignant neoplasm of interest in the analysis.

For SMNs, we calculated standardized incidence ratios (SIRs) as the ratio of observed to expected number of malignancies. The expected number was estimated by multiplying the

person-years at risk with age-, sex-, and calendar year-specific matched cancer references rates of the general population from the NCR (1989+) and the Eindhoven Cancer Registry (up to 1988)[24,26]. We calculated the absolute excess risk (AER) as the absolute difference between the observed and the expected numbers of subsequent neoplasms per 10,000 person-years.

For both SMNs and SNMNs, we calculated the cumulative incidence of subsequent neoplasms in the presence of death as a competing risk. Effects of potential risk factors were analyzed by Fine-Gray[27] competing risk regression analyses, with death as competing risk and attained age as the time scale, because cancer incidence varies by age[28].

The base model included sex, age at neuroblastoma diagnosis, Iodine-metaiodobenzylguanidine (^{131}I MIBG) treatment, and radiotherapy other than ^{131}I MIBG. We also tested the effects of chemotherapy and stem cell transplantation in addition to the base model. For chemotherapy we evaluated the following groups: alkylating agents, anthracyclines, epipodophyllotoxins/platinum agents (groups were combined, because those were often administered together), vinca alkaloids, and antimetabolites. We also analyzed chemotherapy agent dose for agents with at least five exposed cases. We categorized the dose into lower or higher than median dose of all patients exposed to the specific agent. Sensitivity analyses included a model where we reduced the number of variables to check for possible overfitting and a model excluding thyroid neoplasms, to exclude potential screening bias effects. The proportional subdistribution hazard assumption was tested in all models and was not violated. All analyses were conducted using SPSS v26.0 or R studio v1.3 or STATA/SE 13.1.

Results

Patient characteristics

This study included 563 childhood neuroblastoma survivors, of whom 50.1% were male (Table 1). The median age at diagnosis was 11 (range:0–204) months with 66% diagnosed under the age of two. Based on the reconstructed risk group, we assume our cohort consists of 39.3% low-risk, 11% intermediate-risk and 35.5% high-risk patients. The neuroblastoma was mostly

located in the abdomen (47%)(Appendix C). Of all survivors, 25.0% had surgery alone, 17.8% had stem cell transplantation, 34.1% had chemotherapy without radiotherapy, 6.6% had radiotherapy without chemotherapy, and 27.7% received both chemotherapy and radiotherapy. In total, 33 five-year survivors were deceased at end of follow-up. Among the 506 patients for whom we had additional treatment details, 15.5% were treated with ^{131}I-MIBG, of whom 5.1% also received other radiotherapy and 12.1% did not (Table 1, Appendix K). ^{131}I-MIBG therapy was administered between 1988-2015, with 38 patients receiving ^{131}I-MIBG before 2000 and 49 patients after.

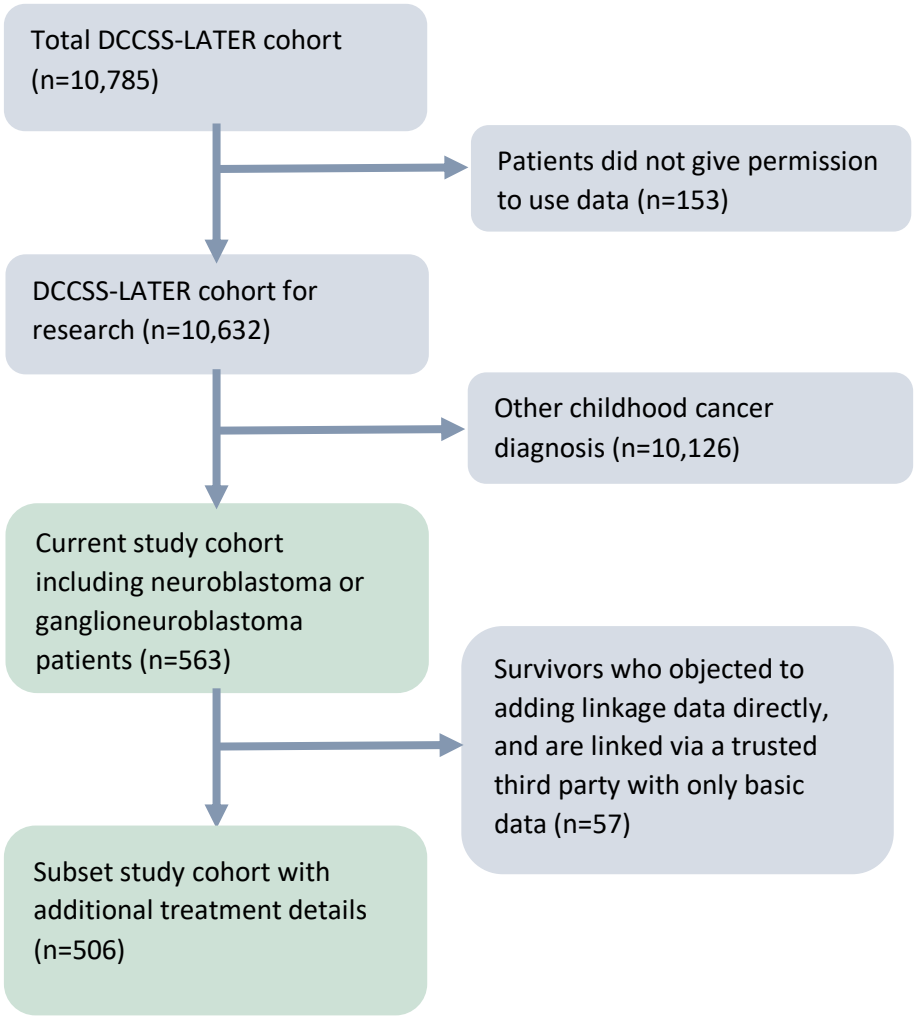

Figure 1 Flow diagram of Dutch Childhood Cancer survivor study (DCCSS)-LATER cohort and the selection of the neuroblastoma (International Classification of Disease for Oncology, Third Edition (ICD-D-O-3)[23] (ICD-O-3 morphology code 9500/3 and ganglioneuroblastoma (ICD-O-3 morphology code 9490/3)

In total, 75 survivors developed at least one subsequent neoplasm. Among those, 23 developed an SMN and 60 an SNMN (8 developed both; Table 1). Furthermore, three survivors developed multiple SMNs and 11 survivors developed multiple SNMNs.

Subsequent malignant neoplasms

After a median follow-up time of 23.7 (range:5.0-56.3) years since neuroblastoma diagnosis, with a total of 11,372 person-years, 23 neuroblastoma survivors developed an SMN. Median latency period between neuroblastoma diagnosis and SMN was 27.4 (range:5.7-44.6) years. We observed 11 carcinomas, 4 sarcomas, 4 hematological neoplasms, 1 melanoma and 3 other malignant neoplasms (Appendix D). The most common malignancies were thyroid (n=2; SIR:13.1, 95%CI:1.6-47.4) and bladder carcinoma (n=3; SIR:147.6, 95%CI:30.4-431.3). Both survivors who developed thyroid carcinoma were ^{131}I MIBG-treated. Of the survivors who developed bladder carcinoma, one was treated with only surgery, one with only chemotherapy and one with both chemo- and abdominal radiotherapy. Both survivors treated with chemotherapy received cyclophosphamide.

The overall SMN risk was significantly elevated compared to the age- and sex- matched general population (SIR:4.0, 95%CI:2.5-5.9; AER:15.1/10,000 person-years, Table 2). The AER increased with longer follow-up time after diagnosis and was 30.4/10,000 person-years for follow-up time beyond 35 years. Among the 61 patients who received ^{131}I MIBG without any additional radiation, five developed an SMN, including two thyroid carcinomas, one B-lymphoblastic leukemia/lymphoma, one peritoneal mesothelioma, and a retroperitoneal sarcoma (Appendix D). SIRs and AERs were higher among ^{131}I MIBG-treated survivors (SIR ^{131}I MIBG only:27.7 (95%CI:9.0-64.7); AER:57.2 per 10,000 person-years and SIR ^{131}I MIBG plus other radiotherapy:25.5 (95%CI:0.6-141.8); AER:44.6 per 10,000 person-years) compared to non-^{131}I MIBG treated survivors (SIR no radiotherapy:1.9 (95%CI:0.7-4.2); and SIR radiotherapy other than ^{131}I MIBG:4.6 (95%CI:0.6-141.8)). The cumulative incidence of SMNs 30 years after childhood diagnosis was 3.4% (95%CI:1.9-6.0%) (Figure 2).

Subsequent non-malignant neoplasms

In total 60 neuroblastoma survivors developed a histologically confirmed SNMN with a median latency period of 28.3 (range:6.0–50.1) years. We observed 11 adenomas, 7 lipomas, 2 fibromas, 2 fibro-sarcomas, 8 leiomyoma's, 5 cervical neoplasms, 15 central nervous system neoplasms, 5 bone neoplasms, 5 BCCs and 1 other non-malignant neoplasm (Appendix E). The cumulative incidence of SNMN 30 years after childhood diagnosis was 10.6% (95%CI:7.3-14.8%) (Table 2; Figure 2). At 40 years, the cumulative incidence of SNMN was increased to 22.2% (95%CI:17.0-28.7%)(Figure 2).

Risk factors for subsequent neoplasms

Our multivariable analyses were restricted to the subset of 506 survivors of whom we had additional treatment details. Neuroblastoma survivors treated with ^{131}I MIBG had a statistically significantly higher risk of developing SMNs compared to survivors treated without ^{131}I MIBG (subdistribution hazard ratio[SHR]:5.7, 95%CI:1.8-17.8)(Table 3), also after adjusting for chemotherapy groups (SHR:4.9, 95%CI:1.5-15.7)(Appendix F). The median cumulative ^{131}I MIBG dose was 200 (95%CI:100-448) mCi for those who developed an SMN and 150 (95%CI:50-700) mCi for those without an SMN. Radiotherapy other than ^{131}I MIBG was not significantly associated with SMN risk (SHR:1.7, 95%CI:0.6-4.5). Risk of SNMN was significantly increased in women compared to men (SHR:3.0, 95%CI:1.6-5.8), although the risk was attenuated when excluding sex-specific tumors (SHR:2.1; 95%CI:1.1-4.2; data not shown). Furthermore, radiotherapy other than ^{131}I MIBG was associated with a significantly higher risk of developing SNMNs (SHR:2.5, 95%CI:1.2-5.6). ^{31}I MIBG treatment was significantly associated with risk of developing SNMNs (SHR:2.6, 95%CI:1.2-5.6), also after adjusting for chemotherapy groups (SHR:2.7, 95%CI:1.1-6.5)(Appendix F). The median cumulative ^{131}I MIBG dose was 150 (95%CI:100-300) mCi for those who developed an SNMN and 150 (95%CI:50-700) mCi for those without an SNMN.

Additional analyses among high-risk neuroblastoma survivors only, showed a slightly attenuated effect of ^{131}I MIBG on SMN (SHR:3.6, 95%CI:0.9-15.2) and SNMN risk (SHR:1.5, 95%CI:0.7-3.6)(Table 3). After adjusting for chemotherapy groups ^{131}I MIBG was significantly

associated with both SMN (SHR:4.7, 95%CI:1.1-20.4) and SNMN (SHR:2.6, 95%CI:1.1-6.5) (Appendix F).

We did not find significant effects on SMN and SNMN risk for any of the chemotherapy groups (Appendix F), chemotherapy agent dose (Appendix G), nor stem cell transplantation (Appendix H). In a sensitivity analysis, with reduced number of variables, ^{131}I-MIBG was still a significant risk factor for SMN and risk was slightly attenuated for SNMN (Appendix I). In an additional sensitivity analysis excluding thyroid neoplasms, ^{131}I-MIBG was still associated with SMN and SNMN risk (Appendix J).

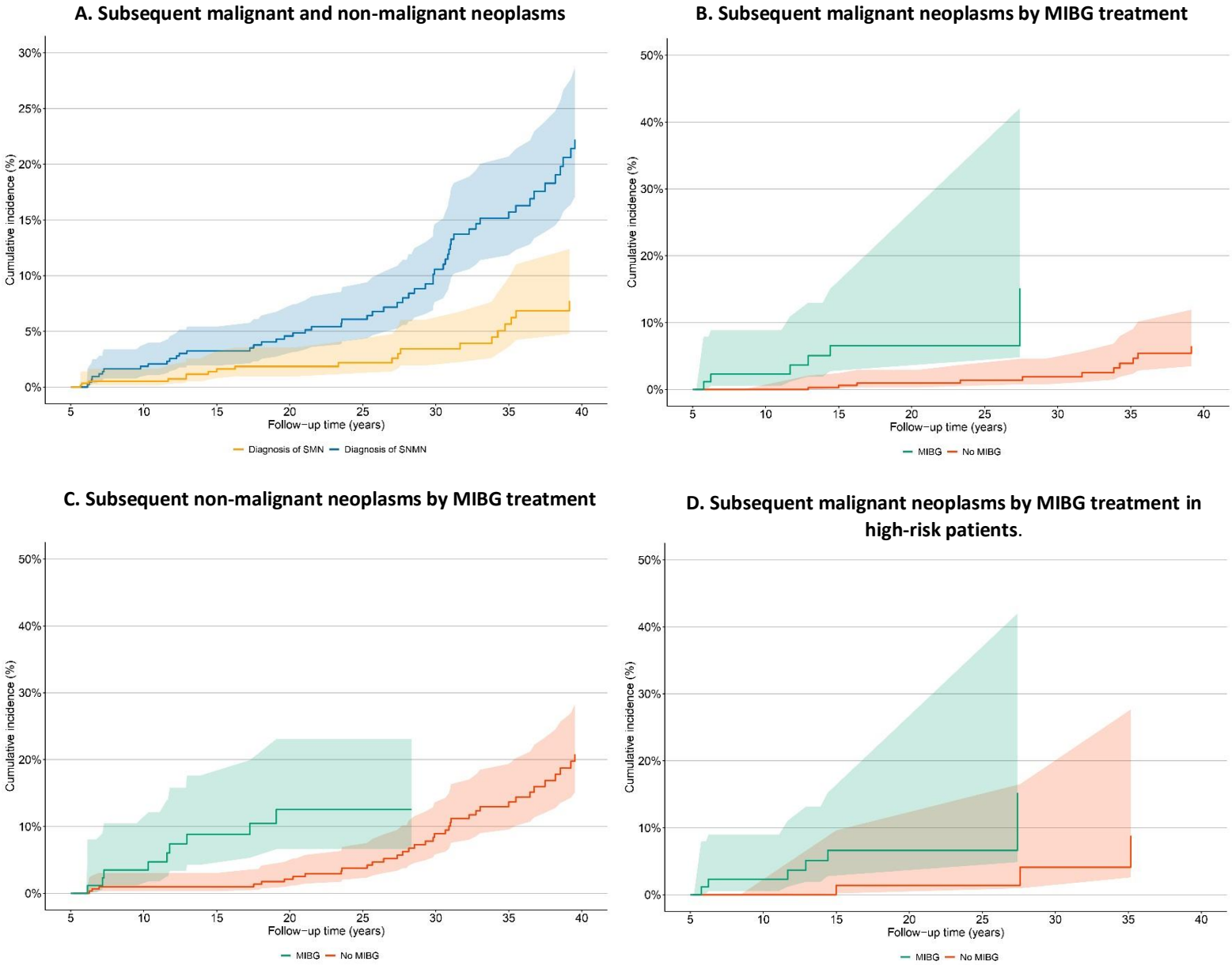

Figure 2 Cumulative incidence of subsequent neoplasms in the DCCSS (Dutch Childhood Cancer Survivor Study) LATER cohort up to 40 years after neuroblastoma diagnosis, with death as competing risk **A.** Cumulative incidence of subsequent malignant neoplasms (SMN) and subsequent non-malignant neoplasms (SNMN). Three SMNs and 18 SNMNs developed after 40 years after neuroblastoma diagnosis and are therefore not shown in this figure. **B.** Cumulative incidence of SMNs for survivors treated with metaiodobenzylguanidine (^{131}I MIBG) and without ^{131}I MIBG **C.** Cumulative incidence of SNMNs for survivors treated with ^{131}I MIBG and without ^{131}I MIBG. **D.**. Cumulative incidence of SMNs for high-risk neuroblastoma survivors treated with ^{131}I MIBG and without ^{131}I MIBG

Table 1. Characteristics of all five-year childhood neuroblastoma survivors from the DCCSS-LATER cohort and those with and without a subsequent neoplasm

Characteristics	Total cohort (%)	Without SN	With SN		
			With any SN	With SMN	With SNMN
Total cohort	**563 (100%)**	**488 (86.7%)**	**75 (13.3%)**	**23 (4.1%)[1,5]**	**60 (10.7%)[1,6]**
Sex					
Male	282 (50.1%)	260	22	10	13
Female	281 (49.9%)	228	53	13	47
Vital status					
Alive	530 (94.1%)	462	68	17	59
Deceased	33 (5.9%)	26	7	6	1
Period of NB diagnosis					
<1980	80 (14.2%)	53	27	10	20
1980-1989	128 (22.7%)	108	20	4	18
1990-1999	126 (22.4%)	106	20	5	18
2000-2009	147 (26.1%)	140	7	4	3
2010-2014	82 (14.6%)	81	1	0	1
Age at NB diagnosis (months)					
<12	310 (55.1%)	268	42	13	34
12-18	63 (11.2%)	58	14	5	11
18 – 48	123 (21.8%)	122	12	1	12
48+	67 (11.9%)	63	7	4	3
Risk group					
Low-risk	221 (39.3%)	199	22	7	17
Intermediate-risk	62 (11%)	59	3	0	3
High-risk	200 (35.5%)	168	32	11	25
Unknown	80 (14.2%)	62	18	5	15
Chemotherapy					
No	214 (38.0%)	195	19	6	15
Yes	348 (61.8%)	292	56	17	45
Unknown	1 (0.2%)	1	0	0	0
Radiotherapy					
No	329 (65.5%)	336	33	8	27
Yes	193 (34.3%)	151	42	15	33
Unknown	1 (0.2%)	1	0	0	0
Treatment groups					
Only surgery	141 (25.0%)	132	9	3	7
CT, no RT	192 (34.1%)	169	23	5	19
RT, no CT	37 (6.6%)	28	9	3	7
Ct +Rt	156 (27.7%)	123	33	12	26
No recorded treatment	41 (7.3%)	39	2	1	2
Stem cell transplantation					
No	458 (81.3%)	397	61	17	49
Yes	100 (17.8%)	87	13	5	10
Unknown	5 (0.9%)	4	1	1	1
Subset with additional details*	**506 (100%)**	**442 (87.4%)**	**64 (12.6%)**	**20 (4.0%)[2]**	**50 (9.9%)[2]**
Chemotherapy					
Alkylating [3]	304 (60.1%)	258	46	14	36
Anthracyclines[3]	195 (38.5%)	179	16	4	14
Epipodophyllotoxins [3]	214 (42.3%)	190	24	7	19
Platinum[3]	218 (43.1%)	194	24	7	19
Vinca alkaloids[4]	222 (43.9%)	190	32	12	24
Antimetabolites[4]	14 (2.8%)	10	4	2	3
Radiotherapy type [3]					
No	330 (65.2%)	305	25	6	20
Radiotherapy other than MIBG	88 (17.4%)	63	25	8	21
MIBG only	61 (12.1%)	50	11	5	7
Radiotherapy other than MIBG plus MIBG	26 (5.1%)	23	3	1	2
Radiotherapy, other than MIBG					
No	391 (69.4%)	355	36	11	27
Yes	114 (20.2%)	86	28	9	23
Unknown	1	1	0	0	0
MIBG					
No	418 (74.2%)	368	50	14	41
Yes	87 (15.5%)	73	14	6	9
Unknown	1	1	0	0	0

DCCSS : Dutch Childhood Cancer Survivor Study NB: neuroblastoma SN: subsequent neoplasm, SMN: subsequent malignant neoplasm; SNMN: subsequent non-malignant neoplasm, CT: chemotherapy, RT: radiotherapy, ^{131}MIBG: metaiodobenzylguanidine labeled with radioactive iodine * From 506 patients we had additional treatment details available.[1] Eight survivors were diagnosed with a primary SMN

and SNMN, [2] Four patients were diagnosed with both an SMN and SNMN. [3] One patient with unknown therapy [4] Two patient had missing data on type of chemotherapy. [5] Three patients developed and SMN within five years after neuroblastoma diagnosis (myelodysplasia, embryonal rhabdomyosarcoma of the endocervix and embryonal tumor in the cerebellum). [5] Twenty patient developed an SNMN within five years after neuroblastoma diagnosis.

Table 2. Standardized ratios, Absolute excess risk and 30-year cumulative incidence for subsequent neoplasms according to patient characteristics

	SMN				SNMN	
Characteristics	**Observed**	**SIR (95%CI)**	**AER / 10,000 PY**	**30-year Cumulative incidence (95%CI)**	**Observed**	**30-year Cumulative incidence (95%CI)**
Total cohort (n=563)	**23**	**4.0 (2.5 - 5.9)***	**15.1**	**3.4% (1.9 – 6.0%)**	**60**	**10.6% (7.6 - 14.6%)**
Sex						
Male	10	4.9 (2.4 – 9.1)*	14.9	3.5% (1.5 – 8.1%)	13	4.6% (2.3 – 9.2%)
Female	13	3.4 (1.8 – 5.9)*	15.3	3.5% (1.6 – 7.3%)	47	16.0% (11.2 – 22.7%)
Age at NB diagnosis (months)						
<12	13	4.1 (2.2 - 7.1)	14,8	4.3% (2.1 – 8.7%)	34	11.1% (7.1 – 16.9%)
12-18	5	7.5 (2.4 – 17.5)	30.8	1.9% (0.3 - 12.6%)	11	19.0% (9.4 – 36.3%)
18-48	1	0.8 (0.0 – 4.4)	-0.1	0.8% (0.1 – 5.6%)[1]	12	22.1% (11.8 – 39.2%)
48+	4	5.3 (1.4 – 13.5)	30.7	5.7% (1.9 – 16.8%)	3	5.7% (1.5 – 21.1%)
Time since NB diagnosis (years)						
5-15						
15-25	8	13.4 (5.8 – 26.4)	15.1		14	
25-35	2	1.9 (0.2 – 6.8)	2.7		10	
35+	7	4.1 (1.6 – 8.5)	26.6		22	
	6	2.4 (0.9 – 5.3)	33.1		14	
Attained age						
5-15	6	11.7 (4.3 – 5.4)	12.3		13	
15-25	4	4.0 (1.1 – 10.3)	8.6		9	
25-35	7	4.4 (1.8 – 9.0)	26.1		22	
35+	6	2.4 (0.8 – 4.9)	28.3		16	
Chemotherapy						
No	6	2.3 (0.8 – 4.9)	7.0	1.8% (0.6 – 5.5%)	15	6.0% (3.0 – 11.9%)
Yes	17	5.4 (3.1 - 8.6)	21.0	4.5% (2.4 – 8.6%)	45	13.7% (9.4 – 19.6%)
Chemotherapy agents*						
Alkylating	14	5.5 (3.0 – 9.2)	20.9	4.2% (2.1 – 8.7%)	36	13.0% (8.5 – 19.6%)
Anthracyclines	4	4.8 (1.3 – 12.4)	12.1	1.8% (0.4 – 6.8%)	14	15.3% (8.0 – 28.0%)
Epipodophyllotoxins	7	7.0 (2.8 – 14.4)	19.7	4.8% (1.9 – 11.6%)	19	14.4% (8.1 – 24.6%)
Platinum	7	6.8 (2.7 – 14.0)	19.0	4.6% (1.9 – 11.2%)	19	13.8% (7.8 – 23.6%)
Vinca alkaloids	12	7.1 (3.7 – 12.4)	28.5	5.1% (2.4 – 10.7%)	24	13.8% (8.3 – 22.4%)
Antimetabolites	2	6.4 (0.8 – 23.0)	50.0	7.1% (1.0 – 40.9%)	3	0
Radiotherapy						
No	8	2.2 (1.0 – 4.4)	5.8	1.2% (0.4 – 4.1%)	27	7.4% (4.5 – 12.0%)
Yes	15	6.7 (3.8 – 11.1)	33.9	7.7% (4.1 – 14.4%)	33	17.1% (11.1 – 25.8%)
Radiotherapy groups*						
No	6	1.9 (0.7 – 4.2)	4.0	1.1% (0.3 – 4.7%)	20	6.7% (3.8 – 11.7%)
RT, no MIBG	8	4.6 (2.0 – 9.0)	28.6	4.8% (1.6 – 14.2%)	21	14.4% (7.4 – 26.8%)
MIBG only	5	27.7 (9.0 – 64.7)	57.2	17.8% (5.3 – 50.7%)[1]	7	13.6% (6.6 – 26.9%)[3]
MIBG plus other RT	1	25.5 (0.6 – 141.8)	44.6	5.5% (0.8 – 32.8%)[2]	2	9.4% (2.4 – 33.0%)[2]
Stem cell transplantation						
No	17	3.1 (1.8 – 5.0)	11.4	2.3% (1.1 – 4.7%)	49	9.6% (6.5 –13.9%)
Yes	5	17.7 (5.7 – 41.3)	43.0	14.2% (5.2 – 35.5%)[1]	10	15.4% (7.3 – 30.8%)
By type of SMN[5]						
Thyroid carcinoma	2	13.1 (1.6 – 47.4)	1.9			
Bladder carcinoma	3	147.6 (30.4 – 431.3)	3.0			

NB: neuroblastoma, SMN: subsequent malignant neoplasm; SNMN: subsequent non-malignant neoplasm, SIR: standardized incidence ratio, AER: absolute excess risk, PY: person-years, CI: confidence interval, RT: radiotherapy, ^{131}I-MIBG: metaiodobenzylguanidine labeled with radioactive iodine *Based on subset of survivors (n=506) for whom we have additional treatment details available. [1] Number represents the 27 year cumulative incidence, which is the longest follow-up time we currently have in this group. [2] Number represents the 13 year cumulative incidence, which is the longest follow-up time we currently have in this group. [3] Number represents the 28 year cumulative incidence, which is the longest follow-up time we currently have in this group. [5] Separate analysis was only done with types of subsequent neoplasms that were found twice or more, overview of all types of subsequent neoplasms can be found in the supplementary.

Table 3. Fine-Gray models for SMNs and SNMNs

All neuroblastoma patients[1]							
		SMN[2]			SNMN[3]		
Variable	**Number of survivors**	**Number of SMN**	**SHR**	**95%CI**	**Number of SNMN**	**SHR**	**95%CI**
Sex							
Male	250	10	1 (ref)		12	1 (ref)	
Female	256	10	0.9	0.4 – 2.2	38	3.0	1.6 – 5.8
Age at diagnosis							
<12m	267	11	1(ref)		27	1 (ref)	
12-18m	59	5	1.7	0.6 – 4.7	11	1.7	0.8 – 3.5
18+ m	180	4	0.4	0.11 – 1.4	12	0.5	0.2 – 1.0
Radiotherapy other than MIBG[3]							
No	391	11	1 (ref)		27	1 (ref)	
Yes	114	9	1.7	0.6 – 4.5	23	2.5	1.4 – 4.5
MIBG[3]							
No	418	14	1(ref)		41	1 (ref)	
Yes	87	6	5.7	1.8 – 17.8	9	2.6	1.2 – 5.6
High-risk patients only							
		SMN[4]			SNMN[4]		
Sex							
Male	106	5	1 (ref)		6	1 (ref)	
Female	88	4	1.1	0.3 – 3.9	15	3.9	1.4 – 10.6
Age at diagnosis							
<12m	68	5	1 (ref)		11	1 (ref)	
12-18m	26	2	0.9	0.2 – 4.6	5	1.3	0.5 – 3.5
18+ m	100	2	0.2	0.04 – 1.5	5	0.2	0.08 – 0.7
Radiotherapy other than MIBG[3]							
No	128	6	1 (ref)		17	1 (ref)	
Yes	66	3	1.7	0.5 – 6.1	4	0.8	0.3 – 2.3
MIBG[3]							
No	108	3	1(ref)		12	1 (ref)	
Yes	86	6	3.6	0.9 – 15.3	9	1.5	0.7 – 3.6

SMN: subsequent malignant neoplasm, SNMN: subsequent non-malignant neoplasm, CI: confidence interval, ^{131I}MIBG: metaiodobenzylguanidine labeled with radioactive iodine, SHR: subdistribution hazard ratio. [1]These analyses were done within a subset of the cohort for whom we had additional treatment details available (n=506) and high-risk only (n=197). [2] One patient was not included in the analysis due to missing data. [3] Nine patients were not included in the analysis due to missing data. [3] 26 patients received both ^{131I}MIBG and other radiotherapy. All survivors who received ^{131I}MIBG were high-risk patients, except for 1 intermediate-risk patient (see Appendix K). [4] Four patients were not included in the analysis due to missing data Of 86 high-risk patients treated with 131IMIBG, 20 (23%) have had a recurrence, 68 (79%) received chemotherapy and 26 (30%) received other radiotherapy. Of the 108 patients treated without 131I MIBG 17 (16%) have had a recurrence, 108 (100%) received chemotherapy and 40 (37%) received other radiotherapy.

Discussion

This study, in a well-characterized cohort of 563 five-year neuroblastoma survivors, shows that neuroblastoma survivors treated with ^{131}I-MIBG may have an increased risk of developing both SMN and SNMN compared to survivors treated without ^{131}I-MIBG. Furthermore, we showed that neuroblastoma survivors had a four times elevated risk of developing an SMN compared to the general population. Thyroid and bladder carcinoma were the most commonly observed malignancies. The 30-year cumulative incidence for SMN was 3.4% (95%CI:1.9-6.0) and for SNMN 10.6% (95%CI:7.6-14.6).

To our knowledge, this is the first study that showed an increased risk of SMN and SNMN after ^{131}I-MIBG treatment in neuroblastoma survivors compared to non-^{131}I-MIBG treated survivors, adjusted for other relevant factors and with long-term follow-up (median:23.7 years since diagnosis). A previous study in a U.S. cohort evaluated SMNs risk among 644 ^{131}I-MIBG-treated neuroblastoma survivors, but only included survivors treated with ^{131}I-MIBG without any comparison group and had a relatively short follow-up period (median:3.6 years after first ^{131}I-MIBG)[29]. This study observed 19 SMNs, primarily hematologic. ^{131}I-MIBG has been used upfront for about three decades in the Netherlands[30-33]. The regimens and doses in our study, with a median dose of 150 (95%CI:50-700) mCi, corresponding to 5.6 GBq and 9.8 mCi/kg, have been fairly similar to the doses prescribed to patient in past and current protocols and trials for relapsed/refractory treatment[34-37] and upfront treatment[38] in other countries. Therefore, we expect that current results are still relevant for current-era protocols.

Furthermore, radiotherapy other than ^{131}I-MIBG, a well-established risk factor for subsequent neoplasms[39], was associated with increased risks of SMNs and SNMNs, although the risk estimate for SMNs was not statistically significant. This could be a result of low power due to the small number of SMN cases.

In our study we found two thyroid carcinomas after ^{131}I-MIBG treatment. Subsequent thyroid carcinoma has a well-recognized association with radiation exposure[40-42]. Radiation via ^{131}I-MIBG treatment by free circulating radio-iodine is also known to damage the thyroid[43], which might contribute to the development of thyroid neoplasms as well as hypothyroidism[44-

[46]. In the Netherlands, neuroblastoma patients receive thyroid protection when exposed to ^{131}I-MIBG. Despite the received protections (Appendix M), these two patients still developed thyroid carcinoma, implying the need for enhanced thyroid protection[47]. In addition to the two thyroid carcinomas, we observed two (para)thyroid adenomas after ^{131}I-MIBG treatment[44,46]. Several reports noted an excess of thyroid cancer among neuroblastoma survivors that could not be solely explained by thyroid radiation exposure; roles of very early age at exposure and a potential genetic susceptibility were hypothesized, but to date not clarified[42,48,49].

Acute myeloid leukemia (AML) has been reported previously as one of the most frequent SMNs after neuroblastoma[12]. In our cohort, only one survivor developed AML. This is likely because we only included five-year survivors and therefore did not include patients who developed subsequent AML within five years, the time frame where most subsequent AML usually develop[50], and deceased before reaching five-year survival.

Noteworthy, one survivor, treated with ^{131}I-MIBG only, developed a peritoneal mesothelioma 14 years after neuroblastoma diagnosis, which is a relatively uncommon malignancy. There is some supportive evidence that radiation exposure might contribute to the risk of developing a mesothelioma in survivors[51-54]. Remarkably, the abovementioned U.S. series of neuroblastoma survivors treated with ^{131}I-MIBG, also observed a case of peritoneal mesothelioma[29]. In both studies, the mesotheliomas were found in close proximity to the primary neuroblastoma site, further strengthening the indication of a possible role of ^{131}I-MIBG.

Furthermore, three survivors developed bladder carcinoma after a long latency period, translating into a more than 100-fold elevated bladder cancer risk in our cohort compared to the general population. Although some therapies, like high-dose cyclophoshapmide[55,56] and abdominal radiation[56,57] have been associated with bladder cancer risk in other studies, our small number of cases did not allow evaluation of treatment effects.

In addition, we observed 3 SMNs and 5 SNMNs in patients who were treated with surgery only. This might suggest that factors other than treatment also contribute to the risk of developing subsequent neoplasms, e.g. genetics. Around 1-2% of all neuroblastoma cases

are familial cases[58,59], and more genetic variants that could be predisposing for neuroblastoma are still being discovered[58,60-63]. Neuroblastoma survivors with genetic predisposition syndromes may face elevated risks of developing multiple neoplasms later in life. Examples include Costello syndrome, LEOPARD syndrome and Li-Fraumeni (Appendix L). We did not have complete information on genetic predisposition syndromes within our cohort, but one survivor with confirmed Turner syndrome developed a mesothelioma, a tumor type not reported in relation to Turner syndrome before, and one survivor with neurofibromatosis type 1 developed multiple neurofibromas.

Based on the reconstructed risk groups, ^{131}I-MIBG-treated survivors were mostly high-risk patients, who usually receive intensified treatment. However, in our analyses adjusted for chemotherapy groups and stem cell transplantation, we still observed an increased SMN and SNMN risk in the total cohort. In high-risk patients only, ^{131}I-MIBG was significantly associated with SMN and SNMN risk when adjusted for chemotherapy groups. Without chemotherapy groups adjustment, the risk associated with ^{131}I-MIBG was still elevated in the high risk group, but the increase was not statistically significant, possibly due to differences in chemotherapy treatments between those with and without ^{131}I-MIBG.

Major strengths of our study are the large cohort size, the long follow-up time, availability of detailed individual treatment information, and the complete follow-up of subsequent neoplasms by linking to nationwide registries. Most non-malignant tumors are usually not covered by cancer registries. By linking our cohort to nationwide pathology database Palga, we were able to have objective information on histologically-confirmed SNMNs.

We also need to consider some limitations when interpreting our findings. First, we did not have data on SNMNs assessed without pathological confirmation, e.g. thyroid nodules identified on ultrasounds imaging without fine-needle aspiration cytology. This might cause a slight underrepresentation of SNMN incidence. Second, because there were no reference rates for SNMNs of the general population, we could not calculate SIRs and AERs. Third, there is potential screening bias for thyroid neoplasms, as ^{131}I-MIBG-treated patients are

recommended to undergo surveillance for thyroid carcinomas[64]. Therefore, the likelihood of identifying a thyroid neoplasm among ^{131}I-MIBG-treated patients might be higher than among non-^{131}I-MIBG treated survivors. However, after exclusion of thyroid neoplasms, we still observed a significantly increased risks among ^{131}I-MIBG -treated survivors. Fourth, it should be noted that the number of SMNs were small leading to wide confidence intervals, especially in the sub-analysis including chemotherapy, stem cell transplantation, and in the high-risk patients only analysis. Lastly, due to the retrospective nature of this study, patients with and without ^{131}I-MIBG treatment were not homogenous regarding other treatments received. We, however, adjusted for other treatments in our analyses.

We suggest validation of the role of ^{131}I-MIBG treatment in future prospective clinical trials and collaborative studies with larger cohort sizes, e.g. pooled analysis of data of various cohorts. Collaborative studies would allow for a more detailed assessment of ^{131}I-MIBG treatment characteristics, e.g. ^{131}I-MIBG dose, and for analyzing risks of specific SMNs after ^{131}I-MIBG treatment, which could be useful as we observed a variety of subsequent neoplasms and ^{131}I-MIBG effects might differ between different types. Moreover, non-^{131}I-MIBG treatments have changed over time and prospective studies that focus on specific risk groups and individual impact for specific therapies among homogenously treated patients is necessary. More information on the role of MIBG in SMN risk will emerge in the future from an ongoing COG-study (NCT03126916). In this study, high-risk patients are randomized to either receive or not receive MIBG during induction, while receiving identical chemotherapy, stem cell transplantation, and immunotherapy. Furthermore, future studies should evaluate the role of cancer predisposition syndromes on the risk of subsequent neoplasms.

In conclusion, our results show that neuroblastoma survivors have an elevated risk of developing SMNs and SNMNs. ^{131}I-MIBG may be a treatment-related risk factor. The precise role of ^{131}I-MIBG needs further validation. The current results emphasizes the need for awareness of subsequent neoplasms and the importance of follow-up care in neuroblastoma survivors, especially for those who were treated with ^{131}I-MIBG or other radiotherapy, and informs the development of future treatment protocols.

Supplementary materials chapter 4

Appendix A. Informed consent procedure

Informed consent was obtained for most survivors who had been invited for active participation DCCSS-LATER research projects. For survivors who had been invited for active participation in DCCSS-LATER research projects, but did not respond after repeated requests via a standardized protocol, and for survivors who had not yet been invited for active participation in any DCCSS-LATER research projects, specific consent was not needed in accordance with Dutch legislation. For 57 survivors who objected to adding linkage data directly to the DCCSS-LATER registry, we anonymized a minimal dataset via a trusted third party. Survivors who declined use of their health care data for research purposes were excluded from the eligible study cohort.

Appendix B. Classification risk groups

Low-risk	Intermediate-risk	High-risk
INSS stage 1	INSS stage 3	INSS Stage 4
INSS stage 2A or 2B and age of diagnosis <12months	Doxorubicin without cyclophosphamide or etoposide	Distant metastases
Alternating Vincristine, endo	Treatment protocol: 'POG A3961', 'POG9243', 'POG 8743'	Chemotherapy using busulfan, melphalan
Watchful waiting		Total body irradiation
Only surgery		Stem cell transplantation
Treatment protocol: VAC, DES 2008		^{I131}MIBG treatment (excluding period 1992 – 2008)
		Treatment protocol: OPEC, COG A397, POG 9640, VECI, NB88, OPEI, AMRO NBL 99
		Treatment protocol POG 9049

To reconstruct risk groups of our cohort a combination of the partially available data about stage and treatment protocols information was used in combination with data on age at diagnosis and other treatment details. Most often a patient was classified based on multiple information.

Appendix C. Topography of neuroblastoma

Primary body site	Number of neuroblastoma diagnosis
Head and neck	27
Thorax	58
Abdomen	240
Trunk, not abdomen	22
Upper extremities	3
Lower extremities	3
Pelvis	21
Peripheral nervous system, not specified	87
Central nervous system, not specified	2
Other / unknown	17
Multiple locations	26
Total	506

The topography the childhood neuroblastoma (n = 506).

Appendix D. Primary neuroblastoma treatment and first subsequent malignant neoplasms

	Neuroblastoma			Subsequent malignant neoplasia		
Topography neuroblastoma	**Year diagnosis**	**Treatment**	**Radiotherapy site**	**Histology**	**Site neoplasm**	**Latency (years)**
				Carcinomas		
Subcutaneous, connective and soft tissue	1998	MIBG, CT	Only MIBG	Papillary carcinoma, follicular variant	Thyroid	13
Adrenal medulla	2004	MIBG, CT	Only MIBG	Papillary adenocarcinoma, NNO	Thyroid	6
Adrenal	1975	RT, CT	Abdominal	Urothelial carcinoma[1]	Bladder	45
Peripheral nerves abdominal	1980	CT	NA	Papillary urothelial carcinoma	Bladder	40
Peripheral nerves / autonomic nervous system - NS	1977	Only surgery	NA	Urothelial carcinoma	Bladder	34
Peripheral nerves / autonomic nervous system - NS	1972	RT	Abdominal	Hepatocellular carcinoma (HCC)	Liver primary	45
Peripheral nerves / autonomic nervous system - NS	1980	CT	NA	Mixed germ cell tumor	Testis	28
Peripheral nerves / autonomic nervous system - NS	1980	Only surgery	NA	Adenosquamous carcinoma	Cervix	36
Adrenal	1978	RT , CT	Abdominal	Keratinized squamous cell carcinoma	Skin trunk	32
Adrenal medulla	1975	Surgery , CT	NA	Invasive Ductal carcinoma	Breast right	34
Unknown	Unknown	Unknown	Unknown	Ductal carcinoma	Breast medial	Unknown
				Sarcomas		
Adrenal medulla	1993	MIBG , CT , SCT	Only MIBG	Epithelioid sarcoma	Retroperitoneal	27.4
Adrenal	1974	RT , CT	Abdominal	Chondrosarcoma, NNO	Pelvic area	16.3
peripheral nerves abdominal	1973	RT	Para aortal	Liposarcoma, NNO	Retroperitoneal	23.3
Unknown	Unknown	Unknown	Unknown	Gastrointestinal stromal tumor (GIST)	Duodenum	Unknown
				Hematological		
Peripheral nerves / autonomic nervous system - NS	1977	Only surgery	NA	Nodular sclerosis Hodgkin lymphoma (NSHL)	Lymph nodes head/neck	12.9
Subcutaneous, connective and soft tissue	1998	MIBG	Only MIBG	B-lymphoblastic leukemia[2] (ALL)	Bone marrow	6.3
peripheral nerves pelvis	2004	Surgery, RT, MIBG, CT[3], SCT	MIBG + shoulder blade + lower extremities	Acute myeloid leukemia (AML)	Bone marrow	12
Unknown	Unknown	Unknown	Unknown	Myelodysplastic syndrome, excess blasts	Bone marrow	Unknown
				Melanoma		
Peripheral nerves head and neck	1972	RT, CT	Cervical (neck)	Melanoma	Skin upper extremity	44
				Other / unknown		
Adrenal	1984	RT, CT	Lever	Gastrinoma[4]	Duodenum	35
Pelvis	2000	MIBG, CT	Only MIBG	Epithelioid mesothelioma	Peritoneal	14
Adrenal	2003	RT, CT, SCT	Abdominal	Neoplasm, NNO	Unknown	15

NS: not specified, ^{131}IMIBG: metaiodobenzylguanidine labeled with radioactive iodine, CT: Chemotherapy, RT: Radiotherapy. Overview of all subsequent malignant neoplasms (SMN) found in the Dutch Childhood Cancer Survivor Study (DCCSS) LATER cohort after childhood neuroblastoma, including details on the childhood neuroblastoma treatment. [1]Also developed an adenocarcinoma of the kidney and prostate as second and third SMN [2]Also developed a superficial spreading melanoma as second SMN [3]Chemotherapy included alkylating agents and epipodophyllotoxins, which are known to be related to AML risk. [4]Also developed a differentiated liposarcoma as second SMN.

Appendix E. Primary neuroblastoma treatment and subsequent non-malignant neoplasms

	Neuroblastoma			Subsequent non-malignant neoplasia		
Topography Neuroblastoma	**Year diagnosis**	**Treatment**	**Radiotherapy site**	**Histology**	**Site neoplasm**	**Latency (years)**
				Adenoma's		
Thorax	1982	Surgery , RT, CT	Thorax	Tubular adenoma	Colon	35
Abdomen	1992	Surgery , CT	NA	Tubular adenoma	Colon	20
Parathyroid	1991	Surgery ,CT	NA	Tubular adenoma	Colon	30
Peripheral nerves – head and neck	1973	CT	NA	Tubular adenoma	Colon	48
Parathyroid	1984	Surgery , RT, CT	Liver	Micro follicular adenoma	Thyroid	28
Parathyroid	2010	Surgery , RT, CT, SCT	MIBG + Abdominal	Follicular adenoma	Thyroid	7
Subcutaneous, connective and soft tissue	1998	MIBG, CT	Only MIBG	Adenoma	Parathyroid	13
Peripheral nerves / autonomic nervous system - NS	1980	Only surgery	NA	Adenoma	Cervix	35
Peripheral nerves / autonomic nervous system - NS	1970	Only surgery	NA	Mucineus cystadenoma	Ovary	30
Subcutaneous, connective and soft tissue – head and neck	1975	Surgery, RT, CT	Liver	Fibro-adenoma	Breast	38
				Fibroma		
Peripheral nerves / autonomic nervous system - NS	1972	Surgery , RT	Abdominal	Mucineus adenofibroma	Ovary	36
Peripheral nerves – thorax	1999	CT	NA	Myofibroma	Colon	7
Parathyroid	1996	Only surgery	NA	Neurofibroma	SCST – upper extremity	21
				Fibro-sarcoma		
Parathyroid	1978	Surgery, RT, CT	Abdominal	Fibrosarcoma	Adnex uterine	29
Peripheral nerves / autonomic nervous system - NS	1993	MIBG, Surgery	Only MIBG	Fibrosarcoma	Mucous membrane oral cavity	17
				Lipoma		
Peripheral nerves / autonomic nervous system - NS	1984	Surgery , CT	NA	Lipoma	SCST – back	36
Parathyroid	1983	Surgery , CT	NA	Lipoma	SCST - head and neck	24
Peripheral nerves / autonomic nervous system - NS	1984	CT	NA	Lipoma	SCST - abdominal	20
Peripheral nerves – abdomen	1973	Surgery , RT	Abdominal + para aortal	Lipoma	SCST – lower extremity	40
Pelvis	1979	Surgery , RT, CT	Pelvic	Angiolipoma	SCST – upper extremity	31
Abdomen	1976	Surgery , RT, CT	Abdominal	Angiolipoma	SCST – upper extremity	29
Unknown	Unknown	Unknown	Unknown	Angiolipoma	SCST – upper extremity	Unknown
				Leiomyoma		
Peripheral nerves – abdomen	1979	Surgery , RT, CT	Abdominal	Leiomyoma	Esophagus	31
Peripheral nerves – abdomen	1982	Surgery , CT	NA	Leiomyoma	SCST – lower extremity	39
Unknown	Unknown	Unknown	Unknown	Leiomyoma	Endometrium	Unknown
Spinal cord	1982	Surgery , RT	Head + spinal cord	Leiomyoma	Uterus	26
Not specified	1972	RT, CT	Abdominal	Leiomyoma	Uterus	48
Medulla	1980	Surgery , CT, SCT	NA	Leiomyoma	Uterus	42
Unknown	1985	Unknown	NA	Leiomyoma	Uterus	31
Unknown	1979	Unknown	NA	Leiomyoma	Uterus	31
				Cervical neoplasm		
Peripheral nerves – thorax	1973	Surgery , RT, CT	Thorax	Cervical intra-epithelial neoplasia, grade 3	Cervix	37
Parathyroid	1991	Surgery , CT	NA	Cervical intra-epithelial neoplasia, grade 3	Cervix	26
Parathyroid	1993	None	NA	Cervical intra-epithelial neoplasia, grade 3	Cervix	25
Peripheral nerves – abdomen	1981	Only surgery	NA	Cervical intra-epithelial neoplasia, grade 3	Cervix	32
Peripheral nerves / autonomic nervous system - NS	1986	Only surgery	NA	Cervical intra-epithelial neoplasia, grade 3	Cervix	31
				Central nervous system		
Parathyroid	1996	Surgery ,RT, CT	Abdominal	Schwannoma	Brain	18
Peripheral nerves – head and neck	1966	Surgery , RT	Brain	Menigioma (mixed)	Brain	50

Peripheral nerves / autonomic nervous system - NS	1998	Surgery , MIBG, CT	Only MIBG	Desembryoplastic neuroepethelia tumor (DNET)	Brain	10
Peripheral nerves / autonomic nervous system - NS	1989	Surgery , RT, CT	MIBG + bones spinal cord	Ganglioneuroma	Spinal cord	12
Parathyroid	1996	Surgery , CT, SCT	NA	Schwannoma	Peripheral nerves / autonomic nervous system – head and neck	18
Medulla	2003	Surgery, MIBG	Only MIBG	Schwannoma	SCST – upper extremity	19
Unknown	Unknown	Unknown	Unknown	Schwannoma	SCST – upper extremity	Unknown
Parathyroid	1996	Surgery ,RT, CT	Abdominal	Schwannoma	Brain	17.5
Unknown	Unknown	Unknown	Unknown	Schwannoma	Spinal cord	Unknown
Parathyroid	1989	Surgery, CT, SCT	NA	Giant cell tumor	SCST – upper extremity	24
Head and neck	1979	Surgery, RT, CT	Bone, head	Ganglioneuroma	SCST - Head and neck	28
Peripheral nerves – abdomen	1993	Only surgery	NA	Ganglioneuroma	SCST	6
Unknown	Unknown	Unknown	Unknown	Ganglioneuroma	Eyelid	Unknown
Not specified	2003	MIBG, CT	Only MIBG	Ganglioglioma	SCST	7
Parathyroid	1988	Surgery, RT, CT	Abdomen	Ganglioneuroma	Bone thorax	6
				Bone		
Medulla	1983	Surgery , CT, SCT	NA	Osteochondroma	Pelvic area	33
Unknown	Unknown	Unknown	Unknown	Osteochondroma	Pelvic area	Unknown
Unknown	Unknown	Unknown	Unknown	Osteochondroma	Bone – lower extremities	Unknown
Peripheral nerves / autonomic nervous system - NS	1993	Surgery , MIBG, CT, SCT	Only MIBG	Odontoma	Mandibula	12
Unknown	Unknown	Unknown	Unknown	Anarismatic bone cyst	Nose	Unknown
				Skin and Other		
Medulla	2002	Surgery , MIBG	Only MIBG	Myxoma	Mucous membrane oral cavity	7
Peripheral nerves / autonomic nervous system - NS	1968	Surgery , RT, CT	Neck / cervical + thorax	BCC	Skin - lower extremity	38
Medulla	1968	Surgery , RT, CT	Abdominal	BCC	Skin – trunk	41
Peripheral nerves - head and neck	1972	Surgery , RT, CT	Neck / cervical	BCC	Skin - head and neck	31
Parathyroid	1978	Surgery , RT, CT	Abdominal	BCC	Skin - upper extremity	32
Trunk	1979	Surgery , RT, CT	thorax	BCC	Skin -head and neck	27

SCST: Subcutaneous, connective and soft tissue; BCC Basal Cell Carcinoma; CNS Central Nervous System; NS: not specified , ^{131}I MIBG: metaiodobenzylguanidine labeled with radioactive iodine, CT: chemotherapy, RT: radiotherapy, SCT: Stem cell therapy. Overview of all subsequent non-malignant neoplasms (SNMN) found in the Dutch Childhood Cancer Survivor Study (DCCSS) LATER cohort after childhood neuroblastoma, including details on the childhood neuroblastoma treatment.

Appendix F. Fine-Gray Analysis for SMN and SNMN including chemotherapy groups

All neuroblastoma patients							
		SMN[1]			SNMN[2]		
Variable	**Number of survivors**	**Number of neoplasms**	**SHR**	**95% CI**	**Number of neoplasms**	**SHR**	**95% CI**
Sex							
Male	250	10	1 (ref)		12	1 (ref)	
Female	256	10	0.9	0.3 – 2.1	38	3.4	1.8 – 6.5
Age at diagnosis							
<12m	267	11	1(ref)		27	1 (ref)	
12-18m	59	5	1.8	0.6– 5.3	11	1.8	0.9 – 3.5
18+ m	180	4	0.4	0.1 – 1.8	12	0.4	0.2 – 1.0
Chemotherapy							
Alkylating agents	304	14	1.1	0.2 – 6.8	36	2.6	1.1 – 6.4
Anthracyclines	195	4	0.7	0.2 – 2.7	14	0.7	0.3 – 1.7
Epipodophyllotoxins and/or platinum agents[3]	219	7	0.8	0.3 – 2.6	19	1.5	0.6– 3.8
Vinca alkaloids	222	12	2.0	0.4 – 9.0	24	0.5	0.2 – 1.1
Antimetabolites	14	2	1.2	0.3 – 5.4	3	0.7	0.2 – 2.3
Radiotherapy other than MIBG[4]							
No	391	11	1 (ref)		27	1 (ref)	
Yes	114	9	1.4	0.5 – 4.2	23	2.6	1.5– 4.5
MIBG[4]							
No	418	14	1(ref)		41	1 (ref)	
Yes	87	6	4.9	1.5 – 15.7	9	2.7	1.1– 6.5
High-risk patients only[4]							
		SMN[5]			SNMN[5]		
Variable	**Number of survivors**	**Number of neoplasms**	**SHR**	**Variable**	**Number of survivors**	**Number of neoplasms**	**SHR**
Sex							
Male	106	5	1 (ref)		6	1 (ref)	
Female	88	4	1.0	0.2 – 4.1	15	4.1	2.0 – 8.6
Age at diagnosis							
<12m	68	5	1(ref)		11	1 (ref)	
12-18m	26	2	0.9	0.2– 4.0	5	1.3	0.6 – 2.9
18+ m	100	2	0.3	0.03 – 1.8	5	0.4	0.2 – 1.0
Chemotherapy							
Alkylating agents	173	8	5.3	0.05 – 530.5	18	1.8	0.7 – 5.1
Anthracyclines	113	3	0.7	0.09 – 5.0	7	0.7	0.3 – 1.6
Epipodophyllotoxins and/or platinum agents[2]	159	7	1.0	0.08 – 10.4	16	2.0	0.8– 5.2
Vinca alkaloids	155	7	0.5	0.04 – 7.1	15	0.6	0.2 – 1.4
Radiotherapy other than MIBG[4]							
No	128	6	1 (ref)		17	1 (ref)	
Yes	66	3	2.0	0.4 – 9.1	4	2.0	1.1 – 3.7
MIBG[3]							
No	108	3	1(ref)		12	1 (ref)	
Yes	86	6	4.7	1.1 – 20.4	9	2.6	1.1 – 6.5

SMN: subsequent malignant neoplasm, SNMN: subsequent non-malignant neoplasm, CI: confidence interval, ^{131I}MIBG: metaiodobenzylguanidine labeled with radioactive iodine, SHR: subdistribution hazard ratio. These analyses were done within a subset of the cohort for whom we had additional treatment details available (n=506) [1] Three patients were not in analysis due to missing data [2] 13 patients were not in analysis due to missing data [3] These chemotherapy agents were combined for this analysis because these agents were often administered together [4]26 survivors received both ^{131I}MIBG and other radiotherapy. [4] This model does not contain antimetabolites because no cases were observed.[5] Five patients were not in analysis due to missing data

Appendix G. Fine-Gray Analysis for SMN and SNMN including selected chemotherapy agents and doses

		SMN[1]			SNMN[2]		
Variable	**Number of survivors**	**Number of neoplasms**	**SHR**	**95% CI**	**Number of neoplasms**	**SHR**	**95% CI**
Sex							
Male	250	10	1 (ref)		12	1 (ref)	
Female	256	10	0.8	0.3 – 2.6	38	3.5	1.7 – 7.2
Age at diagnosis							
<12m	267	11	1(ref)		27	1 (ref)	
12-18m	59	5	2.8	0.7– 11.4	11	1.6	0.7 – 3.6
18+ m	180	4	0.7	0.1 – 2.4	12	0.4	0.2 – 1.0
Carboplatin							
None	345	14	1 (ref)		39	1 (ref)	
≤1680 mg/m2	77	2	0.8	0.09 – 6.2	5	1.4	0.4 – 4.6
>1680 mg/m2	78	4	2.2	0.5 – 9.1	6	1.6	0.5 – 5.5
Cyclophosphamide							
None	263	8	1 (ref)		20	1 (ref)	
≤5000 mg/m2	118	3	0.6	0.1 – 2.5	10	1.7	0.6 – 4.8
>5000 mg/m2	102	4	1.0	0.3 – 3.2	13	1.7	0.8 – 4.0
Vincristine							
None	*282*	*8*	1 (ref)		26	1 (ref)	
≤7.43 mg/m2	*85*	*3*	2.0	*0.4 – 10.6*	6	0.9	0.3 – 2.8
>7.43 mg/m2	*109*	*5*	*1.8*	*0.5 – 6.3*	11	0.6	0.2 – 1.6
Radiotherapy other than MIBG[3]							
No							
Yes	391	11	1 (ref)		27	1 (ref)	
	114	9	1.4	0.4 – 5.3	23	2.2	1.2 – 4.3
MIBG[3]							
No	418	14	1(ref)		41	1 (ref)	
Yes	87	6	4.5	0.9 – 22.4	9	2.8	0.9 – 8.9

SMN: subsequent malignant neoplasm, SNMN: subsequent non-malignant neoplasm, CI: confidence interval, ^{131}I MIBG: metaiodobenzylguanidine labeled with radioactive iodine, SHR: subdistribution hazard ratio. These analyses were done within a subset of the cohort for whom we had additional treatment details available (n=506) [1] 43 patients were not in analysis due to missing data [2] 49 patients were not in analysis due to missing data [3] 26 survivors received both ^{131}I MIBG and other radiotherapy.

Appendix H. Fine-Gray Analysis for SMN and SNMN including stem cell transplantation

		SMN[1]			SNMN[2]		
Variable	**Number of survivors**	**Number of neoplasms**	**SHR**	**95% CI**	**Number of neoplasms**	**SHR**	**95% CI**
Sex							
Male	250	10	1 (ref)		12	1 (ref)	
Female	256	10	0.8	0.3 – 2.1	38	3.0	1.6 – 5.5
Age at diagnosis							
<12m	267	11	1(ref)		27	1 (ref)	
12-18m	59	5	1.4	0.4 – 4.4	11	1.6	0.7 – 3.4
18+ m	180	4	0.4	0.1 – 1.5	12	0.6	0.2 – 1.1
Stem cell transplantation[3]							
No	407	16	1 (ref)		43	1 (ref)	
Yes	94	3	0.9	0.2 – 4.4	6	0.9	0.2 – 3.4
Radiotherapy other than MIBG[4]	391	11	1 (ref)		27	1 (ref)	
No	114	9	1.5	0.5 – 4.3	23	2.4	1.3 – 4.4
Yes							
MIBG[4]							
No	418	14	1(ref)		41	1 (ref)	
Yes	87	6	6.1	1.5 – 24.1	9	2.7	1.0– 7.5

SMN: subsequent malignant neoplasm, SNMN: subsequent non-malignant neoplasm, CI: confidence interval, ^{131}I MIBG: metaiodobenzylguanidine labeled with radioactive iodine, SHR: subdistribution hazard ratio. These analyses were done within a subset of the cohort for whom we had additional treatment details available (n=506) [1] Three patients were not in analysis due to missing data [2] 13 patients were not in analysis due to missing data [3] Among the 94 patients who received SCT 47 also received external beam radiation. [4] 26 survivors received both ^{131}I MIBG and other radiotherapy.

Appendix I. Sensitivity analysis: Fine-Gray Analysis for SMN and SNMN, with reduced number of variables

		SMN[1]			SNMN[2]		
Variable	**Number of survivors**	**Number of neoplasms**	**SHR**	**95% CI**	**Number of neoplasms**	**SHR**	**95% CI**
Radiotherapy other than MIBG[3]							
No	391	11	1 (ref)		27	1 (ref)	
Yes	114	9	1.7	0.6 – 4.5	23	2.3	1.3 – 4.1
MIBG[3]							
No	418	14	1(ref)		41	1 (ref)	
Yes	87	6	4.4	1.7 – 11.7	9	1.9	0.9 – 3.9

SMN: subsequent malignant neoplasm, SNMN: subsequent non-malignant neoplasm, CI: confidence interval, ^{131}I MIBG: metaiodobenzylguanidine labeled with radioactive iodine, SHR: subdistribution hazard ratio . These analyses were done within a subset of the cohort for whom we had additional treatment details available (n=506) [1] Three patients were not in analysis due to missing data [2] 13 patients were not in analysis due to missing data [3] 26 survivors received both ^{131}I MIBG and other radiotherapy

Appendix J. Sensitivity analysis: Fine-Gray Analysis for SMNs and SNMNs, without thyroid neoplasms

		SMN[1]			SNMN[2]		
Variable	**Number of survivors**	**Number of SMN**	**SHR**	**95% CI**	**Number of SNMN**	**SHR**	**95% CI**
Sex							
Male	250	9	1 (ref)		12	1 (ref)	
Female	256	9	0.9	0.3 – 2.2	35	2.9	1.6 – 5.5
Age at diagnosis							
<12m	267	9	1(ref)		26	1 (ref)	
12-18m	59	5	1.9	0.6 – 5.9	10	1.8	0.9 – 3.7
18+ m	180	4	0.5	0.1 – 1.7	11	0.5	0.2 – 1.0
Radiotherapy other than MIBG[3]							
No							
Yes	391	9	1 (ref)		26	1 (ref)	
	114	9	1.9	0.7 – 5.5	21	2.6	1.5 – 4.6
MIBG[3]							
No	418	14	1(ref)		40	1 (ref)	
Yes	87	4	3.8	1.1 – 13.5	7	2.3	1.1 – 5.1

SMN: subsequent malignant neoplasm, SNMN: subsequent non-malignant neoplasm, CI: confidence interval, ^{131}I MIBG: metaiodobenzylguanidine labeled with radioactive iodine, SHR: subdistribution hazard ratio. These analyses were done within a subset of the cohort for whom we had additional treatment details available (n=506) [1] One patient was not included in the analysis due to missing data. [2] Nine patients were not included in the analysis due to missing data. [3] 26 survivors received both ^{131}I MIBG and other radiotherapy.

Appendix K. MIBG information subset cohort (n=506)

	No ^{131}I MIBG	^{131}I MIBG	p-value
Total cohort [1]	418 (82.8%)	87 (15.5%)	
SMN			0.13
Yes	14	6	
No	404	81	
SNMN			0.85
Yes	41	9	
No	377	78	
Sex			0.045
Male	198	52	
Female	220	35	
Vital status			<0.001
Alive	401	72	
Deceased	18	15	
Period of NB diagnosis			<0.001
<1980	67	0	
1980-1989	109	4	
1990-1999	74	30	
2000-2009	102	38	
2009 +	66	15	
Risk group			*<0.001*
Low-risk	205	*0*	
Intermediate-risk	61	*1*	
High-risk	108	*86*	
Unknown	44	*0*	
Age at NB diagnosis (months)			0.025
<12	231	35	
12-18	48	11	
18 – 48	94	23	
48+	45	18	
Radiotherapy other than MIBG			0.090
No	330	61	
Yes	88	26	
Chemotherapy			0.001
No	171	19	
Yes	247	68	
Stem cell transplantation			<0.001
No	366	41	
Yes	48	46	

^{131}I MIBG: metaiodobenzylguanidine labeled with radioactive iodine SMN: subsequent malignant neoplasm, SNMN: subsequent non-malignant neoplasm, NB: Neuroblastoma, [1] For one patient radiotherapy treatment details were unknown. p-value were calculated using Fisher's Exact Test square test comparing differences between the two groups.

Appendix L. Possible genetic predispositions for neuroblastoma and other neoplasms

Syndrome	Pre-disposed tumours
Neurofibromatosis type 1	Neuroblastoma, neurofibroma, malignant peripheral nerve sheath tumor, brain tumors, optic glioma
Li-fraumeni	Neuroblastoma, breast cancer, osteosarcoma, brain tumors, leukemia, adrenocortical carcinoma, soft tissue sarcoma
Congentical Central Hypoventilation Syndrome (CCHS)	Neuroblastoma, ganglioneuroma, Ganglioneuroblastoma
Noonan syndrome	Neuroblastoma, leukemia, low grade glioma, rhabdosarcoma
LEOPARD syndrome (Moonan syndrome with multiple lentigines)	Neuroblastoma, leukemia, melanoma
Costello syndrome	Neuroblastoma, papilloma, rhabdomyosarcoma, transactional cell carcinoma, bladder carcinoma
Weaver syndrome	Neuroblastoma
ROHHAD	Neuroblastoma, ganglioneuroma, Ganglioneuroblastoma (neural crest tumors)
Beckwith-Wiedemann	Neuroblastoma, Wilms tumor, hepatoblastoma
Familial Paraganglioma	Neuroblastoma, paraganglioma, pheochromocytoma
Fanconi anemia	Neuroblastoma, leukemia, wilms tumor, medulloblastoma, embryolnal tumors, sarcomas, nephroblastoma
Cardiofaciocutanous syndrome	Still unclear, possibly similar to LEOPARD
Turner syndrome	Neuroblastoma and related tumors

ROHHAD, rapid-onset obesity, hypothalamic dysfunction, hypoventilation and autonomic dysfunction, Overview of possible genetic predispositions for neuroblastoma as well as other neoplasms. Table based on information from multiple articles [58-63]

Appendix M. Additional information thyroid protection

Neuroblastoma treatment	Observed neoplasms	Received thyroid protection
MIBG, CT	Thyroid carcinoma	KI
MIBG, CT	Thyroid carcinoma	KI, thiamazole, thyroxine

^{131}I MIBG: metaiodobenzylguanidine labeled with radioactive iodine, CT: Chemotherapy. In the Netherlands, neuroblastoma patients receive thyroid protection when exposed to MIBG. Until 2003 protection was done with potassium-iodine (KI) alone, and thereafter with a combination of KI, thiamazole and thyroxine [43,45]

Acknowledgement

We thank all physicians, research nurses, data managers, research assistants, the Dutch National Childhood Cancer Association, and participating patients for their contribution. Furthermore, the authors thank the registration team of the Netherlands Comprehensive Cancer Organization (IKNL) for the collection of data for the Netherlands Cancer Registry. Also, we thank the staff from Palga Foundation for providing linkage data on subsequent non-malignant neoplasms from their registry.

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Chapter 5

Increased Risk of Subsequent Neoplasm after Hematopoietic Stem Cell Transplantation in 5-year Survivors of Childhood Acute Lymphoblastic Leukemia

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Provisionally accepted, Blood Cancer Journal, 2024

Abstract

Acute lymphoblastic leukemia (ALL) survivors are at risk for developing subsequent neoplasms, but there is limited information on long-term risks and risk factors for both subsequent malignant neoplasms (SMNs) and subsequent non-malignant neoplasms (SNMNs). We analyzed long-term risk and risk factors for SMNs and SNMNs among 3,291 five-year ALL survivors from the Dutch Childhood Cancer Survivor Study-LATER cohort (1963-2014). We calculated standardized incidence ratios (SIRs) and cumulative incidences, and used multivariable Cox proportional hazard regression analyses for analyzing risk factors. A total of 97 survivors developed SMNs and 266 SNMNs. The 30-year cumulative incidence was 4.1%(95%CI:3.5-5.3) for SMNs and 10.4%(95%CI:8.9-12.1) for SNMNs. Risk of SMNs was elevated compared to the general population (SIR:2.6,95%CI:2.1-3.1). Survivors treated with hematopoietic stem cell transplantation (HSCT) with total body irradiation (TBI) (HR:4.2,95%CI:2.3-7.9), and without TBI (HR:4.0,95%CI:1.2-13.7) showed increased SMN risk versus non-transplanted survivors. Cranial radiotherapy (CRT) was also a risk factor for SMNs (HR:2.1, 95%CI:1.4-4.0). In conclusion, childhood ALL survivors have an increased SMN risk, especially after HSCT and CRT. A key finding is that even HSCT-treated survivors without TBI treatment showed an increased SMN risk, possibly due to accompanied chemotherapy treatment. This emphasizes the need for careful follow-up of HSCT and/or CRT-treated survivors.

Introduction

Acute lymphoblastic leukemia (ALL) is the most common type of childhood cancer(1-3), with a five-year survival rate currently exceeding 90%(4). However, ALL survivors are at risk for long-term adverse health outcomes including the development of subsequent neoplasms(5, 6). Compared to the general population, childhood ALL survivors have a 2.6 to 13.5 times higher risk of developing subsequent malignant neoplasms (SMNs)(5-8). The most frequently observed SMNs in ALL survivors are central nervous system (CNS) tumors(6, 9). In addition to SMNs, some types of subsequent non-malignant neoplasms (SNMNs) can also cause serious morbidity, such as subsequent meningiomas (5, 10).

Treatment protocols for ALL patients have changed over time. Major adjustments in the Netherlands were (1) the substitution of cranial radiotherapy (CRT) by CNS prophylaxis with intrathecal high-dose methotrexate since to the DCOG-ALL VI protocol in December 1984(11, 12) and (2) trials with replacing TBI with a chemotherapy conditioning regimen for HSCT between 2011 and 2021(13, 14) Several studies examined treatment-related risk factors for subsequent neoplasms in ALL survivors(5, 15-17). Although many studies were limited by short follow-up times (15-17) or the limited availability of specific treatment data(5, 15-17), several risk factors have been suggested. The risk of developing a subsequent neoplasm was found to be higher in patients who were treated with radiotherapy(18), especially CRT(7, 16, 19). Furthermore, patients who received HSCT also showed an increased risk of subsequent neoplasms as compared to non-transplanted leukemia survivors(20-23), which is often suggested to be due to TBI(17, 21, 24, 25). However, the separate impact of HSCT and TBI are not fully clear.

In the current study, we aimed to analyze the long-term risk and associated risk factors for developing SMNs and SNMNs in 5-year survivors of childhood ALL diagnosed between 1963 and 2014.

Methods

Patients

In this multicenter study, 11 704 five-year survivors diagnosed under the age of 18 in any of the seven former pediatric oncology/stem cell centers in the Netherlands, in the period 1963-2014 were included in the Dutch Childhood Cancer Survivor Study (DCCSS)-LATER cohort(26, 27). Data collection from both the original cohort (1963-2001)(26) and the expansion cohort (2002-2014) has been previously documented(28). In the current study we included 3291 survivors diagnosed with ALL according to the International Classification of Disease for Oncology, Third Edition (ICD-D-O-3)(29) (ICD-O-3 morphology code 9835/3, 9836/3 or 9837/3).

Data collection

Information about demographics, diagnosis, and childhood cancer treatment, including relapses, was collected by trained data managers. For 262 (8%) survivors who objected to adding additional linkage data, we only had basic yes/no treatment data available. For the other 3029 ALL survivors, detailed treatment data were available including type and doses of chemotherapy and radiotherapy and information about hematopoietic allogenic stem cell transplantation (HSCT). For anthracyclines and alkylating agents, cumulative doses were calculated. For anthracyclines we used the doxorubicin isotoxic equivalent (DIE) to sum doses of agents(30)(**Table S1**). For alkylating agents, dose was summed according to the cyclophosphamide equivalent dose (CED)(31)(**Table S2**).

Data on subsequent neoplasms were ascertained by linkages to two nationwide registries: the Netherlands Cancer Registry (NCR)(32), with nationwide coverage since 1989, (although some regional registries attained full local coverage earlier), and the Dutch Nationwide Pathology Databank (Palga)(33), with nationwide coverage since 1991. The linkage procedure for the DCCSS-LATER cohort has been reported previously(26, 28). The NCR data were used as main source for malignant neoplasms. For malignant tumors diagnosed before 1989, we used the partially available NCR data in combination with data from Palga and from the DCCSS-LATER registry, based on medical record data. Pathology reports were reviewed to resolve discrepancies between multiple SMN sources. SMN data was complete up to January 31st,

2022. Palga were used as source for histologically confirmed non-malignant tumors and basal cell carcinomas (BCCs) of the skin. SNMNs were defined as subsequent benign, borderline malignant, or in situ tumors. Non-malignant skin tumors were excluded. Excerpts were manually reviewed to identify and classify non-malignant neoplasms according to the ICDD-O-3(29). Challenging records were discussed with a pathologist (RdK). SNMN data was complete up to April 7th, 2022 for the original cohort and up to November 30th, 2022 for the expansion cohort. BCC data was complete until November 30th, 2022. We included subsequent neoplasms that occurred five years or more after ALL diagnosis and were histologically different from the ALL.

Statistical analyses

Analyses were done separately for SMNs, SNMNs and BCCs, because of the differences in entry time. For SMNs, follow-up started five years after ALL diagnosis and for SNMNs and BCCs follow-up started five years after ALL diagnosis or January 1, 1991 (start nationwide coverage Palga), whichever occurred last. Follow-up ended on the date of diagnosis of the first subsequent neoplasm of interest (e.g. for analyses on malignant CNS tumors, at date of first CNS tumor, irrespective of a prior SMN, SNMN or BCC), date of death, date last known vital status (emigration, loss to follow up), or end of study (January 31st, 2022 for SMNs and April 7, 2022 for SNMNs and BCCs), whichever occurred first.

We calculated standardized incidence ratios (SIRs) and absolute excess risks (AERs) of SNMs. The SIR was calculated by dividing the observed number by the expected number based on age-, sex-, and calendar year-specific general population rates from the NCR. The AER was calculated as the excess number of SMNs per 10,000 person years. SIRs and AERs were calculated for any SMN and for specific subgroups. For SNMNs and BCCs, there are no reference rates for the general population and we could therefore not calculate SIRs and AERs.

For SMNs, SNMNs, and BCCs we calculated cumulative incidences, accounting for death as a competing risk. We also calculated the cumulative incidence for survivors diagnosed before and after 1984. The cut-off of 1984 was based on the switch from protocol ALL-V to ALL-VI, where cranial radiotherapy (CRT) was omitted as standard of care for non-high risk ALL

survivors. Differences between curves were compared using Gray's tests(34). Furthermore, we examined potential risk factors by using multivariable Cox proportional hazard regression models, with attained age as time scale(35). Our base model included sex, age at diagnosis, cranial radiotherapy, HSCT ± TBI as part of the conditioning regimen for HSCT. In addition, we analyzed the following chemotherapy groups and dose categories: alkylating agents, anthracyclines, etoposide. Etoposide was predominantly administered to HSCT patients as part of initial treatment in this high-risk group or/and as conditioning for HSCT and was sparingly administered to patients without HSCT. In order to stratify these risks, mutually exclusive groups were created combining etoposide exposure with HSCT subgroups. Stratification on etoposide exposure was only feasible in the HSCT with TBI group, but not in the HSCT without TBI group due to low numbers. Furthermore, we were not able to analyze effects of platinum agents (not part of standard ALL treatment and therefore only very few patients in our cohort were treated with this), glucocorticoids, vinca alkaloids, antimetabolites and asparaginases (part of ALL treatment in almost all protocols and therefore almost everyone in our cohort had this as part of therapy). Although we adjusted for all treatments in our main analysis, we did conduct a sensitivity analysis including only survivors with a relapsed ALL to evaluate the effect of HSCT in a more homogenous group of survivors with intensive treatments. The proportional hazard assumption was tested in all models and was not violated. All analyses were performed using SPSS v 26.0 or R studio v 1.3.

Results

Patient characteristics

Among the 3 291 childhood ALL survivors, 55.2% were male (**Table 1**). Median age at diagnosis was 4.7 years (range: 0.0-17.8 years). In total, 72.8% were treated with chemotherapy only, 18.0% with a combination of chemotherapy and any radiotherapy, 1.7% with chemotherapy and HSCT and 6.9% with a combination of chemotherapy, radiotherapy and HSCT. Of the 3 029 survivors with additional treatment data, 24.5% were treated with any type of radiotherapy, of whom 17.4% with only CRT, 5.3% with only TBI, 0.9% with CRT and TBI and 0.5% with other types of radiotherapy **(Table 1).** Of the 420 survivors who experienced a relapse, 39.3% received only CRT, 25.2% only TBI, 6.2% CRT and TBI and 2.4% other types of radiotherapy **(Table S3).**

Of all survivors, 430 (13.1%) developed at least one subsequent neoplasms, of whom 97 (2.9%) survivors developed at least one SMN, 266 (8.1%) at least one SNMN and 172 (5.2%) at least one BCC. In total, 21 of the 430 survivors who developed a subsequent neoplasm developed both an SMN and SNMN. Among the 420 survivors with relapsed disease, 24 developed at least one SMN.

Subsequent malignant neoplasms

The median follow-up time for SMN was 21.6 (range: 5.0-54.9) years since ALL diagnosis. In total, 106 SMNs were observed in 97 survivors, with 9 survivors developing multiple SMNs. The median latency between childhood ALL diagnosis and occurrence of an SMN was 26.5 (range: 5.8–46.1) years. 87 SMNs were solid tumors. The most frequently observed SMN sites were CNS (n=15), thyroid (n=13) and skin (13 melanomas and 4 squamous cell carcinomas) **(Table 2).**

Overall SMN risk was significantly increased in ALL survivors compared to the age-, sex-, and calendar-year matched general population with an SIR of 2.6 (95% CI 2.1-3.1) and an AER of 10.0 per 10,000 person-years. The AER increased with follow-up time after diagnosis and was 25.5 per 10,000 person-years for follow-up time beyond 30 years. The highest AERs compared to the general population were observed for CNS tumors (AER: 2.2) and thyroid malignancies (AER: 2.0) **(Table 2).** High SIRs were observed for survivors who were treated with chemotherapy and HSCT (SIR: 8.4, 95%CI: 0.2-47.0) and chemotherapy, HSCT and radiotherapy (SIR: 10.5, 95%CI:6.1- 16.8) **(Table S4**). Types of SMNs after HSCT are shown in **Table S5.** ALL survivors who had a relapse (n=24; SIR: 5.6, 95%CI: 3.6-8.4) had a higher SIR than those without a relapse (n=62; SIR: 2.2, 95%CI: 1.7-2.8) **(Table S4).**

The 30-year cumulative incidence of any SMN was 3.8% (95%CI:2.9-4.9) (**Figure 1).** The cumulative incidence of any SMN was not different between survivors diagnosed ≤1984 and survivors diagnosed >1984 (p=0.64), the year where CRT was omitted as standard of care **(Figure 2).** However, the cumulative incidence of subsequent CNS tumors was significantly lower for survivors diagnosed after 1984 compared to survivors diagnosed in or before 1984 (p=0.005) **(Figure 2).**

Subsequent non-malignant neoplasms

In total, 266 survivors developed histologically confirmed SNMNs, with a median latency time between childhood cancer diagnosis and the first SNMN of 25.7 (range: 5.5–48.3) years. The most frequently observed SNMNs were non-malignant meningiomas (n=81), urogenital system neoplasms (n=42), and lipomas (n=36) **(Table 2).** Types of SNMNs after HSCT are shown in **Table S5.** The 30-year cumulative incidence of any SNMN was 9.9% (95% CI: 8.5-11.5) and highest for SNMN subtypes meningiomas (2.5%, 95% CI: 1.7-3.5) and urogenital neoplasms (1.9% 95% CI: 1.3–2.8) **(Figure 1, Table 2)**. For any SNMN, the cumulative incidence was not different for survivors diagnosed ≤1984 and 1984> (p=0.84), but we did see a significant decrease in the incidence of non-malignant meningiomas for survivors diagnosed after 1984 (p<0.001) **(Figure 2).**

Basal cell carcinoma risk

In total, 172 survivors developed at least one basal cell carcinoma (BCC), with a median latency time of 26.1 (range: 5.6–43.5) years. The 30-year cumulative incidence of BCC was 5.6% (95%CI: 4.5–7.0) **(Figure 1).** Among survivors treated with radiotherapy, the 30-year cumulative incidence for BCC was 10.9% (95% CI:8.6-12.6) compared to 1.2% (95% CI: 0.6-2.4) for survivors treated without radiotherapy **(Figure S1)**. The HR was 19.3 (95%CI:12.2-29.8) for survivors treated with TBI and 7.6 (95%CI:5.5-10.5) for survivors treated with CRT (data not shown).

Risk factors for subsequent neoplasms

We analyzed risk factors for SMNs and SNMNs in multivariable models among 3 029 survivors for whom extensive treatment details were available. ALL survivors treated with cranial radiotherapy (CRT) (n=48 SMNs) had a significantly higher risk of developing any SMN compared to survivors treated without CRT (n=38 SMNs) (HR: 2.3, 95% CI: 1.4-4.0) (**Table 3**). Furthermore, HSCT was significantly associated with increased SMN risk, regardless of whether TBI was included in the conditioning regimen (HR for HSCT with TBI: 4.2, 95% CI: 2.3-7.8; HR for HSCT without TBI: 4.0, 95% CI: 1.2-13.7) (**Table S6**). After adjusting for chemotherapy, we still observed a significant effect of HSCT without TBI (n=3 SMNs) (HR: 3.8, 95% CI: 1.1-13.8) **(Table 3)**. Survivors treated with HCST, TBI, and etoposide (n=11 SMNs) appeared to have a higher risk (HR: 5.7; 95% CI: 2.5-12.8) compared to survivors treated with

HSCT and TBI without etoposide (n=2 SMNs) (HR: 1.5; 95% CI: 0.5-6.5); however, this difference was not significant.

ALL survivors treated with CRT also had a higher risk of developing any SNMN compared to survivors treated without CRT (HR: 1.9, 95% CI: 1.3 - 2.6) (**Table 3**). Furthermore, compared to survivors treated without HSCT, survivors who received HSCT with TBI showed a significantly increased risk of developing SNMN (HR: 6.4, 95% CI: 3.9-10.4), whereas those treated with HSCT without TBI did not show a significant increase (HR: 1.9, 95%CI:0.6-7.7) **(Table S6).** After adjusting for chemotherapy, significant effects were still observed for HSCT with TBI, both with etoposide (HR: 4.9, 95% CI: 2.8-10.3) and without etoposide (HR: 4.9, 95% CI: 2.3-10.3) **(Table 3)**.

ALL survivors treated with radiotherapy were at an increased risk of developing basal cell carcinoma, with HRs of 4.3 (95% CI: 2.8–6.7) for CRT vs. no CRT and 6.4 (95% CI: 3.9-10.4) for HSCT plus TBI vs. no HSCT **(Table S6).**

In a sensitivity analysis including only survivors who experienced a relapse, HSCT remained a significant risk factor for SMNs (HR: 2.5, 95%CI:1.0-3.4), and BCCs (HR: 2.7, 95%CI: 1.4-5.4), but not for SNMNs (HR: 4.9, 95%CI: 0.5-2.0) **(Table S7).**

Discussion

This study demonstrates that five-year survivors of childhood ALL have an increased risk of developing subsequent neoplasms, especially after HSCT. A significant new finding is that ALL survivors treated with HSCT but without TBI also have an increased risk of SMNs compared to ALL survivors not treated with HSCT, possibly due to accompanying chemotherapy. Furthermore, CRT was a significant risk factor for development of both SMNs and SNMNs. The risk of any SMN and any SNMN did not decrease for survivors treated after 1984, when prophylactic CRT was omitted from standard protocols, compared to those threated in or before 1984. However, the risk of malignant CNS tumors and benign meningiomas decreased significantly among those treated after 1984.

In this study, we showed that ALL survivors who received HSCT, both with and without TBI, had an increased risk of SMNs compared to survivors treated without HSCT. Previous studies that reported on subsequent neoplasms after HSCT vs. no HSCT have also shown significantly increased risk for SMNs and SNMNs after HSCT(20, 21) with most studies attributing this increased risk to TBI conditioning(21, 25). Recently, similar findings have also been reported for ALL survivors(17). In our cohort we observed a similar increased risk of SMNs for ALL survivors who received HSCT with TBI and those who received HSCT without TBI, compared to ALL survivors treated without HSCT. This suggests that aspects of HSCT other than TBI contribute to the elevated risk of SMN development after HSCT. In our multivariable model, we observed a suggestive trend with higher risks among survivors receiving etoposide within the HSCT with TBI group. Due to a limited number of cases, we were unable to stratify by etoposide exposure within the HCST without TBI subgroup and can therefore not analyze whether this increased risk might be due to concurrent etoposide treatment. Other factors beyond chemotherapy could also play a role; for instance, an association between chronic graft versus host disease (GVHD) and oral cavity cancers has been implied(36). Although we lacked GVHD information, among the nine ALL survivors with malignant oral neoplasms in our cohort, none had received HSCT, and only one out of four with non-malignant oral neoplasms had received HSCT. We could therefore not confirm this previous observation.

Previous studies have indicated that unfractionated and high-dose TBI seemed to be associated with a higher risk of SMN compared to low-dose TBI(21, 25). Unfortunately, our sample size was too small to further explore the impact of the TBI dose and fractionation. In our cohort, most survivors who received TBI were treated with unfractionated TBI or TBI delivered in 2 fractions.

We observed a significantly lower cumulative incidence of malignant CNS tumors and non-malignant meningiomas among patient diagnosed after 1984. In 1984, CRT was substituted by CNS prophylaxis involving high-dose methotrexate and intrathecal chemotherapy as part of the standard ALL treatment protocols in the Netherlands with the introduction of the DCOG-ALL VI protocol(11, 12). CRT has been shown to be an important risk factor for CNS neoplasms, particularly meningiomas(27, 37-40). We did not observe a decrease in the overall incidence

of SMNs and SNMNs for patients treated 1984> vs. ≤1984, which is consistent with the findings of Ishida et al.(16).

Survivors of ALL might also face an increased risk of subsequent neoplasms due to genetic syndromes that could predispose individuals to both ALL and subsequent neoplasms(41, 42). Well-established associations with childhood leukemia and subsequent neoplasms include conditions such as neurofibromatosis-1 (linked to CNS tumors)(43) and Li-Fraumeni Syndrome (linked to multiple tumors such as sarcomas or breast cancer)(44). Information on predisposition syndromes within our cohort was incomplete, preventing a detailed examination of their role. Based on the partially available data, anecdotal evidence includes cases of two congenital aberrations potentially related to the development of subsequent neoplasms: one patient with Down syndrome who developed a subsequent B-cell leukemia and another with a congenital bone aberration who developed an osteochondroma.

Major strengths of our study include the large cohort size, extensive follow-up duration, and comprehensive treatment data on an individual level. Due to linkage with nationwide registries we ensure complete follow-up data including objective data on both malignant and histologically-confirmed non-malignant neoplasms. We also need to consider some limitations. Firstly, our non-malignant data only includes pathologically-confirmed neoplasms, which might cause a slight underrepresentation of the true SNMN incidence. However, physicians might be more alert in childhood cancer survivors which could lead to increased detection of SNMNs. Secondly, we lacked specific data on protocols and risk groups among survivors, therefore comparison of subsequent neoplasms risks across different protocols could not be conducted. Lastly, we had only limited data on genetic predisposition.

In conclusion, childhood ALL survivors have an increased risk of SMNs. Previous studies have shown that TBI increases SMN risk in HSCT survivors. Our results show that HSCT-treated survivors without TBI conditioning also have increased risk of SMNs. This shows the importance of future studies to further investigate the effects of different conditioning regimes and accompanying therapies in survivors receiving HSCT on the development of SMNs, including more detailed assessments of chemotherapy dose, TBI dose and fractionation

used before and after HSCT. Our results also emphasize the need for careful follow-up of survivors treated with HSCT with or without TBI, or with CRT.

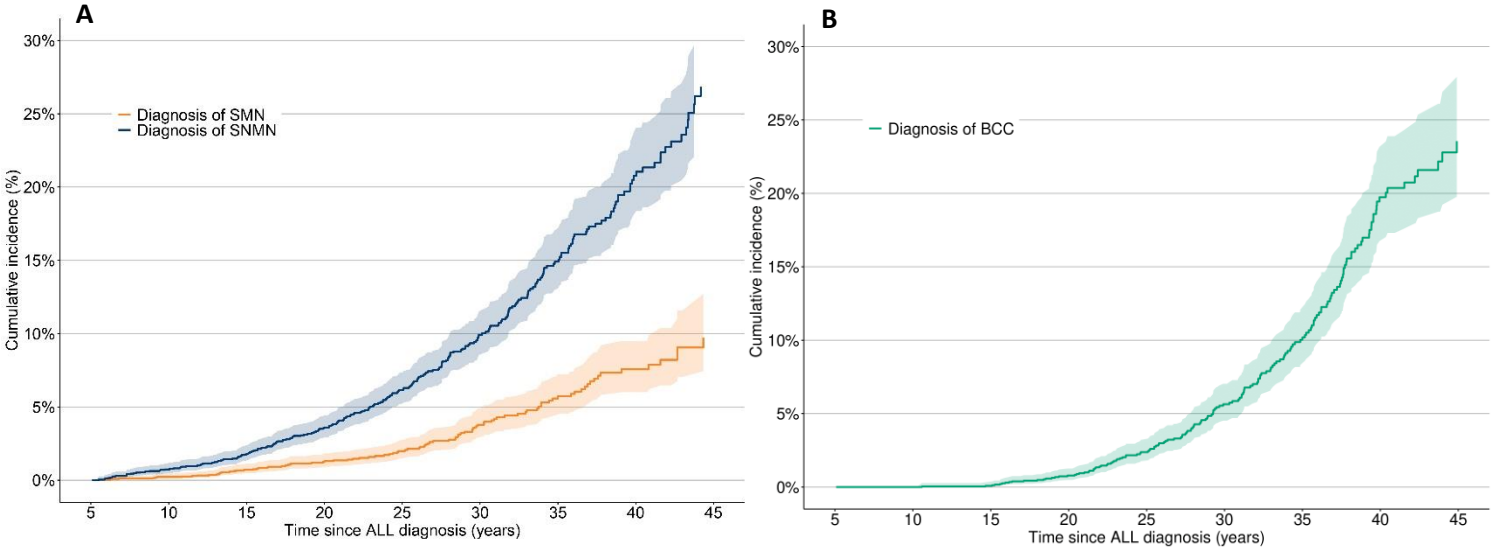

Figure 1. Cumulative incidence of subsequent neoplasms in the Dutch Childhood Cancer survivor study (DCCSS)-LATER cohort with a follow-up time since childhood acute lymphoblastic leukemia diagnosis **A**. Subsequent malignant neoplasms and non-malignant neoplasms Cumulative incidence of subsequent malignant neoplasms (SMN) and subsequent non-malignant neoplasms (SNMNs) **B**. Basal cell carcinomas Cumulative incidence of Basal cell carcinomas (BCCs)

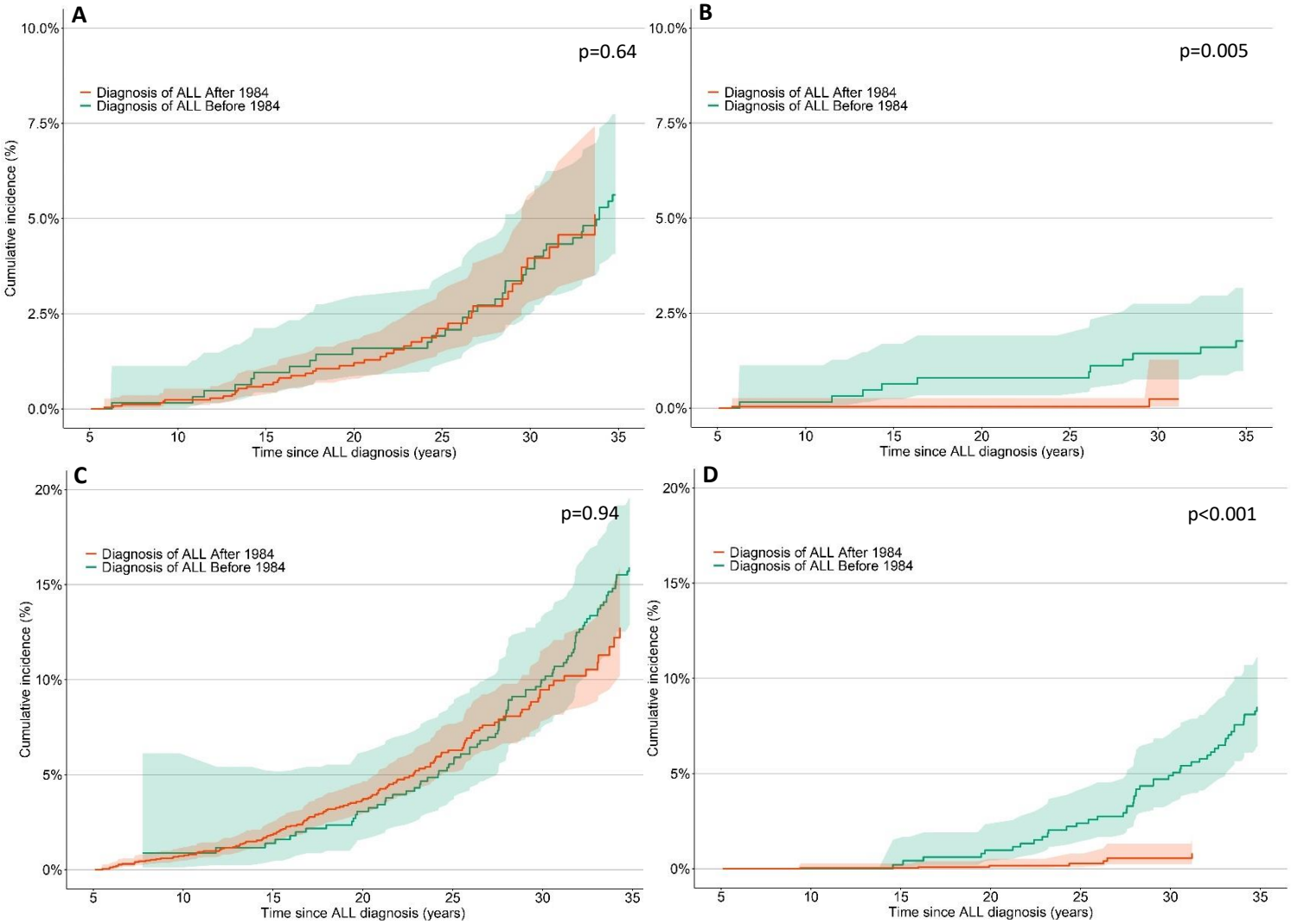

Figure 2. Cumulative incidence of subsequent neoplasms in the Dutch Childhood Cancer survivor study (DCCSS)-LATER cohort with a follow-up time since childhood acute lymphoblastic leukemia diagnosis, stratified by treatment before or in 1984 or after 1984 **A**. Subsequent malignant neoplasms Cumulative incidence of all subsequent malignant neoplasms (SMNs) **B**. Subsequent malignant central nervous system tumors Cumulative incidence of subsequent malignant tumors of the central nervous system **C**. Subsequent non-malignant neoplasms Cumulative incidence of all subsequent non-malignant neoplasms (SNMNs) **D**. Subsequent non-malignant meningiomas Cumulative incidence of subsequent non-malignant meningiomas.

Table 1. Characteristics of ALL survivors from DCCSS-LATER cohort and of survivors with an SMN, SNMN, BCC

Characteristics	Total	Subsequent malignant neoplasms	Subsequent non-malignant neoplasms	Basal cell carcinomas
Total cohort	**3291**	**97**	**266**	**172**
Sex				
Male	1819 (55%)	48 (49%)	114 (43%)	87 (51%)
Female	1472 (45%)	49 (51%)	15 (57%)	85 (49%)
Age at ALL diagnosis				
0-4yrs	1386 (42%)	35 (36%)	108 (41%)	73 (42%)
5-9yrs	1293 (39%)	36 (37%)	110 (41%)	68 (40%)
10-14yrs	471 (14%)	20 (21%)	42 (16%)	27 (16%)
15+yrs	141 (4%)	6 (6%)	6 (2%)	4 (2%)
Year of ALL diagnosis				
1984	629 (19%)	52 (54%)	133 (50%)	128 (74%)
1985-1994	673 (20%)	29 (30%)	71 (27%)	36 (21%)
1995-2004	981 (30%)	10 (10%)	47 (18%)	8 (5%)
2005+	1008 (31%)	6 (6%)	15 (6%)	0 (0%)
Follow up time (years)[1]				
0-9	449 (14%)	7 (7%)	6 (2%)	0 (0%)
10-19	1025 (31%)	25 (26%)	31 (13%)	7 (4%)
20-29	909 (28%)	33 (34%)	65 (24%)	15 (9%)
30+	908 (28%)	32 (33%)	164 (62%)	150 (87%)
Attained age (years)				
5-14	392 (12%)	3 (2%)	6 (2%)	0 (0%)
15-24	987 (30%)	23 (24%)	19 (7%)	5 (3%)
25-34	895 (27%)	30 (31%)	74 (28%)	17 (10%)
35+	1017 (31%)	41 (42%)	167 (63%)	150 (87%)
Relapse				
No	2609 (79%)	62 (64%)	167 (63%)	99 (58%)
Yes	420 (13%)	24 (36%)	48 (18%)	41 (24%)
Vital status				
Alive	3060 (93%)	62 (64%)	240 (90%)	162 (94%)
Deceased	231 (7%)	35 (36%)	26 (10%)	10 (6%)
Treatment for ALL				
CT no HSCT	2395 (73%)	28 (29%)	87 (33)%)	15 (9%)
RT + CT	593 (18%)	51 (53%)	129 (48%)	130 (76%)
CT + HSCT (no RT)	57 (2%)	1 (1%)	1 (0.4%)	0 (0%)
RT + CT + HSCT	226 (7%)	17 (18%)	48 (18%)	27 (16%)
Unknown	20 (1%)	0 (0%)	1 (0.4%)	0 (0%)
Radiotherapy type*				
No radiotherapy	2285 (69%)	27 (28%)	72 (72%)	15 (9%)
CRT no TBI	528 (16%)	45 (46%)	102 (38%)	104 (60%)
TBI no CRT	160 (5%)	10 (10%)	31 (12%)	16 (9%)
CRT + TBI	28 (1%)	3 (3%)	8 (3%)	5 (3%)
Other radiotherapy [2]	16 (0.5%)	1 (1%)	2 (1%)	0 (0%)
Chemotherapeutic agents*				
Alkylating agents [3]	1984 (60%)	46 (47%)	115 (43%)	64 (37%)
Anthracyclines [4]	2084 (63%)	57 (59%)	131 (49%)	75 (44%)
Epipodophyllotoxins [5]	509 (15%)	27 (28%)	56 (21%)	35 (20%)
Platinum agents [6]	2 (0.06%)	0 (0%)	0 (0%)	0 90%)
Vinca alkaloids [7]	3003 (91%)	86 (89%)	215 (81%)	140 (81%)
Antimetabolites[8]	3003 (91%)	86 (89%)	215 (81%)	140 (81%0
Asparaginase [9]	2827 (86%)	77 (79%)	186 (70%)	114 (66%)
Alkylating agents (cumulative dose; CED) mg/m2*				
None	1021 (31%)	40 (41%)	99 (37%)	76 (44)%
0-2000	1125 (34%)	13 (13%)	29 (11%)	12 (7%)
2000+	809 (25%)	32 (33%)	81 (30%)	46 (27%)
Unknown	336 (10%)	1 (1%)	6 (2%)	6 (3%)
Anthracyclines (cumulative dose) mg/m2*				
None	923 (28%)	29 (30%)	83 (31%)	65 (38%)
<200	1288 (39%)	39(40%)	91 (34%)	58 (34%)
200+	752 (23%)	17 (18%)	35 (13%)	15 (9%)
Unknown	66 (2%)	1 (1%)	6 (2%)	2 (1%)
Epipodophyllotoxins agents (cumulative dose) mg/m2*				
None	2495 (76%)	59 (61%)	158 (59%)	105 (61%)
<1485	241 (7%)	12 (12%)	19 (7%)	19 (11%)
1485+	246 (7%)	15 (15%)	34 (13%)	15 (9%)

Unknown	47 (1%)	0 (0%)	2 (1%)	1 (1%)

SMN: subsequent malignant neoplasm, SNMN: subsequent non-malignant neoplasm, BCC: Basal cell carcinoma, ALL: Acute lymphocytic leukemia (ALL) CRT: cranial radiotherapy, TBI: Total body irradiation *This is part of subset of the data (n=3029) of whom we had additional treatment details available with 86 SMNs, 215 SNMNs and 140 BCCs. In total 79 survivors received unfractionated TBI, 62 survivors in 2 fractions and 38 survivors in 3 or more fractions. [1] Follow-up time since ALL diagnosis [2] Seven Mantle field, six testis, one mantle + testis, one Neck/Cervical and one unknown [3] 286 survivors with unknown data of therapy [4.] 22 survivors with unknown data of therapy [5] 25 survivors with unknown data of therapy [6] 33 survivors with unknown data of therapy [7] 16 survivors with unknown data of therapy [8] 15 survivors with unknown data of therapy [9] 17 survivors with unknown data of therapy.

Table 2: Cumulative Incidence, SIRs, EARs, and latency of subsequent neoplasms

Type of subsequent neoplasm	ICD-O-3 code	Cases	SIR (95% CI)	AER/ 10.000 PY	30-year Cumulative Incidence (95%-CI)	Median latency (range), years[1]
Total SMNs[2]		**97**	**2.6 (2.1-3.1)**	**10.0**	**3.8 (2.9-4.9)**	**26.5 (5.8–46.1)**
Solid tumors	**C000-C809**	**87**	**2.7 (2.2 – 3.4)**	**9.3**	**3.4 (268 – 4.4)**	**26.5 (5.8 – 46.1)**
Head and neck	C000-149, C300-C329, C690-C699	10	11.1 (5.3 – 20.4)	1.5	0.2 (0.07 – 0.5)	31.2 (9.3 – 41.6)
Digestive organs[3]	C150-C269	9	24.8 (11.3 – 47.0)	1.4	0.4 (0.2 – 1.0)	29.5 (19.9 – 39.0)
Pulmonary[4]	C339-C349, C384, C390-C399	2	1.5 (0.2 – 5.5)	0.3	0.05 (0.007 – 0.4)	30.8 (19.0 – 42.7)
Bone	C400-C419	5	6.6 (2.2 – 15.5)	0.1	0.1 (0.06 – 0.4)	13.4 (6.8 – 33.0)
Soft tissue[5]	C470-C499	6	8.0 (2.9 – 17.5)	0.9	0.3 (0.1 – 0.7)	22.1 (9.0 – 31.1)
Female breast[6]	C500-C509	10	1.3 (0.6 – 2.3)	0.4	0.4 (0.2 – 0.9)	29.2 (13.1 – 39.8)
Female genital organs[7]	C510-C589	4	1.5 (0.4 – 4.0)	0.2	0.07 (0.01 – 0.5)	35.2 (23.8 – 46.1)
Male genital organs	C600-C639	3	0.7 (0.1 – 2.0)	-0.2	0.07 (0.01 – 0.5)	17.3 (6.3 – 44.3)
Testis	C620-C629	2	0.5 (0.06 – 1.8)	-0.4	0.08 (0.02 – 0.3)	11.8 (6.3 – 17.3)
Central nervous system	C700-C729	15	9.2 (5.1 – 15.1)	2.2	0.6 (0.4 – 1.2)	26.2 (5.8 – 37.6)
Brain	C710-C719	7	4.6 (1.8 – 9.4)	0.9	0.3 (0.1 – 0.8)	28.6 (11.5 – 37.8)
Meninges	C700-C709	5	360 (117.0 – 841.1)	0.8	0.2 (0.06 – 0.7)	28.0 (16.3 – 37.6)
Thyroid	C730-C739, C323,	13	10.9 (5.8 – 18.5)	2.0	0.6 (0.3 – 1.2)	28.4 (10.8 – 33.9)
Melanoma[8]	C44, C69 - M8720-M8790	12	2.4 (1.3 – 4.0)	1.3	0.5 (0.3 – 1.1)	26.5 (11.8 – 40.8)
Nonmelanoma skin (BCC excluded)	C44 excluding M8720-M8790	4	4.0 (1.1 – 10.4)	0.5	0.09 (0.02 – 0.4)	25.4 (12.6 – 30.8)
Hematological	**C42, C77 - M9591-M9948**	**10**	**1.7 (0.8 - 3.2)**	**0.7**	**0.4 (0.2 – 0.9)**	**25.2 (15.7 – 42.7)**
Leukemias	C42 - M9800-M9948	2	1.1 (0.1 – 3.8)	0.02	0.05 (0.006 – 0.3)	26.2 (17.7 – 34.7)
Myeloid	M9840-M9948	1	1.3 (0.03 – 7.5)	0.04	0	34.7 (34.7 – 34.7)
Lymphoblastic	M9811-M9837	1	1.0 (0.03 – 5.7)	0.01	0.05 (0.006 – 0.3)	17.7 (17.7 – 17.7)
Lymphomas	C77 - M9591-M9738	7	3.8 (1.5 – 7.7)	0.9	0.4 (0.2 – 0.8)	25.2 (15.7 – 37.3)
Non-Hodgkin lymphoma	M9591, M9670-M9738	5	2.9 (0.9 – 6.7)	0.5	0.2 (0.09 – 0.6)	25.2 (15.8 – 37.3)
Hodgkin-lymphoma	M9650-M9667	2	1.0 (0.1 – 3.7)	0.0	0.1 (0.03 – 0.5)	21.2 (15.7 – 26.7)
Other hematologic	M9732	1	6.3 (0.2 – 24.9)	0.1	0	42.7 (42.7 – 42.7)
Total SNMNs		**267**	**NA**	**NA**	**9.9 (8.5-11.5)**	**25.6 (5.5 - 48.3)**
Colorectal	C18-C21	27			0.9 (0.5 – 1.6)	29.3 (14.8 – 44.2)
Liver adenoma	C22.0	1			0.04 (0.006 – 0.3)	15.4 (15.4 – 15.4)
Thyroid/parathyroid adenomas	C73.9, C75.0	12			0.7 (0.4 – 1.4)	24.7 (12.3 – 34.3)
Lipomas	M8850-M8881	35			1.7 (1.1 – 2.5)	25.8 (10.3 – 43.3)
Fibromas	M8391, M8810-M8836, M8965, M9013-M9030, M9321, M9540-9550	11			0.4 (0.2 – 0.8)	23.9 (12.6 – 38.8)
Neurofibromas	M9540-M9550	3			0.09 (0.02 – 0.3)	16.9 (12.6 – 33.9)
Head and Neck	C00-C14, C30-C32, C69, C76.0	15			0.7 (0.4 – 1.3)	23.8 (5.9 – 48.3)
Oral (squamous)	C03.0-C06.0 / M8050-M8070, M808-M8081, M8560	4			0.2 (0.05 – 0.6)	23.6 (9.5 – 48.3)
Bone neoplasms	C40.0-41.9	18			0.7 (0.4 – 1.1)	13.4 (6.4 – 41.6)
Osteoma	M9180-M9200	2			0	40.2 (38.7 – 41.6)
Osteochondroma	M9210	2			0.5 (0.3 – 0.9)	10.7 (6.4 – 17.3)
Chondroma	M9220-M9241	2			0.09 (0.02 – 0.3)	14.2 (11.8 – 16.7)
Giant Cell tumor	M9250	2			0.09 (0.02 – 0.4)	16.7 (15.6 – 17.9)

Female breast	C50.0-50.9	28			1.0 (0.6 – 1.7)	27.7 (23.5 – 38.0)
Fibroadenoma	M8392, M9010-M9011	21			0.8 (0.5 – 1.3)	14.5 (6.4 – 38.9)
Ductal carcinoma in situ	M8500-M8505, M8507-M8522	7			0.2 (0.08 – 0.7)	32.9 (8.8 – 43.6)
Urogenital neoplasms	C51.0-C68.9	41			1.9 (1.3 – 2.8)	25.0 (7.3 – 41.6)
Bladder	C67	1			0.08 (0.01 – 0.6)	25.7 (25.7 – 25.7)
Female reproductive system	C51 – C58	38			1.7 (1.2 – 2.6)	25.6 (7.3 – 41.6)
Leiomyoma	M8890 – M8898	8			0.2 (0.7 – 0.5)	18.8 (7.5 – 40.5)
Cervical intra-epithelial neoplasm	M8077	22			1.3 (0.8 – 2.1)	25.8 (16.5 – 41.6)
Male reproductive system	C60-C63	2			0.1 (0.03 – 0.4)	18.5 (15.0 – 22.0)
Central nervous system	C70, C71, C72	89			2.8 (2.0 – 3.9)	31.6 (6.1 – 46.2)
Meningioma	M9150	81			2.5 (1.7 – 3.5)	32.2 (9.4 – 46.2)
Schwannoma/neurinoma	M9560	3			0.1 (0.03 – 0.5)	25.1 (17.5 – 31.9)
Peripheral schwannoma	M9560 excluding CNS	6			0.3 (0.1 – 0.7)	18.6 (13.5 – 23.6)
Vascular, excluding CNS [9]		7			0.3 (0.1 – 0.7)	22.0 (5.5 – 38.1)
Hemangioma	C49.0-49.9, C71.0-71.9 / M9120-M9136, M9141. M9142	5			0.2 (0.04 – 0.6)	27.0 (7.5 – 38.1)
Angioleiomyoma	C49.0-49.9 / M8894	2			0.5 (0.09 – 1.0	15.1 (7.5 – 22.6)
Subcutaneous, other	C49.0-49.9	9			0.3 (0.1 – 0.8)	28.9 (6.3 – 39.2)
Unspecified	M8000	1			0.04 (0.005 – 0.3)	7.3 (7.3 – 7.3)
Basal cell carcinoma		**172**	**NA**	**NA**	**5.6 (4.5 – 7.0)**	**26.1 (5.6 – 43.5)**

ICD-O-3: International Classification of Disease for Oncology, Third Edition, SMN: subsequent malignant neoplasm, SNMN: subsequent non-malignant neoplasm, SIR: standardized incidence ratio, AER: absolute excess risk, PY: Person years, CNS: Central nervous system. [1] Time since childhood ALL diagnosis [2] Nine survivors developed multiple SMNs [3] One digestive tumor occurred as second SMN after a first thyroid carcinoma. [4] One pulmonary occurred as second SMN after a first squamous cell carcinoma. [5] One soft tissue occurred as second SMN after a first squamous cell carcinoma[6] One mamma carcinoma occurred as second SMN after a first ductal carcinoma in the other breast and one mamma carcinoma occurred after a thyroid carcioma[7] One female genital organ tumor occurred as second SMN after a first mamma carcinoma [8] One melanoma occurred as second SMN after another melanoma and one melanoma after stomach carcinoma [9] Vascular neoplasms in the brain were not included in this category, but in the "Central nervous system" category.

Table 3. Multivariable Cox Proportional Hazard Regression Analysis for SMNs and SNMNs

		SMN[1]			SNMN[2]		
Variable	**Number of survivors**	**Number of SMN**	**HR**	**95% CI**	**Number of SNMN**	**HR**	**95% CI**
Sex							
Male	1672	45	1 (ref)		93	1 (ref)	
Female	1357	41	1.1	0.7 – 1.8	122	**1.7**	**1.3 – 2.3**
Age at diagnosis (years)							
0-4	1249	31	1(ref)		83	1 (ref)	
5-9	1210	31	0.7	0.4 – 1.2	90	0.8	0.7 – 1.2
10-14	442	18	0.9	0.5 – 1.7	36	0.4	0.5 – 1.0
14+	128	6	1.4	0.6 – 3.8	6	0.2	0.2 – 1.1
Cranial radiotherapy							
No	2356	38	1 (ref)		105	1 (ref)	
Yes	446	48	**2.3**	**1.4 – 4.0**	110	**1.9**	**1.3 – 2.6**
Other RT							
No	3013	85	1 (ref)		14	1(ref)	
Yes	16	1	4.7	0.6 – 38.4	2	**5.9**	**1.4 -25.1**
HSCT, TBI, etoposide[3] (mutually exclusive groups)							
No HSCT							
HSCT, no TBI with and without etoposide	2728	70	1 (ref)		171	1 (ref)	
HSCT, TBI, without etoposide	83	3	**3.8**	**1.1 – 13.8**	4	1.7	0.6 – 4.8
HSCT, TBI, with etoposide	25	2	1.5	0.5– 6.5	**9**	**4.9**	**2.3 – 10.5**
	159	11	**5.7**	**2.5 – 12.8**	**29**	**6.3**	**3.8 – 10.3**
Anthracyclines mg/m2							
None	923	29	1 (ref)		83	1 (ref)	
<200	1288	39	1.4	0.8 – 2.5	91	1.1	0.8 – 1.6
200+	752	17	1.5	0.7 – 3.2	35	1.0	0.6 – 1.6
Alkylating agents mg/m2							
None	1021	40	1 (ref)		99	1 (ref)	
<2000	1125	13	1.0	0.5 – 2.1	29	0.9	0.6 – 1.5
2000+	809	32	1.0	0.5 – 1.9	81	1.2	0.8 – 1.7

SMN: subsequent malignant neoplasm, SNMN: subsequent non-malignant neoplasm, CI: confidence interval, HR: hazard ratio HSCT: Hematopoietic stem cell transplantation, TBI: Total body irradiation. These analyses were done within a subset of the cohort for whom we had additional treatment details available (n=3029) etoposide was stratified based on the median dose 1423 mg/m2 [1]109 observations deleted due to missing information [2]150 survivors were not included in the analysis due to missing data [3]Etoposide as part of pre-treatment and conditioning regimen.

Supplementary materials chapter 5

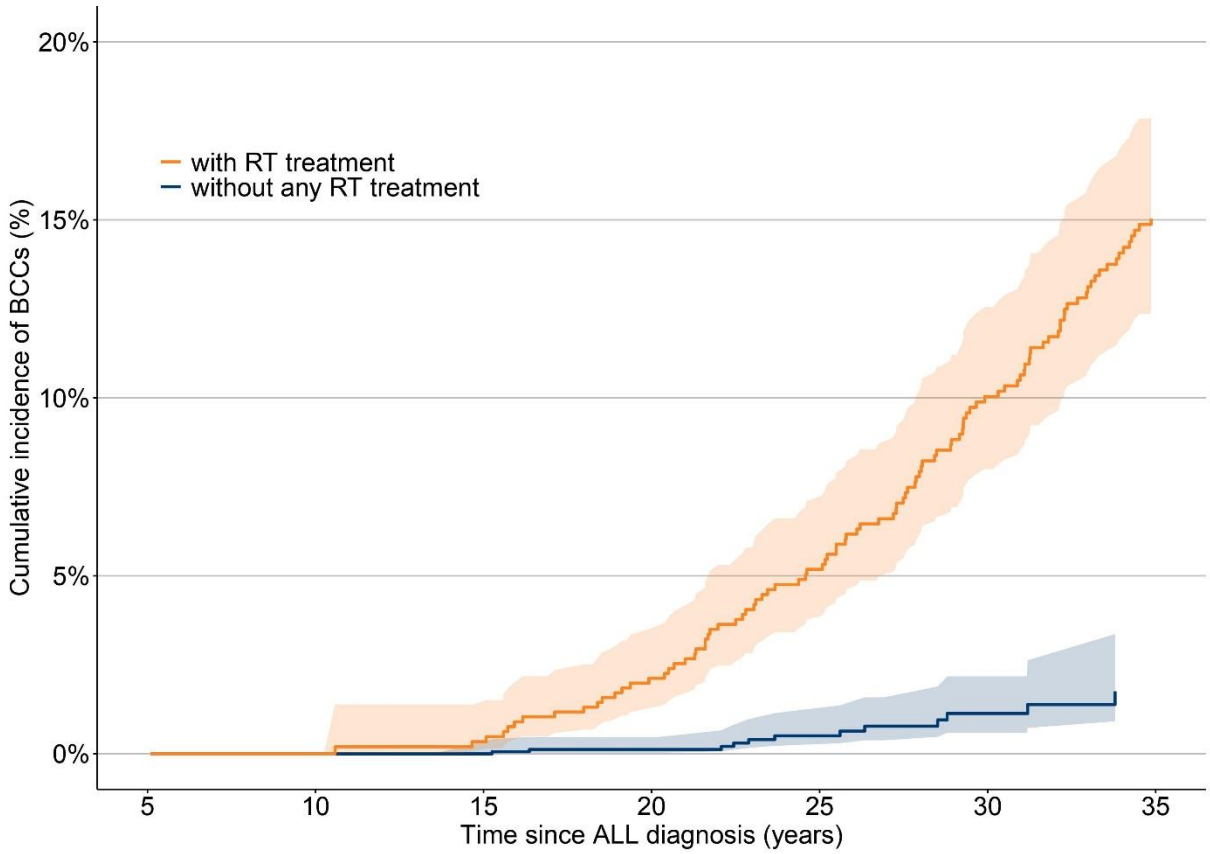

Figure S1: Cumulative incidence of basal cell carcinomas (BCCs) in the Dutch Childhood Cancer Survivors Study (DCCSS)-LATER cohort with follow-up time since childhood acute lymphoblastic leukemia (ALL) diagnosis, stratified by ALL survivors who were treated with radiotherapy (RT) or treated without RT.

Table S1. Conversion factors for doses of individual anthracyclines agents (all in mg/m²) into doxorubicin isotoxic equivalent dose

Chemotherapeutic agent*	Conversion factor
Doxorubicin	1
Daunorubicin	0.6
Epirubicin	0.8
Idarubicin	1
Mitoxantrone	10.5

Conversion factors are based on Feijen et al. (30)

Table S2. Conversion factors for doses of individual alkylating agents (all in mg/m²) into cyclophosphamide equivalent dose

Chemotherapeutic agent*	Conversion factor
Cyclophosphamide	1
Ifosfamide	0.244
Procarbazine	0.857
Chlorambucil	14.286
Carmustine (BCNU)	15.0
Lomustine (CCNU)	16.0
Melphalan	40
Thiotepa	50
Chlormethine (nitrogen mustard)	100
Busulfan	8.823

Conversion factors are based on Green et al. (31)

Table S3. Treatment characteristics non-relapsed and relapsed 5-year ALL survivors

	Non-relapse (n=2609)			Relapse (n=420)		
Variable	**Total non-relapse**	**SMN**	**No SMN**	**Total relapse**	**SMN**	**No SMN**
Total	2609	62 (2.4%)	2547 (97.6%)	420	24 (5.7%)	396 (94.3%)
HSCT						
No	2512 (96.3%)	58	2454	216 (51.4%)	12	204
Yes[1]	90 (3.7%)	4	86	179 (48.6%)	12	167
Radiotherapy groups						
No radiotherapy	2179 (83.5%)	26	2153	106 (25.2%)	1	106
CRT no TBI	363 (13.9%)	33	330	165 (39.3%)	12	153
TBI no CRT	54 (2.1%)	2	52	106 (25.2%)	8	98
CRT + TBI	2 (0.1%)	0	2	26 (6.2%)	3	23
Other radiotherapy [2]	6 (0.2%)	1	5	10 (2.4%)	0	10

SMN: subsequent malignant neoplasm, HSCT: hematopoietic stem cell transplantation, CRT: Cranial radiotherapy, TBI: Total body irradiation. These analyses were done within a subset of the cohort for whom we had additional treatment details available (n=3029) [1] Of the 90 patients with HSCT in the non-relapse group 55 (61%) patients received TBI. Of the 179 patients with HCST in the relapse group, 131 (73%) patients received TBI. [2] Seven Mantle field, six testis, one mantle + testis, one neck/cervical and one unknown

Table S4. SIRs and EARs for any SMN by patient and treatment characteristics

Characteristic	SMNs (n = 97)	SIR (95% CI)	AER
Sex			
Male	48	3.0 (2.2 – 3.9)	9.9
Female	49	2.3 (1.7 – 3.0)	10.0
Age at ALL diagnosis (years)			
0-4	35	2.8 (2.0 – 3.0)	8.7
5-9	36	2.5 (1.7 – 3.4)	9.3
10-14	20	2.3 (1.4 – 3.5)	13.6
15+	6	2.8 (1.0- 6.0)	20.0
Year of ALL diagnosis			
1984	52	2.5 (1.9 – 3.3)	16.3
1985-1994	29	2.8 (1.9 – 4.0)	11.0
1995-2004	10	1.9 (0.9 – 3.4)	2.9
2005+	6	4.1 (1.5 – 8.8)	6.5
Follow up time (years)[1]			
0-9	7	3.5 (2.1 – 5.4)	5.0
10-19	25	2.6 (1.7 – 3.8)	8.1
20-29	33	3.0 (2.2 – 4.3)	26.9
30+	32	1.5 (0.8 – 2.5)	15.2
Attained age (years)			
5-14	3	1.8 (0.4 – 5.4)	1.0
15-24	23	3.7 (2.4 – 5.6)	7.5
25-34	31	2.9 (2.0 – 4.1)	14.3
35+	40	2.1 (1.5 – 2.8)	23.8
Recurrence			
No	62	2.2 (1.7 – 2.8)	7.4
Yes	24	5.6 (3.6 – 8.4)	30.3
Vital status			
Alive	62	1.9 (1.4 – 2.4)	5.4
Deceased	35	41.3 (28.8 – 57.4)	18.7
Rt			
No	29	1.7 (1.1 – 2.4)	3.1
Yes	68	3.3 (2.6 – 4.2)	22.3
CT			
No	0	NA	NA
Yes	97	2.6 (2.1 – 3.1)	10.0
HSCT			
No	79	2.2 (1.7 – 2.7)	7.8
Yes	18	10.3 (6.1 – 16.3)	43.5
Treatment for ALL			
CT no HSCT	28	1.7 (1.1 – 2.4)	3.0
RT + CT	51	2.7 (2.0 – 3.5)	17.8
CT + HSCT (no RT)	1	8.4 (0.2 – 47.0)	18.3
RT + CT + HSCT	17	10.5 (6.1 – 16.8)	47.2
Unknown	0		
Radiotherapy type*			
CRT no TBI	45	2.9 (2.1 – 3.8)	19.3
TBI no CRT	10	10.0 (4.8 – 18.3)	39.9
CRT + TBI	3	9.4 (1.9 – 27.3)	58.4
Other radiotherapy [2]	1	2.5 (0.2 – 47.6)	47.1
Chemotherapeutic agents*			
Alkylating agents	46	3.3 (2.4 – 4.4)	11.5
Anthracyclines	57	3.4 (2.6 – 4.4)	13.2
Epipodophyllotoxins	27	6.2 (4.1 – 9.0)	29.2
Platinum agents	0	0	-5.3
Vinca alkaloids	86	2.7 (2.1 – 3.3)	10.3
Antimetabolites	86	2.7 (2.1 – 3.3)	10.3
Asparaginase	77	2.9 (2.3 – 3.6)	10.5
Antitumor antibiotics	5	6.5 (2.1 – 15.1)	46.2
Alkylating agents (cumulative dose) mg/m2*			

None	40	2.2 (1.5 -3.0)	8.8
0-2000	13	2.6 (1.4 – 4.4)	6.0
2000+	32	4.0 (2.8 – 5.7)	17.4
Anthracyclines (cumulative dose) mg/m2*			
None	29	1.8 (1.2 – 2.7)	6.1
0-200	39	3.3 (2.4 – 4.6)	13.2
200+	17	3.9 (3.3 – 6.3)	13.4
Epipodophyllotoxins agents (cumulative dose) mg/m2*			
None	59	2.1 (1.6 – 2.7)	7.0
<1485	12	4.9 (2.5 – 8.6)	22.9
1485+	15	8.2 (4.6 – 13.6)	38.7

SIR: Standardized incidence risk, AER: absolute excess risk, per 10,000 person years compared to the general population, SMN: subsequent malignant neoplasms, NA: Not applicable. CT: chemotherapy, RT: radiotherapy, including all types, HSCT: Hematopoietic stem cell transplantation *This is part of subset of the data (n=3029) of whom we had additional treatment details available [1]Follow-up time since ALL diagnosis [2] Seven Mantle field, six testis, one mantle + testis, one neck/cervical and one unknown

Table S5. Types of neoplasms after hematopoietic stem cell transplantation

Year diagnosis ALL	TBI conditioning	Histology	Site neoplasm
Malignant (n=16)			
1984	Yes	Adenocarcinoma	Colon
1993	Unknown	Squamous Cell carcinoma [1]	Tongue
1995	Yes	Squamous Cell Carcinoma	Skin
1995	Yes	Differentiated liposarcoma	Retroperitoneal
1975	No	Hemangiopericytoma	Cerebral meninges
1987	Unknown	Papillary adenocarcinoma	Thyroid
1988	Yes	Papillary adenocarcinoma	Thyroid
1981	Yes	Leiomyosarcoma	Soft tissue
2001	Yes	Synovial sarcoma	Soft tissue
1986	Yes	Ductal carcinoma	Mamma
1993	Yes	Ductal carcinoma	Mamma
2006	No	Ductal carcinoma	Mamma
1991	Yes	Lymphnode cancer (B-cell)	Lymph nodes
1994	No	Lymphnode cancer	Bone marrow
1983	Yes	t-AML / MDS	Bone marrow
1990	Yes	Osteosarcoma	Bone
1990	Yes	Pleomorphic sarcoma	Bone
2007	Yes	Osteosarcoma (chondroblast)	Bone
Non-malignant (n = 49)			
1984	Yes	Adenoma	Colon
1984	Yes	unknown type/p	Colon
1985	Yes	Adenoma	Colon
1987	Yes	Adenoma	Colon
1995	Yes	Adenoma	Colon
1995	Yes	Adenoma	Colon
2003	Yes	Adenoma	Colon
1992	Yes	Adenoma	Rectal
1991	Unknown	Papilloma	Head and Neck
1975	No	Meningioma	Brain
1982	Yes	Meningeoma[2]	Brain
1983	Yes	Meningeoma[3]	Brain
1987	Unknown	Meningioma	Brain
1990	Yes	Meningeoma[4]	Brain
2005	Yes	Meningioma (atypical)	Brain
1995	Unknown	Neurinoma/schwannoma[5]	Spinal cord
1987	Unknown	Follicular adenoma	Thyroid
1989	Unknown	Follicular adenoma [6]	Thyroid
1989	Yes	Adenoma	Peripheral nervous system
2005	Yes	Vestibular schwannoma	Peripheral nervous system
1983	Yes	Adenoma	Parathyroid
2006	Yes	Adenoma[7]	Liver
1981	Yes	Angiolipoma[8]	SCST
1983	Yes	Vestibular schwannoma[9]	SCST
1983	Yes	Lipoma[10]	SCST
1988	Yes	Lipoma	SCST
1991	Yes	fibro lipoma	SCST
1981	Yes	Angiolipoma[11]	SCST
1993	Yes	Lipoma	SCST
1993	Unknown	Neurofibroma	SCST
1999	Yes	Leiomyoma	SCST
1985	Yes	Fibroadenoma	Mamma
1987	Yes	Ductal carcinoma in situ	Mamma
2005	Yes	Fibroadenoma	Mamma
2006	No	Ductal carcinoma in situ	Mamma
2011	Yes	Fibroadenoma , NNO	Mamma
1992	Yes	Fibrous tumor, NNO/p	Abdominal

1976	Yes	Leiomyoma	Cervix
2003	Yes	Adenoma	Ovary
2012	No	Cystadenoma	Ovary
2000	Yes	Leiomyoma	Testis
1988	Yes	Osteochondroma	Bone
1990	Yes	Osteochondroma	Bone
1991	Yes	Osteochondroma	Bone
1995	Yes	Osteochondroma[12]	Bone
2003	Yes	Osteochondroma[13]	Bone
2006	Yes	Osteochondromatosis[14]	Bone
2008	Yes	Osteochondromatosis	Bone
2010	No	Osteochondromatosis	Bone

ALL: Acute lymphoid leukemia, TBI: Total body irradiation, SCST: Subcutaneous, connective and soft tissue. [1] Also developed a osteosarcoma after the adenocarcinoma [2]Developed another meningioma [3] Also developed a colon adenoma and a oral papilloma [4] Also developed a Cervical intraepithelial neoplasia [5] Also developed a schwannoma in peripheral nervous system and a cystic adenoma in the testis [6] Also developed a colon adenoma [7] Also developed a cervical hemangioma [8] Also developed a subcutaneous lipoma [9] Developed another neurinoma [10] Also developed rectal lipoma [11] Also develop a rectal adenoma and an endometrial leiomyoma [12] also developed a thyroid adenoma [13] Developed another osteochondroma [14] Also develop a peripheral neurofibroma and one in central nervous system.

Table S6. Multivariable Cox Proportional Hazard Regression Analyses for SMN, SNMN, and BCC

Base model										
		SMN[1]			SNMN[2]			BCC[3]		
Variable	**Number of survivors**	**Number of SMN**	**HR**	**95% CI**	**Number of SNMN**	**HR**	**95% CI**	**Number of BCC**	**HR**	**95% CI**
Sex										
Male	1672	45	1 (ref)		93	1 (ref)		73	1 (ref)	
Female	1357	41	1.1	0.7 – 1.7	122	**1.7**	**1.3 – 2.3**	67	1.0	0.7 – 1.5
Age at diagnosis (years)										
0-4	1249	31	1(ref)		83	1 (ref)		58	1 (ref)	
5-9	1210	31	0.8	0.5 – 1.3	90	0.9	0.6 – 1.2	58	0.8	0.5 – 1.1
10-14	442	18	1.0	0.5 – 1.8	36	0.7	0.4 – 1.0	22	0.5	0.4 – 0.9
14+	128	6	1.4	0.5 – 3.4	6	0.4	0.2 – 0.9	2	0.3	0.5 – 0.9
Other RT										
No	3013	85	1 (ref)		14	1 (ref)				
Yes	16	1	4.8	0.6 – 38.7	2	**6.5**	**1.6 – 27.1**			
Cranial radiotherapy										
No	2356	38	1 (ref)		105	1 (ref)		31	1 (ref)	
Yes	446	48	**1.9**	**1.2 – 3.1**	110	**1.8**	**1.3 – 2.4**	109	**4.3**	**2.8 – 6.7**
HSCT and TBI										
No HSCT	2728	70	1 (ref)		171	1 (ref)		117	1 (ref)	
HSCT, no TBI	83	3	**4.0**	**1.2 – 13.7**	4	1.7	0.6 – 4.8	2	1.9	0.6 – 7.7
HSCT + TBI	186	13	**4.2**	**2.3 – 7.8**	39	**6.6**	**4.5 – 9.5**	21	**6.4**	**3.9 – 10.4**

SMN: subsequent malignant neoplasm, SNMN: subsequent non-malignant neoplasm, CI: confidence interval, HR: hazard ratio HSCT: Hematopoietic stem cell transplantation, TBI: Total body irradiation. These analyses were done within a subset of the cohort for whom we had additional treatment details available (n=3029) [1] 39 patients were not included in the analysis due to missing data. [2] 99 patients were not included in the analysis due to missing data. [3] 99 patients were not included in the analysis due to missing data.

Table S7. Multivariable Cox Proportional Hazard Regression Analyses for SMN, SNMN, and BCC among survivors who had experiences a relapse

Base model										
		SMN[1]			SNMN[2]			BCC[3]		
Variable	**Number of survivors**	**Number of SMN**	**HR**	**95% CI**	**Number of SNMN**	**HR**	**95% CI**	**Number of BCC**	**HR**	**95% CI**
Sex										
Male	260	10	1 (ref)		24	1 (ref)		24	1 (ref)	
Female	160	14	**2.7**	**1.2 – 6.3**	24	**1.9**	**1.0 – 3.5**	17	1.2	0.6 - 2.3
Age at diagnosis (years)										
0-4	163	8	1(ref)		27	1 (ref)		21	1 (ref)	
5-9	184	12	1.3	0.5 – 3.2	17	0.4	0.2 – 0.8	14	0.5	0.3 – 1.1
10+	73	4	1.0	0.3 – 3.4	4	0.3	0.06 – 0.7	6	0.5	0.2 – 1.3
Cranial radiotherapy										
No	222	9	1 (ref)		22	1 (ref)		16	1 (ref)	
Yes	191	15	1.3	0.5 – 3.4	26	**1.0**	**2.5 – 9.7**	25	1.4	0.7 – 2.9
HSCT										
No	216	12	1 (ref)		17	1 (ref)		20	1 (ref)	
Yes	179	12	**2.5**	**1.0 – 3.4**	30	4.9	0.5 – 2.0	21	**2.7**	**1.4 – 5.4**

SMN: subsequent malignant neoplasm, SNMN: subsequent non-malignant neoplasm, CI: confidence interval, HR: hazard ratio HSCT: Hematopoietic stem cell transplantation, TBI: Total body irradiation. These analyses were done within a subset of the cohort for whom we had additional treatment details available (n=3029) [1] 28 patients were not included in the analysis due to missing data. [2] 79 patients were not included in the analysis due to missing data. [3] 79 patients were not included in the analysis due to missing data.

Acknowledgement

The authors thank all physicians, research nurses, data managers, research assistants, the Dutch National Childhood Cancer Association, and participating patients for their contribution. We would also like to thank the registration team of the Netherlands Comprehensive Cancer Organization (IKNL) for the collection of data for the Netherlands Cancer Registry. Moreover, we thank the staff from Palga Foundation for providing linkage data on subsequent non-malignant neoplasms from their registry.

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Chapter 6

Summary and general discussion

The survival rate of childhood cancer has increased, resulting in a growing number of survivors who are at risk of late adverse effects. This thesis aimed to enhance our understanding regarding the risk of and risk factors for subsequent malignant neoplasms (SMNs) and subsequent non-malignant neoplasms (SNMNs) after childhood cancer treatment. This Chapter will highlight the main findings and discuss the strengths and limitation of the studies, also in context of other important childhood cancer survivors studies worldwide. This is followed by discussing the clinical implications of our main findings and recommendations for future studies. Finally, we conclude this Chapter with an overview of the key messages of this thesis.

Main findings

To investigate the risk of developing subsequent neoplasms we used data from the Dutch Childhood Cancer Survivor Study (DCCSS) LATER cohort. This cohort included five-year childhood cancer survivors, diagnosed before the age of 18, in one of the seven original pediatric oncologic/hematopoietic stem cell centers in the Netherlands. The total DCCSS-LATER cohort (1963-2014) included 11,548 survivors. Data on subsequent neoplasms was ascertained by linkages with two nationwide registries: the Netherlands Cancer Registry (NCR)[1] and the Dutch Nationwide Pathology Databank (Palga)[2].

Temporal trends of subsequent malignant neoplasms in childhood cancer survivors and the impact of treatment changes

Chapter 2 describes, to our knowledge, the first study in Europe examining temporal trends in the risk of developing SMNs among five-year childhood cancer survivors, and relate these to treatment shifts over the past five decade. After a median follow-up of 21.1 years, in total, 550 survivors developed an SMN, translating into a 3.5 (95%CI: 3.2-3.8) times higher risk compared to the general population. Our findings indicate that the risk of developing SMNs among five-year childhood cancer survivors decreased over time (pre-1980, 1980-1989, 1990-1999, and 2000 onwards). A mediation analysis showed that this decrease was associated with a reduction in the use of radiotherapy. In contrast, changes in the use chemotherapy seemed to have the opposite effect, mainly due to the use of anthracyclines and epipodophyllotoxins.

The effect of chemotherapy is supported by observations among the irradiated survivors, where we observed an increased risk over time, associated with higher doses of chemotherapy. This suggests that the temporal decline of SMN risk due to decreased radiotherapy treatments, was counteracted by chemotherapy. The use of several groups of chemotherapy in more recent diagnosis periods was also increased, especially the use of anthracyclines and epipodophyllotoxins. Furthermore, this study shows that, although the risk decreased, childhood cancers survivors remain at an increased risk of developing SMNs across all decades, with a significantly higher risks for survivors treated with radiotherapy, anthracyclines, and epipodophyllotoxins compared to survivors who did not receive those treatments.

Subsequent neoplasms among childhood neuroblastoma survivors

In **Chapter 3 and 4** we explored the risk on and risk factors for subsequent neoplasms in neuroblastoma survivors. In **Chapter 3** we conducted a systematic review and reviewed and appraised all literature on this topic. Among the 13 included articles, neuroblastoma survivors were shown to have an 2.8 to 10.4 times elevated risk of developing SMNs compared to the general population. There was limited evidence on risk factors. A study using univariate analyses suggested a higher SMN risk for patients treated with radiotherapy, women, and high-risk neuroblastoma patients. Furthermore, only few studies reported on SNMNs. We recommended that future studies should focus more on potential risk factors. This information is important to enhance risk stratification. In **Chapter 4**, we capitalized on this knowledge and analyzed the long-term risk and associated risk factors for developing SMNs and SNMN among the 563 five-year neuroblastoma survivors from the DCCSS-LATER cohort (1963-2014)**.** In total, 24 survivors developed at least one SMN, resulting in a 30-year cumulative incidence of 3.4% (95% CI: 1.9-6.0%), and 60 survivors developed at least one SNMN, resulting in a 30-year cumulative incidence of 10.4% (95% CI: 7.3-14.8%). Our multivariate models showed that neuroblastoma survivors who were treated with Iodine-metaiodobenzylguanidine (^{131}IMIBG) had a significantly higher risk of developing SMNs compared to survivors treated without ^{131}IMIBG, also after adjusting for chemotherapy groups.

Subsequent neoplasms among childhood acute lymphoblastic leukemia survivors

In **Chapter 5** we analyzed the long-term risk of and risk factors for subsequent neoplasms in 3,291 five-year survivors of childhood acute lymphoblastic leukemia (ALL). After a median follow-up of was 21.6 years, we observed 97 survivors with at least one SMN and 266 survivors with at least one SNMN. In addition, 172 survivors developed a basal cell carcinoma (BCC). ALL survivors who were treated with cranial radiotherapy (CRT) had an increased risk to develop SMN, SNMN, and BCC. We also compared patients treated before or in 1984 to patients treated after 1984, corresponding to the year when prophylactic CRT was omitted from standard protocols. We did not observe a decrease in risk of any SMN or any SNMN for survivors treated after 1984. However, the risk of malignant CNS tumors and benign meningiomas significantly decreased for survivors diagnosed after 1984. In multivariable analyses, we found that survivors who were treated with allogenic hematopoietic stem cell transplantation (HSCT) with total body irradiation (TBI) as conditioning regimen had a higher risk of SMN, SNMN, and BCC compared to the survivors treated without HSCT. A significant new insight of this study is that we also observed a higher risk for developing SMNs in HSCT-treated survivors without TBI conditioning, which might be due to the accompanying chemotherapy treatment, but due to the limited number of cases, we were unable to analyze this further.

Strengths and limitations

The DCCSS-LATER 3 studies described in this thesis combined, for the first time, the original DCCSS-LATER cohort (diagnosed between January 1, 1983 and December 31, 2001)[3] with the expansion DCCSS-LATER cohort (diagnosed up to December 31, 2014), encompassing over 11,000 childhood cancer survivors. This substantial cohort size is a major strength, enabling a more precise estimation of risks and allowing for subgroup analyses on specific childhood cancers, as was done in **Chapter 4 & 5**. Furthermore, by including survivors over a time span of five decades and a median follow-up of more than 20 years for all studies, we were able to investigate very long-term risks of subsequent neoplasms and compare risks across different decades, as explored in **Chapter 2.**

Our DCCSS-LATER dataset has unique aspects compared to the other childhood cancer survivor studies. Specifically, the DCCSS-LATER data contains comprehensive and objective follow-up information for both malignant and non-malignant neoplasms by linking our data to nationwide registries. Most cancer registries do usually not cover non-malignant tumors. By linking our data to Palga, we obtained objective information on histologically-confirmed SNMNs. These linkages, combined with our detailed individual treatment data for primary childhood cancer and all recurrences, sets our dataset apart. Our treatment data includes specifics on chemotherapy agents and their cumulative doses, radiotherapy types, fields and doses, and other treatments, such as stem cell transplants and surgery for both the primary tumor and all recurrences. Other multicenter childhood cancer survivors studies do not have this exceptional combination of complete baseline and follow-up data. For example, the CCSS relies on patient-reported SMN outcomes validated by pathology or medical records. The BCCSS and studies from the Nordic Countries use linkage with cancer registries, but lack detailed data the childhood cancer treatment. Our unique combination of comprehensive treatment information and objective follow-up data on SMN and SNMNs allows a thorough evaluation of potential risk factors for subsequent neoplasms.

We should also take into account some limitations. Firstly, there are some limitations from our linkage with Palga for SNMNs. Because our source for SNMNs was Palga, we only had data on histologically confirmed SNMNs. This might have led to a slight underrepresentation of the true SNMN incidence. Furthermore, it is important to note that physicians might be more alert in monitoring childhood cancer survivors, which could result in an increased detection rate of SNMNs. Additionally, there are no reference rates for SNMNs in the general population, which prevented us from comparing SNMN rates among survivors to the general population. Secondly, Palga has a nationwide coverage since 1991. To adjust for this we left-truncated this data, to ensure that only reliable and complete data was used. Only small percentage (<10%) of the follow-up data occurred before 1990, predominantly involving survivors under the age of 30, a group with a relatively low risk for subsequent tumors. Therefore, we consider the impact to be limited. The NCR has a nationwide coverage since 1989. For the pre-1989 era we used the partially available data both NCR, Palga and we supplemented this data with SMN data from medical records, making it unlikely that many SMNs were missed. Thirdly, in our

analyses in specific childhood cancer groups in **Chapter 4 & 5,** we had a relatively low number of events. We were therefore limited in some of our models to evaluate detailed aspect of treatments, e.g. ^{131}I-MIBG dose or effects of chemotherapy conditioning in HSCT-survivors. As a result, some of our risk estimates had large confidence intervals. Likewise, the number of events was low for most subtypes of subsequent neoplasms, which limited us to perform risk factor analyses for specific subsequent neoplasm types. A fourth limitation is the lack of genetic information. Although we had some limited data on genetic predisposition syndromes, it was incomplete and could therefore not be accounted for in the analyses. Certain cancer susceptibility syndromes can play a role in the development of subsequent neoplasms. Approximately 8.5% of the children newly diagnosed with cancer have a pathogenic germline mutation[4], which could contribute to development subsequent neoplasms[5]. This may have had some impact on the treatment-related risk estimates in our (and other) studies on subsequent neoplasms among childhood cancer survivors.

Clinical point of view

The findings presented in this thesis have implications for current childhood cancer survivors as well as for newly diagnosed childhood cancer patient.

General childhood cancer survivors

Clinical conclusions

In **Chapter 2**, we observed that childhood cancer survivors treated with radiotherapy had a significant higher risk of developing SMNs. The positive aspect is the reduced use of radiotherapy over time has led to a decrease in the SMN risk over time. This is further supported by our findings in **Chapter 5,** where we observed a decrease in CNS-related tumors among ALL survivors diagnosed after 1984, corresponding to the year where cranial radiotherapy was omitted from standard protocols. These results confirm that minimizing the use of radiotherapy in treatment protocols are indeed a helpful strategy in reducing the development of subsequent neoplasms. However, in **Chapter 2**, we also saw that the temporal decline of SMN risk, due to this decreased radiotherapy treatments, is counteracted by an

increased use of chemotherapy, in particular of anthracyclines and epipodophyllotoxins. Furthermore, we observed a significant higher risk of any SMN among childhood cancer survivors treated with either anthracyclines and/or epipodophyllotoxins. This concerning effect of chemotherapy is supported by an increasing risk of developing SMNs over time among the subgroup of irradiated childhood cancer survivors, which was attributable to higher doses of chemotherapy. Anthracyclines and epipodophyllotoxins have previously been reported to increase the risk of secondary leukemia's[6]. Additionally, anthracyclines have been reported to increase the risk for subsequent female breast cancer[7], and other solid cancers[3].

Clinical implications

Altogether, while efforts to reduce radiotherapy usage effectively lowered the long-term risk of SMNs, further evaluation is needed regarding the role of chemotherapy in SMN risk. Current chemotherapy protocols require reevaluation to better balance treatment efficacy and long-term health outcomes for newly diagnosed childhood cancer survivors. Although anthracyclines and epipodophyllotoxins are crucial in childhood cancer treatment, ongoing research into alternative treatment strategies aims to minimize these risks while maintaining effective cancer control. In addition, a continuous effort to limit radiation exposure and explore alternative treatments is also warranted. Moreover, ongoing monitoring of survivors, especially for irradiated survivors, is essential. Overall, these results underscore the crucial need for careful, long-term follow-up of childhood cancer survivors, even decades after treatment due to the persistent risks on subsequent neoplasms.

Childhood neuroblastoma survivors

Clinical conclusions

Our findings also have more specific implications for neuroblastoma. The systematic review in **Chapter 3** shows a well-established risk of neuroblastoma survivors to develop SMNs, with a 2.8 to even 10.4 times higher risk compared than the general population. Although a wide variety of types of subsequent malignant and non-malignant neoplasms were observed, thyroid carcinoma and acute myeloid leukemia are the most frequently reported subsequent

neoplasms after neuroblastoma. These findings emphasize the importance of survivorship care for neuroblastoma patients. Furthermore, our study in **Chapter 4** demonstrates, for the first time, that neuroblastoma survivors treated with ^{131}IMIBG have a higher risk of developing SMN compared to those treated without ^{131}IMIBG.

Clinical implications

^{131}IMIBG has been used upfront for some decades in the Netherlands and regimens and doses have been similar to past and current protocols for relapsed/refractory treatment and upfront treatment in other countries. Therefore, this key finding is still relevant for the current era protocols. This important result could help inform the development of future treatment strategies and survivorship care protocols in neuroblastoma survivors who were treated with ^{131}IMIBG. Additionally, we observed two survivors with a thyroid carcinoma after ^{131}IMIBG, despite having received thyroid protection. In the Netherlands, neuroblastoma patients receive thyroid protection when exposed to ^{131}IMIBG, to minimize the risk of radiation-induced thyroid complications. In addition to the two thyroid carcinomas, we observed two (para)thyroid adenomas after ^{131}IMIBG treatment. The development of these thyroid neoplasms after ^{131}IMIBG may raise concern of the effectiveness of the thyroid protection and potential improved strategies to protect the thyroid gland from exposure might need to be evaluated.

Childhood acute lymphoblastic leukemia survivors

Clinical conclusions

Chapter 5 presents new information on treatment-related risks of subsequent neoplasms in ALL survivors. Our results showed that childhood ALL survivors treated with HSCT had an increased risk of developing SMNs compared to those treated without HSCT. Previous research has primarily linked the increased risk among HSCT-treated survivors to TBI conditioning[8-11]. However, our results indicate that HSCT aspects other than TBI also contribute to an elevated risk of SMN development after HSCT, as we also found an increased risk in HSCT-treated patients without TBI conditioning. Our multivariable model suggested

that this may be attributed to the accompanied chemotherapy treatment. Between 2011 and 2021, several trials were conducted in which TBI was replaced by a chemotherapy conditioning regimen[12,13]. However, TBI (in combination with etoposide) was reintroduced as conditioning regimen for HSCT in ALL, because of superior survival of this group compared to HSCT-survivors conditioned without TBI in the FORUM study[12].

Clinical implications

These novel insights underscore the complexity of treatment decisions for ALL survivors considering HSCT conditioning and highlight the ongoing need for research on long-term complications following HSCT to make balanced decisions on future protocols that consider both survival rates and side effects. Furthermore, it is important for clinicians to be aware that ALL survivors treated CRT and/or with HSCT, even without TBI conditioning, face an elevated risk of developing SMNs. This underscores the importance of monitoring and personalized risk management strategies for survivors.

Future recommendations for research

To conduct more robust analysis of risk factors for the development of subsequent neoplasms, future studies with a higher number of events are essential. This would allow us to improve our assessment of treatment-related interactions and to investigate treatments in greater detail, such as radiotherapy doses and specific chemotherapy agents. For instance, this will enable further validation on the role of ^{131}I-MIBG in the development of SMNs, including ^{131}I-MIBG dose, and allow for analyses of risks of specific SMNs after MIBG treatment. Moreover, larger event numbers will facilitate subgroup analysis, particularly within specific childhood cancer groups and different risk groups. This would provide the opportunity to further investigate the effect of various conditioning regimes and accompanying therapies on the development of SMNs in ALL survivors treated with HSCT. A more detailed assessment of factors, such as chemotherapy dose, TBI doses and fractionations used before HSCT is essential for improving our understanding of the risk of developing subsequent neoplasms.

A crucial way for achieving these higher numbers of events is by pooling data internationally. By combining datasets, the total number of events will increase, thereby strengthening statistical power. Moreover, this will also enhance the generalizability of findings across diverse populations and protocols.

Future studies should aim to obtain more comprehensive information on genetic predisposition for subsequent neoplasms. For example, research into underlying mechanism by which MIBG treatment contributes to thyroid carcinogenesis, including the role of age at exposure and potential genetic susceptibility. Efforts to obtain more genetic information would enable investigations into possible genetic mutations associated with the risk of developing subsequent neoplasms. Identifying such markers and integrating genomic data with clinical data can also improve individualized risk prediction for survivors, and thereby enhancing personalized care strategies.

Key messages

The studies in this thesis enabled us to answer several research questions, thereby contributing important new knowledge to the existing literature.

- Childhood cancer survivors have a higher risk of developing SMNs **(Chapter 2) .**
- Childhood cancer survivors treated with radiotherapy have a significant higher risk of developing any type of SMN than survivors treated without radiotherapy (**Chapter 2).**
- Childhood cancer survivors treated with anthracyclines or epipodophyllotoxins have a significantly higher risk of SMNs than those treated without these chemotherapeutics (**Chapter 2).**
- The risk of developing SMNs has slightly decreased over time, with lower risks among survivors diagnosed in more recent eras. This is mainly attributable to a reduced use of radiotherapy **(Chapter 2).**
- The temporal decline of SMN risk due to decreased radiotherapy treatments, is counteracted by chemotherapy. Mainly due to increase use of anthracyclines and epipodophyllotoxins **(Chapter 2).**
- Among irradiated childhood cancer survivors, there is an increased risk of SMN over time, which is attributable to higher chemotherapy doses in recent eras **(Chapter 2).**
- There is only little evidence on risk factors for developing SMNs in neuroblastoma survivors **(Chapter 3).**
- Neuroblastoma survivors who were treated with Iodine-metaiodobenzylguanidine (^{131}I MIBG) have an increased risk of developing SMNs **(Chapter 4).**
- Acute lymphoblastic leukemia survivors who were treated with cranial radiotherapy have an increased risk to develop SMNs (especially CNS malignancies), SNMNs (especially meningiomas), and BCCs **(Chapter 5).**
- Acute lymphoblastic leukemia survivors who were treated after 1984, the year when cranial radiotherapy was omitted from the standard ALL protocol, have shown a significantly lower risk of developing malignant CNS tumors and non-malignant meningiomas **(Chapter 5).**

- Acute lymphoblastic leukemia survivors who were treated with allogeneic hematopoietic stem cell therapy have an increased risk of developing SMNs. This is both for HSCT-treated survivors with and without TBI conditioning **(Chapter 5).**

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