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Medical risk factors and late effects of childhood cancer –

Etiological and methodological considerations

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Content

Summary

Background

Every year, about 400,000 children and adolescents are newly diagnosed with childhood cancer worldwide. However, risk factors for the development of most childhood cancers are largely unknown. Rare genetic disorders explain less than 10% of the cases and only few further established risk factors for the development of cancer in childhood and second primary neoplasms occurring later in life are established. Many risk factors originate from the medical field. While they primarily include exposure to antineoplastic agents and high doses of ionizing radiation during cancer therapies, even low doses of ionizing radiation may contribute to the development of radiation-induced first and second primary neoplasms. Moreover, immunological factors were suggested to be associated with the development of childhood cancer. Here, the establishing of adequate immunocompetency through insult by infections or vaccination in particular is assumed to exert protective effects against carcinogenesis. In the strive to elaborate on this, however, most previously conducted retrospective studies were troubled by small sample sizes and inadequate exposure assessments of potential risk factors. This has resulted in an ongoing incomplete mechanistical understanding for the processes underlying development of cancer in childhood. Therefore, improved exposure assessment methods and novel study designs are needed to assess the iatrogenic influence on the occurrence of first and second primary neoplasms in children.

Aims

The five original publications of this present cumulative dissertation aim to improve the knowledge on risk factors for childhood and second primary neoplasms with a particular focus on factors of medical origin.

Methods

The influence of vaccinations on the development of childhood cancer was examined in a systematic literature review that summarized the current state of

research. Within a subsequent meta-analysis, the evidence was quantified by pooling the published risk estimates.

Besides immunological factors, the transcriptomic radiation-response was examined using experimental data from the molecular epidemiological study KiKme (**K**rebs **i**m **K**indesalter und **m**olekulare **E**pidemiologie). To identify conditions for the conducting of irradiation experiments that yield most information, various parameters were compared and the most promising were determined. To identify effects of radiation exposure ongoing beyond the molecular level, the medical exposure to ionizing radiation during cancer therapy was also evaluated. To do so, a novel questionnaire was developed within the framework of the KiKme study. Combining data from this self-administered questionnaire with data from treating hospitals and therapy optimizing studies allowed the validation of the newly developed questionnaire on the lifetime history of radiation exposure and cancer therapies in children. Finally, the KiKme study population was used to examine the adverse late effects of the disease as well as differences in the lifestyle of long-term survivors of childhood cancers with and without second primary neoplasms.

Results

The systematic literature search identified 6,774 articles on vaccination and cancer risk in children. Of these, 34 articles met the inclusion criteria for subsequent meta-analysis. A total of 26 meta-analyses could be performed on eleven cancer outcomes after nine different vaccinations. Four inverse associations were found between Bacillus Calmette-Guérin vaccination and leukemia death, between Haemophilus influenzae type b vaccination and acute lymphoblastic leukemia, and between a high number of unspecified vaccinations and acute lymphoblastic leukemia or leukemia in general.

For the KiKme study, a total of 591 former childhood cancer patients and cancer-free control subjects were successfully recruited. In detail, the final study population consisted of 101 childhood cancer survivors that developed a second primary neoplasm later in life, 340 survivors with a first primary neoplasm only, and 150 cancer-free controls. Moreover, the study was successful in collecting skin

biopsies (N=499), saliva samples (N=511), and information on lifestyle, socioeconomic and anthropometric factors, as well as on medical radiation history, health, and family history of diseases from self-administered questionnaires (n=556).

The collected skin biopsies were used to identify the time point of exposure to ionizing radiation yielding most differentially expressed genes. The largest number of differentially expressed genes was found four hours after exposure to both high and low doses of ionizing radiation. The functional analysis of the differentially expressed genes showed alteration of metabolic pathways that may implicate cellular senescence.

The combination of data from questionnaires of the KiKme study and therapy data from treating hospitals suggested that the newly developed questionnaire was reliable for a retrospective assessment of exposure to these therapies. A high agreement was found between self-reported data and data from medical records regarding exposure to chemotherapy and radiotherapy in long-term survivors with second primary neoplasms. Conducted analyses on received cancer therapies and late adverse health effects showed an association between the exposure to chemotherapeutic agents in and diseases of the thyroid gland as well as the lipid metabolism.

Investigated associations between the occurrence of first or second primary neoplasms and various diseases as well as lifestyle in the later life of long-term survivors of childhood cancer revealed that survivors were more affected by pathologies and may consequently take more medication than cancer-free controls. In detail, it was shown that thyroid diseases and disorders of the lipid metabolism are more common in survivors. Overall, survivors had a healthier lifestyle compared to cancer-free controls, defined primarily by lower consumption of soft drinks and alcohol, lower tobacco use, and a lower body mass index. However, survivors exercised less than cancer-free controls potentially explained by the late effects of cancer and therapy, as well as the resulting physical limitations.

Discussion and conclusions

The results of the present dissertation contribute to the knowledge of the development of childhood and second primary cancers by showing that the risk for the development of these cancers, as well as for the occurrence of late adverse health effects, can be influenced both positively and negatively by medical factors. Overall, these results provide a basis for further elaboration on childhood cancers and its late effects and thus may contribute to a long-term reduction in the burden of the disease by providing options for individualized planning of therapies and identification of individuals in need of more extensive follow-up care.

Zusammenfassung

Hintergrund

Weltweit erkranken jedes Jahr etwa 400.000 Kinder und Jugendliche an einer Kinderkrebserkrankung. Die Risikofaktoren für die Entstehung der meisten Krebserkrankungen im Kindesalter sind jedoch weitgehend unbekannt. Seltene Gendefekte erklären weniger als 10 % der Fälle. Darüber hinaus gibt es nur wenige andere nachgewiesene Risikofaktoren für die Entstehung von Kinderkrebserkrankungen und später auftretenden Folgeneoplasien. Eine Großzahl dieser Risikofaktoren stammt dabei aus dem medizinischen Bereich. Zu den wenigen etablierten Risikofaktoren gehören beispielsweise die Exposition gegenüber antineoplastischen Medikamenten und hohe Dosen ionisierender Strahlung im Rahmen von Krebstherapien. Aber auch niedrige Dosen ionisierender Strahlung werden als Risikofaktoren für die Entstehung strahleninduzierter Erst- und Folgeneoplasien diskutiert. Darüber hinaus wurden immunologische Faktoren mit der Entstehung von Krebs im Kindesalter in Verbindung gebracht. Dabei wird davon ausgegangen, dass insbesondere die Etablierung einer ausreichenden Immunkompetenz durch Infektionen und Impfungen eine protektive Wirkung bei der Krebsentstehung hat. Kleine Stichprobengrößen und eine häufig unzureichende Expositionserhebung potenzieller Risikofaktoren in bisher durchgeführten zumeist retrospektiven Studien erlauben jedoch noch kein vollständiges Verständnis der zugrunde liegenden Mechanismen der Krebsentstehung im Kindesalter. Daher sind verbesserte Methoden zur Expositionserhebung und neuartige Studiendesigns erforderlich, um den Einfluss von verschiedenen medizinisch-bedingten Faktoren auf das Auftreten von Erst- und Folgeneoplasien bei Kindern bewerten zu können.

Ziele

Die vorliegende kumulative Dissertation besteht aus fünf Originalveröffentlichungen und verfolgt das Ziel, das Wissen über Risikofaktoren für das Auftreten von Kinderkrebserkrankungen sowie Folgeneoplasien zu verbessern. Hierbei liegt der Schwerpunkt auf medizinischen Risikofaktoren.

Methoden

Der aktuelle Forschungsstand zum Einfluss von Impfungen auf die Entstehung von Kinderkrebserkrankungen wurde in einer systematischen Literaturübersicht zusammengetragen. Zur Bewertung der Evidenz einzelner Assoziationen wurden in einer anschließenden Meta-Analyse veröffentlichte Risikoschätzer für mögliche Assoziationen quantifiziert.

Neben den immunologischen Faktoren wurden experimentelle Daten aus der molekularepidemiologischen Studie KiKme (**K**rebs **i**m **K**indesalter und **m**olekulare **E**pidemiologie) genutzt, um strahleninduzierte Veränderungen der Genexpression zu identifizieren. Hierzu wurden die besten Versuchsbedingungen zur Durchführung von Bestrahlungsexperimente ermittelt, um in folgenden umfangreichen Genanalysen die bestmöglichen Ergebnisse erzielen zu können. Um nicht nur auf molekularer Ebene Aussagen über den Effekt von Strahlenexposition machen, sondern auch um die Exposition gegenüber medizinischer Strahlung während der Krebstherapie bewerten zu können, wurde im Rahmen der KiKme Studie ein neuer Fragebogen entwickelt. Die Kombination von Selbstangaben aus diesem Fragebogen mit Daten aus behandelnden Kliniken und Therapieoptimierungsstudien ermöglichte darüber hinaus die Validierung des neu entwickelten Fragebogens zur Erfassung von lebenslanger Strahlenexposition und Krebstherapien bei Kindern. Das Studienkollektiv der KiKme-Studie wurde außerdem genutzt, um Zusammenhänge zwischen Krebsstatus und unerwünschten Spätfolgen von Kinderkrebserkrankungen sowie dem Lebensstil von Langzeitüberlebenden zu untersuchen.

Ergebnisse

In der systematischen Literaturrecherche konnten 6.774 Publikationen zu Impfungen und Krebsrisiko bei Kindern gefunden werden. Davon erfüllten 34 Publikationen die Einschlusskriterien für die anschließende Meta-Analyse. Es konnten insgesamt 26 Meta-Analysen zu elf Krebserkrankungen nach neun verschiedenen Impfungen durchgeführt werden. In der Synthese der Ergebnisse wurden vier inverse Assoziationen zwischen Bacillus Calmette-Guérin-Impfung und Leukämietod, zwischen Haemophilus influenzae Typ b-Impfung und akuter

lymphatischer Leukämie sowie zwischen einer hohen Anzahl nicht spezifizierter Impfungen und akuter lymphatischer Leukämie bzw. Leukämie im Allgemeinen gefunden.

Für die KiKme-Studie konnten insgesamt 591 Kinderkrebsüberlebende und krebsfreie Kontrollpersonen erfolgreich rekrutiert werden. Die Studienpopulation setzt sich aus 101 Kinderkrebsüberlebenden mit einer später aufgetretenen Folgeneoplasie, 340 Überlebenden, die nur eine Krebsdiagnose im Kindesalter hatten und 150 krebsfreien Kontrollpersonen zusammen. Darüber hinaus konnten im Rahmen der Studie erfolgreich Hautbiopsien (n=499), Speichelproben (n=511) von Teilnehmenden gesammelt werden. Des Weiteren gaben die Teilnehmenden in selbst ausgefüllten Fragebögen Informationen zu Lebensstil, sozioökonomischen und anthropometrischen Faktoren sowie zu medizinischer Strahlenexposition, Gesundheit und familiärer Krankheitsgeschichte an (n=556).

Mithilfe der gesammelten Hautbiopsien konnte der beste Zeitpunkt für die Durchführung von Genanalysen nach der Exposition gegenüber ionisierender Strahlung ermittelt werden. Die meisten Gene wurden vier Stunden nach der Exposition gegenüber hohen und niedrigen Dosen ionisierender Strahlung differenziell exprimiert. Die unterschiedlich exprimierten Gene standen im Zusammenhang mit Stoffwechselwegen, die auf zelluläre Seneszenz hindeuten könnten.

Der Vergleich der Daten aus den Fragebögen der KiKme-Studie und den Therapiedaten der behandelnden Krankenhäuser konnte zeigen, dass der neu entwickelte Fragebogen für eine retrospektive Bewertung der Exposition gegenüber Krebstherapien geeignet ist. Insbesondere die Exposition gegenüber Chemotherapie sowie Strahlentherapie bei wurde von den Langzeitüberlebenden mit Folgeneoplasien sehr präzise angegeben. Durchgeführte Analysen zu Assoziationen zwischen erhaltenen Krebstherapien und gesundheitlichen Spätfolgen ergaben einen Zusammenhang zwischen dem Erhalt von Chemotherapie und Erkrankungen der Schilddrüse sowie des Fettstoffwechsels.

Die Untersuchung von Assoziationen zwischen dem Auftreten von Erst- oder Folgeneoplasien mit verschiedenen, häufig auftretenden Krankheiten sowie dem Lebensstil von Langzeitüberlebenden ergab, dass Kinderkrebsüberlebende häufiger an verschiedenen Spätfolgen leiden und infolgedessen möglicherweise mehr Medikamente einnehmen als krebsfreie Kontrollen. Im Einzelnen zeigte sich, dass Schilddrüsenerkrankungen und Störungen des Fettstoffwechsels bei Überlebenden häufiger auftreten. Insgesamt haben Überlebende im Vergleich zu krebsfreien Kontrollpersonen einen gesünderen Lebensstil, einschließlich eines geringeren Konsums von Softdrinks und Alkohol, eines geringeren Tabakkonsums und eines niedrigeren Body-Mass-Indexes. Allerdings treiben Überlebende weniger Sport als krebsfreie Kontrollpersonen, was auf die Spätfolgen der Erkrankung und der erhaltenen Therapie sowie auf die daraus resultierenden körperlichen Einschränkungen zurückzuführen ist.

Diskussion und Schlussfolgerungen

Die Ergebnisse der vorliegenden Dissertation tragen zum aktuellen Wissenstand zur Entstehung von Kinderkrebserkrankungen und später auftretenden Folgeneoplasien bei, indem sie zeigen, inwiefern das Risiko für die Entwicklung einer Krebserkrankung sowie für das Auftreten von gesundheitlichen Spätfolgen durch medizinische Faktoren sowohl positiv als auch negativ beeinflusst werden kann. Insgesamt bilden die Ergebnisse eine Grundlage für die weitere Erforschung von Kinderkrebserkrankungen und dessen Spätfolgen und könnten beispielsweise über Möglichkeiten der individuellen Therapieplanung sowie der Identifizierung von Überlebenden, die eine besonders engmaschige Nachsorge benötigen, zu einer langfristigen Verringerung der Krankheitslast beitragen.

Abbreviations

ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
BMI	Body mass index
CT	Computed tomography
DAG	Directed acyclic graph
DNA	Deoxyribonucleic acid
FPN	First primary neoplasm
GLMM	Generalized linear mixed model
Gy	Gray
ISCED	International Standard Classification of Education
κ	Cohen's Kappa
KiKme	Krebs im Kindesalter und molekulare Epidemiologie
RNA	Ribonucleic acid
SPN	Second primary neoplasm
TP53	Tumor protein 53

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Outline of the dissertation

This dissertation aims to investigate medical risk factors for the development of cancer in childhood. While there are only few established risk factors, it is known that through the more elaborated vulnerability of children towards several exogenous carcinogens, which are often found in the field of medical care, the risk for both cancer development and late sequelae can be thus modulated. While, e.g., iatrogenic exposure to ionizing radiation in the context of diagnostics and therapy, or application of antineoplastic agents are established risk factors for the development of first and also second primary neoplasms in childhood, factors modulating the immune system such as vaccinations are discussed regarding their anti-neoplastic effects.

To comprehensively cover the various dimensions of potential medical risk factors, this dissertation is presented as a cumulative dissertation. It includes a total of five original publications (*Appendix, pages 57-145*), three of which have been authored as the first author and two as a co-author. All them were published in international peer-reviewed journals.

The dissertation is composed of a total of five chapters. *Chapter 1* provides an overview of current knowledge on cancer in childhood. In addition to epidemiological measures of the disease, various risk factors for pathogenesis, especially those of iatrogenic origin, and late sequelae in survivors are presented. Elaborating on this, *Chapter 2* highlights the gaps in current knowledge and derives the research questions addressed in this cumulative dissertation. *Chapter 3* then describes the data on which the individual research questions of this dissertation are based, as well as methods for the statistical evaluation of these data. In *Chapter 4*, the results of the five included publications are briefly summarized and then discussed in the first part of *Chapter 5* contextualizing the results in the current state of research and evidence. Finally, after a general discussion and the presentation of potential prevention strategies derived from the results of this work, the strengths and limitations of this dissertation are critically discussed. *Chapter 5* then concludes on implications of this work for future research in the area of medical risk factors and the late effects of childhood cancer.

1 Introduction

The following chapter provides an overview of the epidemiology of childhood cancer and the cancer sites most common for cancer in childhood. In addition, risk factors that contribute to carcinogenesis as well as associated late effects are examined.

1.1 Cancer in childhood

The term *childhood cancer* encompasses a heterogeneous group of cancer types that share onset between birth and the age of 19 [1]. Every year, about 400,000 children and adolescents are newly diagnosed globally [2], with around 2,200 of these being in Germany. Here, marginally higher age-standardized incidence rates have been reported in males (18.4 per 100,000) than in females (15.7 per 100,000) [3], whereas the frequencies of the cancer entities differ vastly depending on the topology. Among children, the diagnostic spectrum is completely different from that in adults. While most new cases in adults are solid tumors of the lung, colon, stomach, liver, prostate, or breast and uterus [4], children are much less likely to develop such solid tumors [3]. Accounting for an estimated 19% of all childhood cancers, acute lymphoblastic leukemia (ALL) is the most common childhood cancer worldwide [1]. However, the incidence underlies age-specific variation (*Figure 1*). Overall, together with leukemia, lymphomas, and tumors of the central nervous system are the three most common entities [5]. With respect to the incidences in Germany, leukemia also represents the largest group (30%), followed by tumors of the central nervous system (23-25%), and lymphomas (13-17%, *Figure 2*) [3].

The majority of leukemia cases occurs before the age of six so that the median age at diagnosis of leukemia is five years with a peak of incidence at two to four years [6]. Lymphomas occur more frequently in young teens and only rarely in young children. Depending on the subtype of the lymphoma, the median age at onset ranges between 9 and 14 years. Tumors of the central nervous system are a very heterogeneous group with different ages of onset [6].

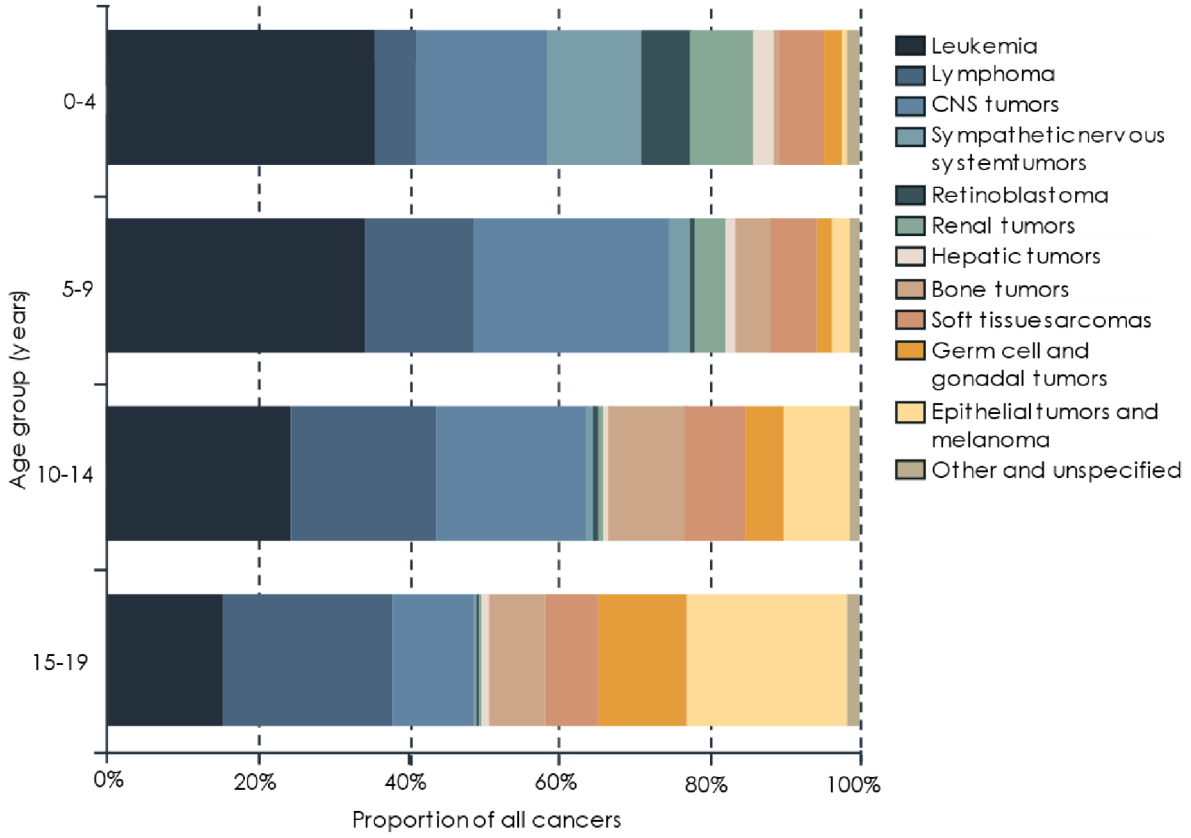

Figure 1: Proportion of childhood cancer worldwide (2001-2010) - adapted from Steliarova-Foucher et al., 2017 [5].

According to current estimates, the survival time for 82% of all children with cancer is at least 15 years post-diagnosis [6]. Nevertheless, childhood cancer remains a leading cause of morbidity and mortality in this age group [7]. Regarding disability-adjusted life-years [8], a metric which accounts for both, morbidity and mortality of cancer in childhood, recent data show that, despite low numbers of cases and deaths, childhood cancers are one of the most prominent causes of the global burden of disease with more than 11.5 million disability-adjusted life-years [9]. A major contribution to this burden is made by the estimated 100,000 childhood cancer deaths per year [1]. Moreover, later-onset diseases (see *Chapter 1.3*) also contribute to the disability-adjusted life-years of childhood cancer survivors, although to a lesser extent.

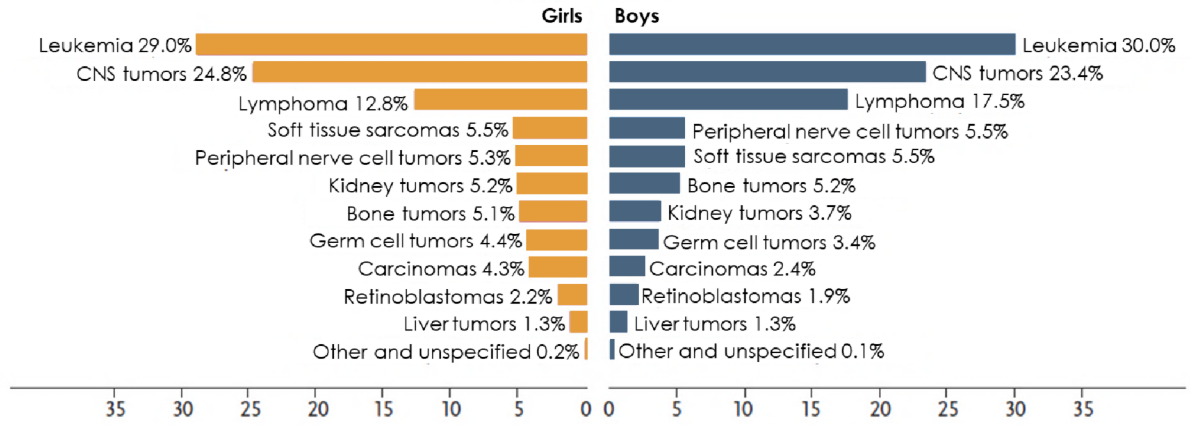

Figure 2: Most common cancer diagnoses in children in Germany (2010-2019) - adapted from Erdmann et al., 2021 [3].

1.2 Risk factors for developing childhood cancer

The factors that modulate the risk for carcinogenesis in adults do not apply for children. In contrast to cancer in adults, where exogenous factors drive carcinogenesis, it is assumed that cancer in children originates in rapidly dividing and proliferating embryonal tissues, giving genetic factors a greater role in the pathogenesis [1]. Nevertheless, risk factors differ between cancer sites and environmental factors can also play a role in children, albeit to a lesser extent than in long-term exposed adults [10]. Factors that modulate the risk for childhood and later-occurring second primary cancers in the fields of medical diagnostics, cancer therapies, as well as immunological modulators such as vaccinations or exposure to infections, have been the subject of research for some time.

1.2.1 Familial predisposition and genetic syndromes

Some rare genetic disorders are associated with cancer in childhood [10]. The cause of such disorders can be either rooted in germline or somatic mutations. While in germline mutations the genetic defect is passed on from the germ cells of the parents (egg or sperm cell) to the children, somatic mutations arise post-fertilization in the course of embryonic or fetal development or even later in life. An example of a germline mutation that prominently increases cancer risk is the

Li-Fraumeni syndrome, a familial predisposition to cancer, leading to a familiar accumulation thereof. It is an autosomal-dominant inherited syndrome defined by a mutation in the tumor suppressor gene *TP53* (*tumor protein 53)* [10]. Due to the mutations in the tumor suppressor gene *TP53*, cancer occurs more frequently and at an earlier age in affected individuals than in the normal population. About half of affected individuals receive their first cancer diagnosis before the age of 30 [11].

Another well-known syndrome that is associated with cancer development is Trisomy 21. As implicated by the name, patients with Trisomy 21 carry three chromosomes at position 21 instead of two in their genome. This genetic defect is caused by an accidental defect during the meiotic division of the maternal egg [12]. Trisomy 21 is associated with a large increase in the risk of both acute myeloid leukemia (AML) and ALL [10]. In contrast, the risk for other cancers is reduced compared to the general population [10].

Together, the Li-Fraumeni syndrome and Trisomy 21, along with other known genetic disorders such as Fanconi anemia (risk for AML), the Backwith-Wiedemann (risk for Wilms' tumor), and Noonan syndrome (risk for juvenile myelomonocytic leukemia), explain only about 10% of the childhood cancer cases [10]. Evidently, factors contributing to the development of childhood cancers can be presumed.

1.2.2 Germline mutations

In addition to mutations that occur in genetic cancer predisposition syndromes [13-15], an increasing number of genetic variants that are not primarily associated with familial predisposition or genetic syndromes have been identified recently. The literature review of six studies on pathogenic and potentially pathogenic germline variants in childhood cancer survivors revealed that 8.5-35.0% of the individuals studied carried at least one such variant [16]. Moreover, these variants were identified in a wide range of genes with functional potential to promote the development of adult cancers, and in the case of childhood cancer survivors, second primary neoplasms. Here, the most frequently reported genes with at least one

reported pathogenic or likely pathogenic germline variant were *TP53*, *BRCA1 and BRCA2 (Breast Cancer Genes 1* and *2)*, *postmeiotic segregation increased 2 (PMS2)*, *DICER*, and *Von Hippel-Lindau (VHL*) [16]. In a study that screened 3,006 childhood cancer survivors for germline mutations in 60 genes with known autosomal dominant cancer predisposition and moderate to high penetrance, 5.8% of childhood cancer survivors with a second primary neoplasm were found to carry pathogenic or likely pathogenic variants. These mutations were associated with an increased risk of second primary neoplasms in childhood cancer survivors who had received radiotherapy [17]. Based on data from the St. Jude Lifetime Cohort, it is assumed that more than 20,000 survivors of childhood cancer living in the United States carry such a pathogenic or likely pathogenic variant in one of 156 cancer predisposition genes [18]. In addition, another recent review concluded that genes promoting childhood cancer in certain germline variants are the same genes that are disrupted by somatic mutations in acute leukemia and myeloid neoplasms [19].

1.2.3 Medical exposures

Immunological factors

Infections and their underlying mechanisms presumed to connect the immune system and cancer development are based on various theories. Kinlens' "population-mixing theory" suggests that children raised in isolated areas are at increased risk of developing leukemia and lymphomas due to a reduced degree of herd immunity [20-22]. Graves, on the other hand, hypothesizes in his "delayed infection hypothesis" that children who have not experienced an infection during the first year of life and thus have not experienced a pathogen-induced activation of the immune system are at increased risk of developing ALL [23]. To date, only the Epstein-Barr virus has been established as a risk factor for the development of lymphoma in childhood, in particular Burkitt's and Hodgkin's lymphomas [10]. The immune response that counteracts pathogens such as the Epstein-Barr virus includes an inflammatory response. However, carcinogenic pathogens evade the host's immune system, leading to unresolved immunostimulants and ultimately a chronic low-grade inflammatory state, which can lead to carcinogenesis in the long

term [24]. This process occurs mainly via the *nuclear factor 'kappa-light-chain-enhancer' of activated B-cells* (*NF-κB*), a transcription factor involved in the inflammatory response. Through this pathway, inflammation is considered a hallmark of cancer and plays a major role in the development and progression of most cancers [25].

Besides the direct exposure to infectious agents, a plethora of factors associated with immunofunction were suggested to be associated with the development of childhood cancer in previous observational studies: natural birth [26], long breastfeeding [27], early day-care attendance [28], allergies [29], and absence of autoimmune diseases [30]. Moreover, vaccinations are assumed to be protective by priming the still largely naïve immune system for future encounters with pathogens and, thus, a more competent homeostatic performance concerning the return to baseline in inflammation that is strongly driving carcinogenesis when deregulated [23, 31-34]. Furthermore, vaccines might regulate cancer risk in children by further improving readiness of certain macrophages and natural killer cells due to a non-specific stimulation, improving their activity directed against tumor cells [33].

Ionizing radiation and cancer therapies

The proportion of childhood cancers caused by factors like ionizing radiation or exposure to genotoxic applications during cancer therapies is low as only a few children are exposed to such substances in such early age or even prenatally [10, 35]. Nevertheless, their importance is rooted in being among the few established causal risk factors for cancer development in children. Among these, antineoplastic agents such as deoxyribonucleic acid (DNA)-alkylating agents and topoisomerase II inhibitors are causal risk factors for the development of acute myeloid leukemia and myelodysplasia in children [10]. In addition, the risk of osteosarcomas later in life is increased post-chemotherapy, but also, and to a larger extent, after exposure to ionizing radiation during radiotherapy. Such application, either for the treatment of a neoplastic or a non-neoplastic disease, increases the risk of tumors of the central nervous system [10]. For instance, these often occur as second

primary neoplasm in survivors of leukemia, as the treatment of this disease can include deposition of ionizing irradiation to tissues in the head. Especially *in utero* and postnatally, exposure to ionizing radiation is associated with an increased risk of developing AML, whereas the exposure can be both of medical or environmental origin [10]. Besides high dose radiation, which is commonly applied in radiotherapy, also low doses are assumed to increase the risk for the development of radiation-induced first [36, 37] and second primary neoplasms [10, 38]. Such low dose radiation is commonly used in medical diagnostics, like computed tomography [39]. A dose-response relationship is already established for the development of ALL and is suspected for the development of central nervous system tumors [10]. Even at the lowest doses, where ionizing radiation itself rarely introduces genotoxic insults, the stimulus may contribute to the development of cancer via a radiation-induced increased number of reactive oxygen species [40]. However, since only a small proportion of children are exposed to ionizing radiation and only a small percentage of childhood cancer survivors develop a second primary neoplasm later in life, general risk factors other than ionizing radiation must play a role. Thus, particularly pronounced genetic defects in the DNA repair or the cell cycle control may predispose to the occurrence of second primary neoplasms [41]. Moreover, it is likely that a combination of exogenous environmental factors and multiple low penetrance genetic variants, rather than a single exogenous or high penetrance genetic factor, is responsible for most childhood cancers and especially second primary neoplasms following childhood cancer.

1.2.4 Other risk factors

Besides the risk factors already mentioned, there are a few other proven factors involved in the development of certain childhood cancers. For example, high birth weight and growth through life are considered to be associated with the development of ALL [10]. Similarly, high birth weight and fetal growth have been recently associated with the risk of developing tumors of the central nervous system in a large Swedish birth cohort [10, 42]. In the past decades, several other risk factors for childhood cancer have been suspected, e.g., gestational age, preconception exposure to chemical and physical mutagens, exposure to infections

during pregnancy, parental smoking, and exposure to pesticides as well as insecticides. However, none of these suspected factors has yet been established.

1.3 Late effects of childhood cancer

Due to enormous advances in the diagnosis and treatment of childhood cancer, survival has increased significantly in recent decades. While the 5-year survival rate in Germany was 67% at the beginning of the 1980s, it has now increased to 86-87% (based on diagnoses between 2009 and 2018) [3]. It should be noted, however, that the probabilities of long-term survival are highly dependent on the underlying cancer site [3]. Due to the increased probability of survival, the proportion of long-term survivors in the population as well as the respective number of adverse late health effects is increasing. The consequences of cancer in childhood lead directly to a lower quality of life for those affected compared to healthy children of the same age group [43, 44]. Moreover, the mental health status of childhood cancer survivors is influenced by the impactful experiences during childhood [45-48]. In addition to psychological stress caused by the illness itself [45-48], many survivors experience long-term adverse health outcomes, which are often of iatrogenic origin [49-54]. The risk of late adverse health effects is related to the dose, dose-rate, and fractioning of the applied radiation as well as the type, amount, and application plan of chemotherapeutics [55-58]. Since cancer therapies do not only affect the tumor itself but also healthy tissue in the surrounding area, the development of a second primary neoplasm is a common late adverse health effect of cancer in childhood [59]. About 6.8% of childhood cancer survivors develop a second primary neoplasm within 30 years after their first diagnosis [6]. Besides this particularly serious late adverse health effect of cancer in childhood, survivors are also at increased risk for infertility as well as chronic cardiovascular or lung diseases [51, 60-62]. Cardiovascular diseases are a prominent cause of morbidity and mortality of long-term survivors of childhood cancer [63-65]. Cardiovascular diseases occurring after cancer therapies might be either directly related to the therapy (e.g., due to radiation exposure) [66], or might be modulated by the occurrence of cardiovascular risk factors like hypertension, dyslipidemia, and

diabetes in this vulnerable group of survivors [67-69]. In addition to the disease- and therapy-related risks for late adverse health effects, exogenous factors, such as the consumption of alcohol and tobacco, may also influence the risk for late effects in long-term survivors of childhood cancer. Both, smoking and alcohol use have been shown to be risk factors for the development of various adverse health outcomes. Although long-term survivors of childhood cancer are generally less likely to be heavy drinkers compared with controls [70-72], single survivors in particular tend to consume alcohol more frequently than those living in a partnership [71]. In particular heavy drinking is associated with increased use of tobacco in survivors [73]. However, without consumption of alcohol, the majority of survivors smoke less overall than healthy controls [72, 74-76]. Due to the toxins and mutagens contained in alcohol, tobacco and its additives, their consumption may have additive or even synergistic effects on pre-existing risk factors for late adverse health effects in childhood cancer survivors [73, 77, 78].

While on one hand lifestyle can influence the risk of the occurrence of late adverse health effects, the presence of concomitant diseases on the other hand can also influence the lifestyle of survivors. In this context, physical activity is a good example: Even though studies have shown that physical activity prevents the occurrence of late adverse health effects after cancer in childhood [79, 80], about 50% of survivors do not reach the recommended daily level of physical activity [81]. This could, among other reasons, be due to a poorer physical condition of former cancer patients, as study results show that, especially in the presence of musculoskeletal diseases, the recommendations are not reached [81].

2 Aims of the dissertation

As the introduction to this dissertation outlines, both the development of cancer itself as well as lifestyle and health after surviving it are determined by multifactorial influences. The overall aim of this dissertation is to improve the knowledge of risk factors for childhood cancer development with a particular focus on those of medical origin (e.g., ionizing radiation in the course of diagnostics and

cancer therapy as well as vaccination in primary prevention), and to investigate their impact on survivors' health and lifestyle after recovery. To tailor the research questions of this dissertation as precisely as possible to the gaps in knowledge in this area that still need to be filled, the following section will first take a closer look at the missing information from and methodological issues of previous research. The specific research questions that this dissertation attempts to answer will then be presented in the second part of this chapter.

2.1 Research gaps

2.1.1 Medical factors and the risk of childhood and second primary cancer

Vaccination

As described before (see *Chapter 1.2.3*), immunological factors such as vaccinations have been the focus of research interest for a long time. Despite a large number of publications, the potential of risk-modulation for the onset of childhood cancer after vaccination has not yet been conclusively clarified. The results of the previously conducted studies are not consistent and, due to the rareness of the disease, are mostly limited by their small sample size and thus insufficient statistical power. In addition, most of the studies rely on data from (parental) self-reports or vaccination cards, which are considered to be not as valid as data from registries or medical records. Moreover, only two small meta-analyses on very specific research questions have been conducted in the past [82, 83]. To date, there has been no meta-analysis of different vaccines and the general risk of childhood cancer as well as of histologic and site-specific subtypes, excluding leukemia. To fill this gap, a comprehensive systematic literature review and meta-analysis was needed to assess the current state of knowledge regarding the putative association of different vaccinations and childhood cancer risk, including stratification by cancer site.

Ionizing radiation, cancer therapies, and genetic susceptibility

The proportion of childhood cancers caused by environmental factors such as ionizing radiation is estimated to be rather small overall. However, exposure to ionizing radiation, whether in the form of environmental exposure or in terms of medical application, is an important risk factor in the development of childhood and second primary cancers. Since probably not a single factor but a combination of exogenous environmental factors and multiple endogenous low-penetrant genetic variants is responsible for the development of such cancers, the risk for carcinogenesis might be modulated by the individual capacities of the radiation response, e.g., in the course of radiotherapy or diagnostics. To date, some molecular biology studies have been performed on this topic, but the underlying mechanisms are still largely unknown, especially regarding exposures to low doses of ionizing radiation [84, 85]. Since different cell types, irradiation doses, and measurements at different time points have been used to perform irradiation experiments in previous studies [85-90], a comprehensive comparison of the results is limited. In addition, often only small case numbers were included in previous studies [84, 89, 91, 92] or they have used skin models [87, 88, 93] or established cell lines [86, 94-96] whose genotypes and phenotypes are not exact copies of human cells [97] or may have already changed through frequent replication [98]. To date, there is still a lack of standardized and evidence-based methods for the experimental design of molecular biological irradiation experiments to be able to investigate the radiation-related risk for the development of childhood cancers and subsequent tumors from irradiation experiments on human cell lines.

However, obtaining resources for such molecular biological investigations is often challenging and laboratory work is conceptually not feasible, especially in large study populations. Therefore, to investigate the influence of radiation exposure, e.g., in the context of medical diagnostics and cancer therapies, on different outcomes, the use of questionnaires to determine live-long medical radiation exposure and a connected epidemiological evaluation is appropriate. Data from a large number of participants can be collected in a simple and cost-effective way using questionnaires. Because data collection via questionnaires presents some challenges, a certain recall bias must be considered. Furthermore, data collection

via self-report in questionnaires is difficult if the exposure being asked about occurred many years ago or at such a young age that the study participants can no longer remember correctly or with sufficient precision. This also applies to the recall of childhood cancer therapies and radiation-based medical diagnostics applied in childhood. Despite a lack of valid data on cancer therapies and radiation exposure in childhood, until now, there is no established questionnaire that retrospectively assesses exposures to cancer therapies or any kind of medical diagnostic using ionizing radiation in childhood among adult survivors.

2.1.2 Late health effects and lifestyle after surviving childhood cancer

Previous studies were able to show that a cancer diagnosis in childhood is often accompanied by adverse health outcomes later in life and has also an influence on survivors' lifestyle (see *Chapter 1.3*). Survivors of childhood cancer differ in many ways from healthy controls. However, none of the previous analyses have examined whether there are also differences between childhood cancer survivors with different numbers of cancer diagnoses. Such differentiated analyzes at the level of number of cancer diagnoses are important since a first cancer diagnosis and its associated sequelae, which include second primary neoplasms as the most harmful sequela, can have a major impact on survivors' health and lifestyle. Nonetheless, studies investigating number of childhood cancer diseases and possible associated late health effects as well as differences in lifestyle are still missing in the research area of long-term childhood cancer survival.

To sum up, the risk factors that contribute to the development of most childhood cancers and to the sequelae of cancer and its treatment later in life remain largely unknown to this day. Moreover, novel study approaches encompassing new designs are needed to avoid the existing methodological issues and to close the gaps in knowledge in this area. A comprehensive overview of different vaccinations and the risk of multiple cancer sites is needed to evaluate the impact of a trained immune system on carcinogenesis. To improve the understanding of underlying mechanisms of cancer development after exposure to ionizing radiation,

standardized experimental setups need to be defined and experiments with large sample sizes need to be performed afterward. To be able to estimate the influence of ionizing radiation, which often occurs in the medical field in the course of cancer therapies and diagnostics, on the development of childhood and second primary cancers, valid questionnaires are needed to effectively collect this information in epidemiological studies. Data collected with such a new instrument can then be used to study late effects of childhood cancer and associated cancer therapies.

2.2 Objectives

The purpose of this dissertation is to address the described research and thus to achieve the overall goal of this dissertation, which is to improve knowledge of medical risk factors for the development of cancer in childhood and to examine the impact of various medical factors on survivors' health and lifestyle after recovery.

To this end, this dissertation first aims to summarize the current state of research on the risk of cancer after vaccinations in childhood. For this purpose, a systematic literature review was conducted to investigate a possible preventive effect of vaccination through early stimulation of the immune system. The results of the identified individual studies were summarized in meta-analyses.

Subsequently, the influence of different medical factors on the development of second neoplasms after cancer in childhood as well as on health and lifestyle after recovery will be considered. This part of the dissertation is based on data from the KiKme (**K**rebs **i**m **K**indesalter und **m**olekulare **E**pidemiologie) case-control study, which is nested into the cohort of the German Childhood Cancer Registry. The study population consists of childhood cancer survivors with one or more diagnoses as well as cancer-free controls. The KiKme study contains both epidemiological information from self-administered questionnaires as well as biological data from irradiation experiments on participants' skin biopsies. Therefore, it provides the possibility of a multidimensional approach to further investigate different risk factors for the development of childhood second primary cancer as well as late adverse health outcomes after recovery. Taken together, using the pooled results

from published studies on vaccination and risk of childhood cancer as well as the unique data from the KiKme study, this dissertation will provide new insights into the field of childhood cancer risk research on immunization, radiation susceptibility and late effects after childhood and second primary cancers by fulfilling the following research objectives:

1. **To advance knowledge on the etiology of childhood cancer by**
   a. assessing evidence on the association between different vaccinations and childhood cancers, including stratification by cancer sites (*Publication 1*).

2. **To advance knowledge of the etiology of second primary cancers and other health outcomes in childhood cancer survivors by**
   a. implementing a nested case-control study with a large collective of childhood cancer survivors and cancer-free controls including the collection of questionnaire information as well as skin biopsies and saliva samples (*Publication 2*),
   b. establishing the best experimental conditions for the identification of differentially expressed genes and corresponding pathways in irradiation experiments using fibroblasts from skin biopsies (*Publication 3*),
   c. investigating associations between cancer status and adverse late health outcomes of childhood cancer and lifestyle parameters (*Publication 4*),
   d. validating a self-administered questionnaire assessing childhood cancer treatments and associated risks for adverse health outcomes (*Publication 5*).

Each of the above-mentioned aspects was addressed in a separate publication to contribute to the two main research questions. The overall conceptual framework of the dissertation and the content of the individual publications are presented in *Figure 3*.

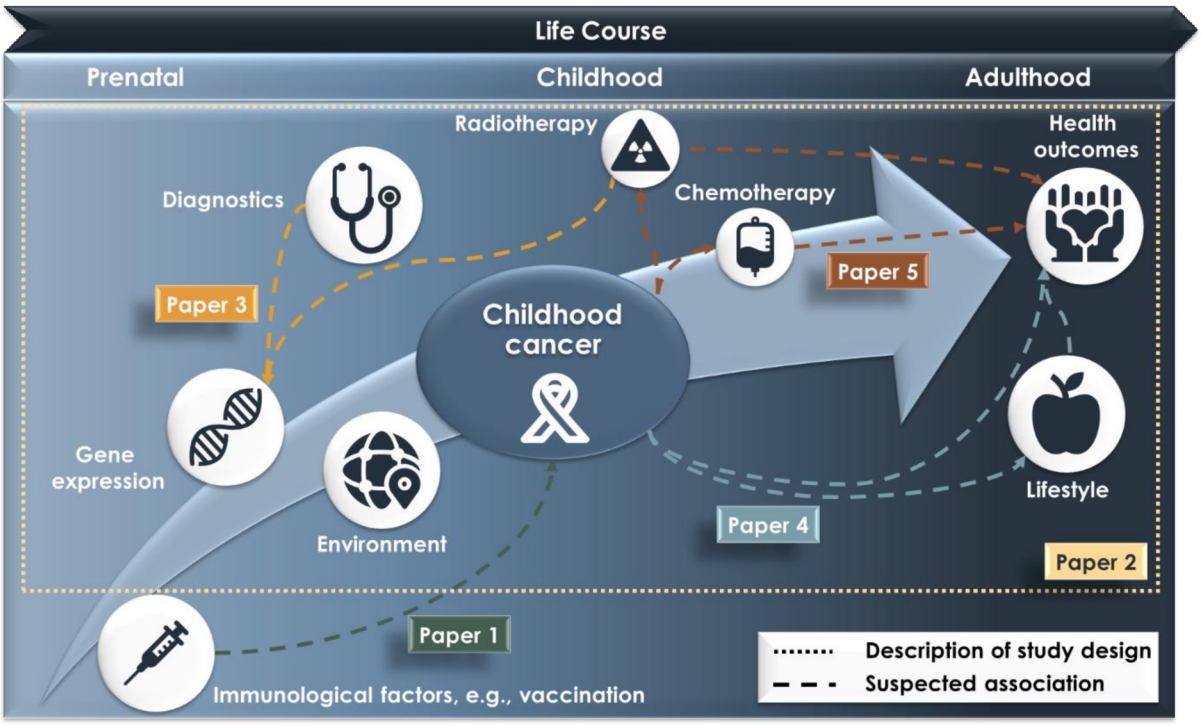

Figure 3: Conceptual framework of the dissertation, including a presentation of the contents of the five included publications and suspected associations.

3 Subjects and methods

The following chapter describes the data basis of this dissertation. The first part of the chapter presents the methods used to conduct the systematic literature review on cancer risk after childhood vaccination and explains the statistical methods used in a meta-analysis of these studies (*Objective 1a*). In the second part of the chapter, the study population of the KiKme study, which builds the ground for the remaining four research objectives of this dissertation, is described in detail. The various data collection instruments, including laboratory work and experimental setups as well as the collection of information from questionnaires, are presented (*Objective 2a*). Furthermore, the section on the KiKme study provides an overview of the multifaceted statistical methods used to establish the best experimental conditions for irradiation experiments with fibroblasts from skin biopsies (*Objective 2b*), to examine late effects and lifestyle after surviving childhood cancer (*Objective 2c*), and to validate a questionnaire on life-long exposure to radiation (*Objective 2d*).

3.1 Systematic literature review and meta-analysis on vaccinations and risk of childhood cancers

To *assess evidence on the association between vaccinations and childhood cancers, including stratification by cancer sites*, which is *Objective 1a* of this dissertation, a systematic literature search was conducted.

3.1.1 Systematic literature search and data extraction

Literature was systematically searched in the three databases MEDLINE, Embase, and the Science Citation index. Studies published before November 2020 were considered for inclusion if they met the following criteria:

1. Original epidemiological study on the influence of vaccination on the risk of childhood cancer or cancer death.
2. An appropriate reference group consisting of individuals without cancer or at least without same cancer as the outcome of interest within the study population.
3. A recommended first application of the investigated vaccine before the age of 10 years.

For inclusion in the subsequently conducted meta-analysis, studies needed to provide any kind of quantitative risk estimates and their variability measures, or at least exact numbers of cases and controls for a crude calculation of risk estimates and corresponding confidence intervals. Data from the included studies were extracted and the quality of included studies was assessed by applying both the Newcastle-Ottawa Scale [99] as well as a self-developed scoring system, which includes the items 'study design', 'sample size', 'outcome assessment', 'exposure assessment', 'confounder control', 'statistical methods', 'other methods', and 'important study characteristics'. Both the quality assessment and the previous selection of the studies with the extraction of the data from them were carried out by two independent reviewers. Disagreements between them were solved with a third reviewer.

3.1.2 Statistical analyses

Pooled analyses were conducted if three or more estimates on the same exposure and outcome were available [100]. If more than one estimate on different numbers of vaccinations with the same cancer outcome was available, additional dose-effect analyses were conducted [101]. Heterogeneity between studies was quantified using the I^2 statistic whose statistical significance was itself assessed using the Q statistic. By excluding individual studies from the analysis, it was then examined whether heterogeneity could be reduced. Furthermore, subgroup analyses were conducted to account for potential sources of heterogeneity. Subgroup analyses consisted of the implementation year of the study (<1964; 1964+), study design (case-control or ecological study; cohort or case-cohort study), quality of exposure assessment (low: self-report or vaccination card; high: trial, registry, or medical documentation) as well as outcome assessment (via registries; other sources) and confounder control (low: basic or no adjustment/matching; high: adjustment or matching for other vaccines or immunological factors), consideration of a latency period (no; yes), quality score on the Newcastle-Ottawa Scale [99] (fourth quintile was used as cut-off to differentiate between low and high levels; low: quality scale <6; high: quality scale 6+) as well as on the developed quality scale (below and above the fourth quintile; low: detailed quality score <24.7; high: detailed quality score 24.7+). If at least five estimates on the same research question were available, publication bias could be assessed using funnel plots [102].

3.2 Nested case-control study KiKme

The goal of the KiKme study was to determine differences in genetic predispositions and radiation sensitivity between former childhood and second primary cancer patients as well as cancer-free control subjects. To achieve this goal, participants for the KiKme study had to be recruited and examined. The following subchapters will address *Objective 2a.* to *implement a nested case-control study with a large collective of childhood cancer survivors and cancer-free controls including the collection of questionnaire information as well as skin biopsies and saliva samples.*

3.2.1 Recruitment and data collection

The recruitment of childhood cancer survivors and cancer-free controls began in 2013. Survivors that were registered at the German Childhood Cancer Registry were contacted to participate in the study and categorized as survivors of a first primary neoplasm (FPN) or survivors of at least one subsequent second primary neoplasm (SPN). The recruitment of cancer-free controls was regionally limited to the areas around the University Hospital of the Johannes Gutenberg-University in Mainz (Germany). Cancer-free controls were admitted to the Department of Orthopedics and Trauma Surgery due to an elective operation. Despite the regional selection of controls, they should be similar to the general population, as accidents and the associated hospitalizations were assumed to be random. Controls with severe diseases (e.g., cancer, hemophilia, Human Immunodeficiency Virus, hepatitis, diabetes) were excluded from participation since underling genetic predispositions to the existing disease could potentially be the same as genetic predispositions to cancer. All participants were individually matched by age and sex, and childhood cancer survivors were additionally matched by cancer site, year of diagnosis, and age at diagnosis. All participants were divided into 101 matching groups, each consisting of one SPN survivor and at least one matched participant from the FPN survivor group as well as one from the cancer-free control group.

Collection of skin biopsies, laboratory work, and irradiation experiments

Skin biopsies were taken from participants by punch biopsy with a diameter of 3 mm on the inside of the cubital region for cases and near the surgery region for cancer-free controls. Immediately after collection, fibroblasts were isolated, cultured, and cryopreserved. In addition to fibroblasts, saliva samples were collected to exclude genetic mosaics.

For the irradiation experiments, fibroblasts were cultured and synchronized by contact inhibition in the G_0/G_1 phase of the cell cycle to exclude cell cycle-dependent effects on gene expression profiles. For this purpose, cells were seeded at a density of 9,000 cells per cm^2 and cultured for 14 to 15 days. Cell cycle arrest in the G_0/G_1

stadium was confirmed by flow cytometry. For the irradiation experiments, the D3150 X-ray therapy system (Gulmay Medical Ltd, Byfleet, UK) was used. The fibroblasts were exposed to radiation doses similar to those used in everyday medical practice: The applied high dose of ionizing radiation of 2 Gray (Gy) corresponds to an average tumor single dose in fractionated radiotherapy [103] and the low dose of ionizing radiation of 0.05Gy, roughly corresponds to an organ dose in computed tomography [104]. In addition, one cell line per subject was sham irradiated (0Gy) and kept under the same conditions as the treated cells in the control room of the irradiator. To avoid batch effects within groups, all cells from a matched triplet including an SPN survivor, an FPN survivor, and a cancer-free control were cultured, treated, and sequenced simultaneously. Experiments were terminated two and four hours after exposure to irradiation, respectively. These time points were identified from preliminary experiments on fibroblasts from three cancer-free controls (two hours) and the literature (four hours, [86]) as potential time points with the highest differential gene expression.

After irradiation, ribonucleic acid (RNA) was isolated from irradiated and sham irradiated fibroblasts and RNA integrity was assessed. RNA sequencing libraries were then pooled, assembled into cBot clusters, and sequenced on a HiSeq2500 instrument (Illumina, San Diego, California, USA) in high-output mode. Afterward, single-end reads of 50 base pairs in length were generated.

Collection of information using self-administered questionnaires

In addition to the molecular biological part of the KiKme study, all participants were asked to provide information on anthropometric and socio-economic factors, medical history, health status, and lifestyle parameters via self-administered questionnaires (*Appendix, pages 146-173*). Of particular interest were the questions about participants' health and health-related behavior, lifelong radiation and cancer therapy exposure, regular medication, as well as severe diseases in their families including cancer.

To assess their medical history and health status, participants answered a self-administered questionnaire and were asked whether they take any regular medication and whether they have been diagnosed with one of the following diseases: diabetes, hypercholesterolemia, hypertension, lung diseases such as asthma or bronchitis, hay fever, inflammatory joint or vertebral diseases including arthrosis and rheumatism, neurodermatitis, heart attack, stroke, thyroid diseases, Epstein-Barr virus infections, HIV, Hepatitis, or any other severe disease. Additionally, age at diagnosis was requested. To assess the formation of medical therapies and lifelong radiation exposure, participants were asked whether they had ever received cancer therapy or whether they ever received any kind of radiation-based diagnostic or intervention. If any of the points were applicable, participants were asked in which year, at which age, how often, and with which doses or medications they were treated. Furthermore, information on affected body regions was inquired. To collect information about the history of cancer in the family, participants were requested to provide information on the cancer site and age at diagnosis for all relatives (children, siblings, nephews and nieces, parents, grandparents, aunts, uncles, and cousins) in an interview.

About the current lifestyle of participants, the extent of physical activity as well as smoking and drinking habits, along with consumption of soft drinks, water, coffee, and other drinks, were requested. Moreover, the body mass index (BMI) of participants was calculated by dividing weight in kilograms by height squared in meters (kg/m^2) based on their self-reported information on weight and height. Here, the range for normal weight was defined as BMI between 18.5 and <25 kg/m^2, overweight as BMI ≥25 kg/m^2, and obesity as BMI ≥30 kg/m^2 according to WHO and NIH standards [105].

Data management and cleaning

All collected study data from questionnaires and interviews were continuously entered into a study database during the project period. This entry was done twice and on separate screens for quality assurance reasons.

All data on the collected biosamples (cultivation time, passage, cryophase, extraction of RNA and DNA, residual amounts) were stored in a biospecimen database. An essential component of data management was the administration of assigned pseudonyms to ensure compliance with data protection and to ensure subject identity. Thus, all questionnaire information and biosamples were maintained separately under different pseudonyms. All data were subjected to regular plausibility checks during the course of the project. Before data analysis, all data were cleaned and analysis datasets were created, which included the creation of new analysis variables.

3.2.2 Statistical analyses

Experimental setups for irradiation experiments on human fibroblasts

To determine the best possible time point for RNA isolation after irradiation and to answer *Objective 2b. Establishment of the experimental conditions for the identification of differentially expressed genes and corresponding pathways in irradiation experiments using fibroblasts from skin biopsies* of this dissertation, a total of 15 participants were selected from the KiKme study population. They were grouped into five matched triplets, each consisting of one survivor of at least one SPN, one survivor of an FPN, and one cancer-free control. Cells for the radiation experiments were from nine male and six female participants with a mean age of 28.27 years (age range at recruitment: 21-40 years). FPN diagnoses were lymphoma (n=6) or leukemia (n=4) and were diagnosed at a mean age of 8.10 years (age at FPN diagnosis: 4-14 years). SPN diagnoses were thyroid/skin cancers (n=2, each) or leukemia (n=1) and occurred at a mean age of 20.00 years (age at SPN diagnosis: 10-36 years).

The best time point for the end of irradiation experiments was defined as the time point with the largest number of differentially expressed genes after radiation exposure. To identify this time point, the RNA sequencing data had to be processed. To this end, the raw data were cleaned so that bases with a quality of less than three were removed and reads were trimmed if the average quality over

four bases was less than 15 [106]. The processed reads were then aligned to the human reference genome (GRCh38) [107] and expression per gene, expressed as the number of aligned reads per gene, was quantified [108]. Only genes with a minimum of ten counts in at least four samples were analyzed. Data were then normalized for sequencing depth [109] and reads were aggregated at the UCSC gene annotation level. Furthermore, residual and principal component analyses were performed [110], with a visual inspection of the correlation of the first three principal components and RNA quality parameters and the number of reads. For differential expression analysis, data were transformed [111, 112], and then gene expression after irradiation was compared with that after sham irradiation for each time point and irradiation dose. Disease status was not considered, but gene expression variability and random variance were considered [112]. Differentially expressed genes with a p-value of less than 0.05 after adjustment for false discovery rate via the Benjamini-Hochberg procedure were flagged as significant and used for subsequent pathway analyses. Finally, pathway analyses were performed using Ingenuity Pathway Analysis (version 1.13, QIAGEN Inc., 2018). Here, negative log (-log10) p-values of at least 1.30 (corresponds to a p-value=0.05) were defined as significant. The threshold for the activating z-score, which indicates the (de)activation of pathways via a comparison of given expression directions of pathway components with information from the dataset entered for analysis, was chosen to be greater than or equal to |2| [113]. To display and compare pathways across all experiments, the comparison analysis was conducted using Ingenuity Pathway Analysis. Moreover, predicted downstream outcomes and upstream regulators were summarized.

Impact of cancer status on adverse late effects of childhood cancer and lifestyle parameters

To answer the *Objective 2c* on *associations between cancer status and adverse late effects of childhood cancer and lifestyle parameter*s of this dissertation, self-reported data from the KiKme participants were used.

To analyze associations between cancer status and late adverse health as well as lifestyle effects, GLMM were applied to the data. Here, associations of the late effects with the cancer status (survivors of at least one SPN vs. survivors of FPN) and with the case-control status (all survivors vs. cancer-free controls) were determined using odds ratios and 95% confidence intervals. In these models, each matching group, consisting of one SPN survivor and at least one matched participant from the FPN survivor group as well as one from the cancer-free control group, was treated as a random effect. Moreover, models were adjusted for birth year and age at recruitment. To account for further confounding, a directed acyclic graph (DAG) [114] was developed based on prior knowledge using DAGitty (version 3.0)[1] to identify further necessary adjustment variables (ethnicity, International Standard Classification of Education (ISCED), exposure to cancer therapy, and genetic predisposition in the family). All outcomes that exceeded a prevalence of more than 5% in the whole study sample were considered for analysis.

In addition to the analysis of single outcomes, an analysis of a created general health score was conducted. Besides factors depicting participants' actual health status, the score also includes resources and healthy behaviors that can contribute to a good health status. The score consists of the items 'number of diseases', 'BMI', 'education' defined via the ISCED [115], 'smoking status', 'alcohol consumption', 'soft drink consumption', 'physical activity', and 'current employment'. If fewer than four items were answered, the health score was set to be missing. The maximum score was 8 points (*Table 1*). The total number of points of each participant was then divided by the number of variables that were not missing and the score was divided into three categories (<0.75 points, 0.75 points, > 0.75 points).

[1] http://www.dagitty.net/dags.html

Table 1: Scoring of the created general health score.

Variable	Points (max. 8)
Number of diseases	
< 3	1
≥ 3	0
BMI	
< 18.5	0
18.5-30.0	1
> 30.0	0
ISCED [115]	
High (upper secondary education or above)	1
Low (lower secondary and primary education)	0
Smoking status	
Never smoker	1
Current or former smoker	0
Alcoholic drinks per day	
< 1	1
≥ 1	0
Consumption of soft drinks	
No consumption	1
Consumption	0
Physical activity per week	
≥ 5 hours	1
< 5 hours	0
Current employment	
Employed or self-employed	1
Incapacitated or retired	0

Abbreviations: BMI, body mass index; ISCED, International Standard Classification of Education

Validation of the questionnaire on childhood cancer treatments

Similar to *Objective 2c* on associations between cancer status and adverse late effects of childhood cancer and lifestyle parameters, *Objective 2d. Validation of a self-administered questionnaire assessing childhood cancer treatments and associated risks for adverse health outcomes* was analyzed using data from the self-administered questionnaires. For a subsample of participants, data on received cancer therapies recorded by their treating hospitals or therapy-optimizing studies were available in addition to the data from the self-administered questionnaire. The available information from the medical records includes binary variables (yes/no) on the exposure to cancer therapies, which was compared to the data that was obtained using self-administered questionnaires within the KiKme study. To

measure the concordance between both data sources, kappa statistic (κ) was used [116]. A possible influence of other factors (e.g., number of neoplasms, socio-demographic factors, comorbidities, time since cancer treatment) on the concordance between the questionnaire and medical records were analyzed using logistic regression. In a subsequent step, generalized linear mixed models (GLMM) were applied to the data to analyze the association between exposure to cancer therapies and the risk of later adverse health effects.

Like the analyses on associations between cancer status and late adverse health effects and lifestyle described earlier in this chapter, the matching group was included as random effect to the model and possible further adjustment variables were identified using DAGs [114]. However, the developed DAG showed that no further adjustment was necessary to estimate the total effect of exposure to cancer therapy on health outcomes. In addition to the GLMM, differences in time between cancer therapy and later occurring adverse health outcomes were analyzed in survival analyses.

4 Main findings

This chapter summarizes the main findings of the five publications (*Appendix, pages 57-145*) that have been incorporated into this cumulative dissertation.

4.1 Association between vaccination and childhood cancer

The systematic literature search identified 6,774 articles in three literature databases of which 60 articles met the inclusion criteria for the systematic literature review. The studies included in the review reported 706 single risk estimates for different childhood cancers after diverse types of vaccinations. Of these effect estimates, 85 showed a decreased risk and 48 showed an increased risk of different childhood cancers. The remaining 576 effect estimates revealed no significant association. Of the 60 studies included in the systematic literature review, 35 articles met the inclusion criteria for the subsequent meta-analysis.

Most of these studies were case-control studies (n=23), followed by retrospective cohort and case-cohort studies (n=11), and ecological studies using aggregated data (n=1). Risk estimates that were extracted from these included original publications allowing for a total of 27 analyses on eleven cancer outcomes (childhood cancer, childhood cancer death, leukemia, leukemia death, ALL, lymphoma, Hodgkin lymphoma, bone cancer, brain cancer, kidney cancer, and skin cancer) after exposure to nine different vaccination indicators (any vaccination, early vaccination, number of any vaccine injections, Bacillus Calmette-Guérin, Haemophilus influenzae type b, poliomyelitis, hepatitis, diphtheria-tetanus-pertussis/-poliomyelitis, and measles-mumps-rubella vaccination). In our pooled analysis, we observed inverse associations between Bacillus Calmette-Guérin vaccination and leukemia death, between Haemophilus influenzae type b vaccination and ALL, and between a high number of unspecified vaccinations and ALL or leukemia, respectively. All other overall pooled analyses did not show any associations. However, the stratified analyses performed showed that factors such as study design and quality had an impact on the results. For example, the observed significant inverse association between Haemophilus influenzae type b vaccination and between a high number total number of vaccinations and ALL were found to be only significant for case-control studies but not for cohort studies after stratification for study design. Similarly, in the stratified analysis for study quality, the association between number of vaccinations and ALL was only found to be present for studies with low quality, but not for those with high study quality.

The number of analyses was in general limited, since often only a few studies (on average four studies per meta-analysis) could be pooled for a common analysis due to different exposures and outcomes. Due to the limited number of studies, which mostly had a small sample size, it was difficult to explore risk factors with small effects, as even pooled estimators often failed to achieve the required statistical power. Furthermore, most studies relied on self-reporting by the children or their parents, a method which was regarded as less valid. In addition, a large part of the studies did not adequately adjust for important confounders and only few studies had a longitudinal design.

4.2 Recruitment of childhood cancer survivors and cancer-free controls

The recruitment for the nested case-control study KiKme started in 2013. A total of 247 survivors of at least one SPN and 1,729 survivors of FPN were invited by the German Childhood Cancer Registry to participate in the study. Of the contacted participants, 92 SPN survivors (37%) and 399 FPN survivors (23%) showed their willingness to participate. During the further recruitment process, some participants withdrew their consent to participate while others decided to participate, despite previous refusal. In addition, participants who were registered as FPN survivors at the German Childhood Cancer Registry but developed an SPN in the meantime were shifted from the FPN to the SPN survivor group. Cancer-free controls were recruited from the Department of Orthopedics and Traumatology of the University Medical Center Mainz when they were hospitalized for elective orthopedic surgery after an accident. In total, 246 potential cancer-free controls were contacted, of which 163 (66%) decided to participate in the study. However, seven of them had to be excluded due to serious illnesses, four withdrew their participation, and another two were excluded for nonresponse. The whole recruitment process is illustrated in *Figure 4*.

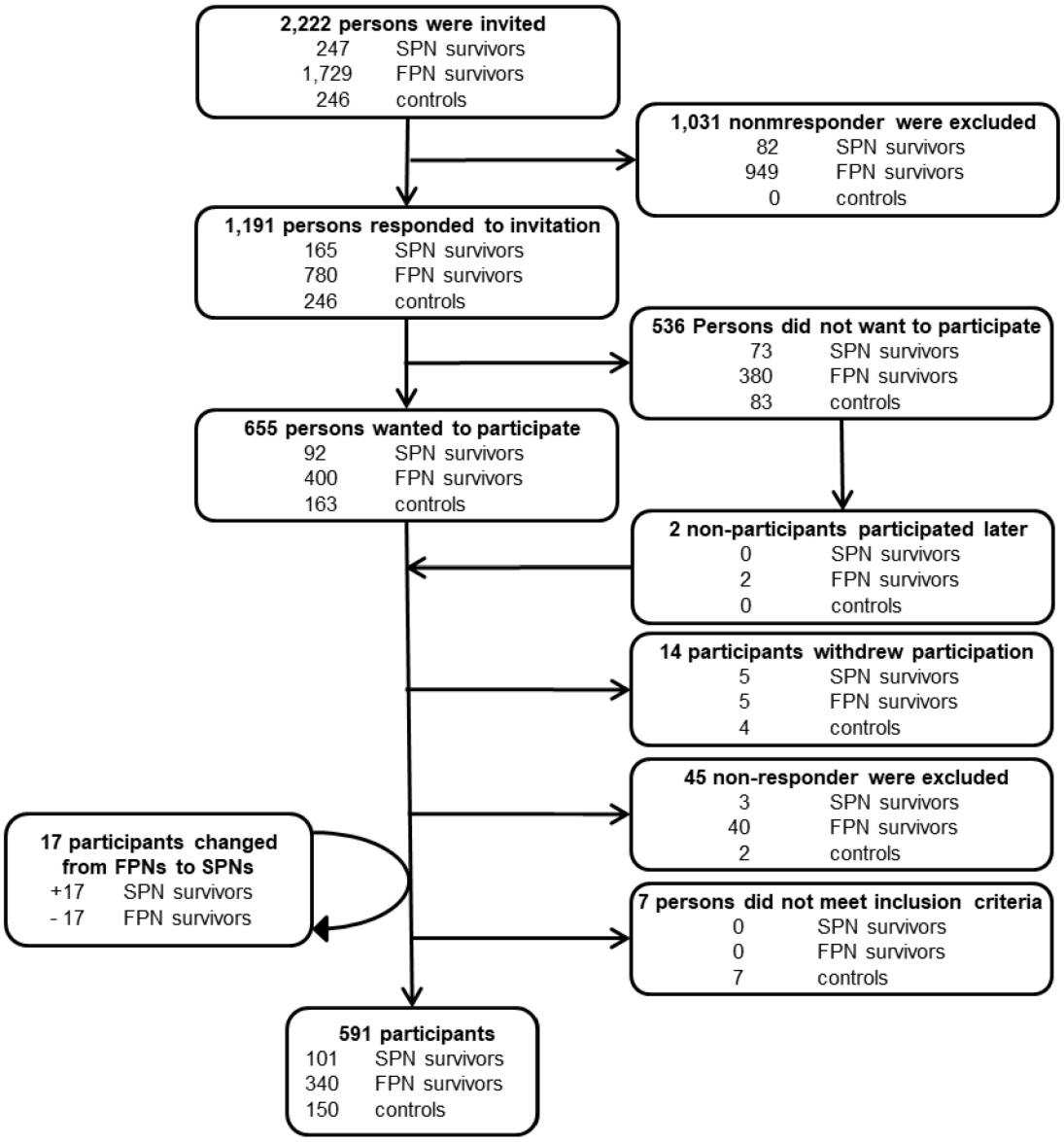

Figure 4: Enrollment of participants in the nested case-control study KiKme - adapted from Marron et al., 2021 [117]. Abbreviations: FPN, first primary neoplasm; SPN, second primary neoplasm.

In total, 591 former childhood cancer patients and cancer-free control subjects aged 19 to 53 years (mean 32 years, 51% women and 49% men) were successfully recruited for the KiKme study. In detail, the final study population consists of 101 childhood cancer survivors that developed an SPN later in life, 340 survivors with an FPN only, and 150 cancer-free controls [117]. Further characteristics of all participants, such as age at diagnosis and tumor morphology, are summarized in *Table 2* and *Table 3*.

Table 2: Characteristics of the KiKme study participants - adapted from Marron et al., 2021 [117].

	SPN survivors	FPN survivors	Cancer-free controls	Total
Total, n	**101**	**340**	**150**	**591**
Female, n (%)	50 (50%)	189 (56%)	62 (41%)	**301 (51%)**
Male, n (%)	51 (50%)	151 (44%)	88 (59%)	**290 (49%)**
Age at recruitment, mean (range)	32 (19-51)	34 (19-53)	29 (18-48)	**32 (19-53)**
< 25 years, n (%)	19 (19%)	44 (13%)	57 (38%)	**120 (20%)**
25-29 years, n (%)	25 (25%)	69 (20%)	40 (27%)	**134 (23%)**
30-34 years, n (%)	19 (19%)	78 (23%)	20 (13%)	**117 (20%)**
≥ 35 years, n (%)	38 (38%)	149 (44%)	33 (22%)	**220 (37%)**
Age at 1st diagnosis, mean (range)	7 (0-14)	8 (0-16)		
Year of 1st diagnosis	1980-2011	1980-2012		
Years between 1st and 2nd diagnosis, mean (range)	16 (2-35)			
Age at 2nd diagnosis, mean (range)	23 (5-46)			
Year of 2nd diagnosis	1986-2018			

Abbreviations: FPN, first primary neoplasm; SPN, second primary neoplasm

Moreover, the study was successful in collecting skin biopsies from 499 (84.4%) study participants (consisting of 92 SPN, 307 FPN, and 100 cancer-free control samples) as well as saliva samples from 511 (86.5%) participants (consisting of 84 SPN, 319 FPN, and 108 cancer-free control samples). Complete information from questionnaires was available for a total of 554 (93.7%) study participants (including questionnaire information from 85 survivors of SPN, 325 survivors of FPN, and 144 cancer-free controls). Study participants were free to participate only in parts of the survey.

Table 3: Cancer diagnoses and cancer therapies of the KiKme study participants - adapted from Marron et al., 2021 [117].

	SPN survivors	FPN survivors
Cancer sites (International Classification of Childhood Cancer 3rd Edition)		
1st Neoplasm (n (%))		
Leukemia (I(a), I(b), I(c), I(d))	41 (41%)	166 (49%)
Lymphoma (II(a), II(b), II(c))	41 (41%)	135 (40%)
Central/peripheral nervous system (III(a), III(b), III(c), III(d), IV(a))	15 (14%)	35 (10%)
Other tumors (V, VI(a), IX(a), IX(e))	4 (4%)	4 (1%)
2nd Neoplasm (n (%))		
Thyroid cancer (XI(b))	30 (30%)	
Skin carcinoma (XI(e))	32 (32%)	
Malignant melanoma (XI(d))	4 (4%)	
Leukemia (I(a), I(b), I(d))	9 (9%)	
Lymphoma (II(a), II(b))	6 (6%)	
Central nervous system (III(a), III(b), III(e))	9 (9%)	
Breast cancer (XI(f))	3 (3%)	
Other unspecific carcinoma (XI(f))	6 (6%)	
Sarcoma (IX(d), IX(e))	2 (2%)	
3rd Neoplasm (n)		
Renal carcinomas (VI(b))	1 (1%)	
Skin carcinoma (XI(e))	2 (2%)	
Breast cancer (XI(f))	1 (1%)	
Other and unspecified carcinomas (XI(f))	2 (2%)	
Other specified intracranial and intraspinal neoplasms (III(e))	2 (2%)	
4th Neoplasm (n)		
Thyroid cancer (XI(b))	1 (1%)	
Cancer therapies		
1st Neoplasm (n (%))		
Chemotherapy	93 (92%)	312 (92%)
Radiotherapy	74 (73%)	225 (66%)
Surgery	25 (25%)	64 (19%)
2nd Neoplasm (n (%))		
Chemotherapy	22 (22%)	
Radiotherapy	21 (21%)	
Surgery	56 (55%)	
3rd Neoplasm (n)		
Chemotherapy	1 (1%)	
Surgery	2 (2%)	
4th Neoplasm (n)		
Surgery	1 (1%)	

Abbreviations: FPN, first primary neoplasm; SPN, second primary neoplasm

4.3 Setups for irradiation experiments to identify differentially expressed genes

In the first step, the best irradiation doses for the irradiation experiments on fibroblasts were identified in experiments performed on cell lines of three cancer-free controls of the KiKme study sample. Due to the highest number of differentially expressed genes, 0.05Gy and 2Gy were identified as the best irradiation doses.

In a second step, the time point of four hours after radiation exposure was identified as the best time point for the termination of the experiments and RNA extraction after irradiation. This result was based on an overall higher number of differentially expressed genes four hours after exposure to low and high doses of ionizing radiation compared to two hours after exposure (*Figure 5*).

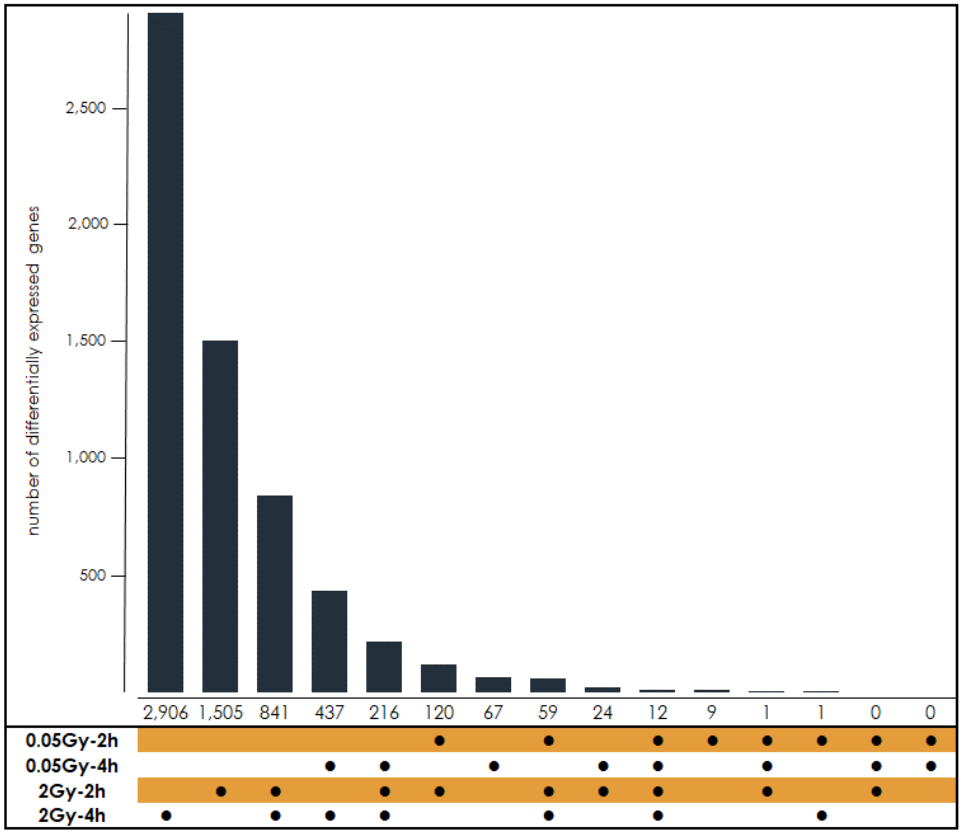

Figure 5: Differentially expressed genes in human fibroblasts two and four hours (h) after exposure to a low (0.05 Gray (Gy)) and a high dose (2Gy) of ionizing radiation (adjusted for false discovery at a rate of 0.05).

Concomitant with the identified differentially expressed genes, inactivation of the pathway *small cell lung cancer signaling* was observed after exposure to high doses of ionizing radiation in the experiments that were terminated after two hours. In contrast, an inactivation of the *FAT10 cancer signaling* pathway as well as activation of the pathways *gluconeogenesis I*, *glycolysis I*, and *prostanoid biosynthesis* was observed four hours after exposure to high doses of ionizing radiation. After exposure to low doses of ionizing radiation, no activated or inactivated pathways were found for both time points.

4.4 Impact of cancer status on adverse late effects of childhood cancer and lifestyle parameters

Childhood cancer survivors differ from cancer-free controls in terms of their health status and lifestyle. Survivors were more affected by thyroid and lipid metabolism disorders than cancer-free controls. This was accompanied with survivors being more likely to report taking medications regularly than cancer-free controls. Survivors showed an overall healthier lifestyle that included lower consumption of sugared-sweetened beverages, alcohol, and tobacco. Overall, childhood cancer survivors in the KiKme study sample had a lower BMI than the cancer-free controls. However, survivors appeared to be less active than the controls. All other analyses performed on cardiovascular, chronic lung, inflammatory bone, allergic, and infectious diseases, as well as on the calculated health-score revealed no association with tumor status.

4.5 Validation of the questionnaire on childhood cancer treatments and associated adverse health outcomes

For a total of 272 (46%, 93 survivors of SPN and 179 survivors of FPN) of the KiKme study participants information on cancer therapies was available from medical records. Comparison with their data from self-administered questionnaires showed that even many years after exposure to cancer therapies,

the newly developed and established questionnaire used in the KiKme study was valid for a retrospective assessment of cancer therapies in childhood. This was particularly the case for chemo- and radiotherapy in childhood cancer survivors with at least one SPN. However, self-reported information on radiotherapy provided by survivors of FPNs was too imprecise and therefore not used for the subsequent analyses of therapy-associated adverse health outcomes.

Using the valid information from self-administered questionnaires on exposure to chemotherapy it was possible to assess differences in late health outcomes between survivors with and without chemotherapy. Survivors exposed to chemotherapy in childhood were found to be more likely to have disorders of the thyroid gland and lipid metabolism. Moreover, they were less likely to be overweight or obese compared to controls without prior chemotherapy. No effect was observed for occurrence of cardiovascular diseases or SPNs.

5 Discussion

In the following chapter, the most important results of the five included publications will be interpreted and placed in the overall context of current research, followed by a general discussion on medical risk factors of childhood cancer and late effects including implications for prevention strategies. Additionally, the strengths and limitations of the dissertation are highlighted. The chapter concludes with suggestions for future research.

5.1 Discussion of results

Most of the associations that were assumed in the conceptual framework of this cumulative dissertation (*Figure 3*) were confirmed by the conducted studies as indicated in *Figure 6*. The following section provides a brief discussion of the results obtained in each paper.

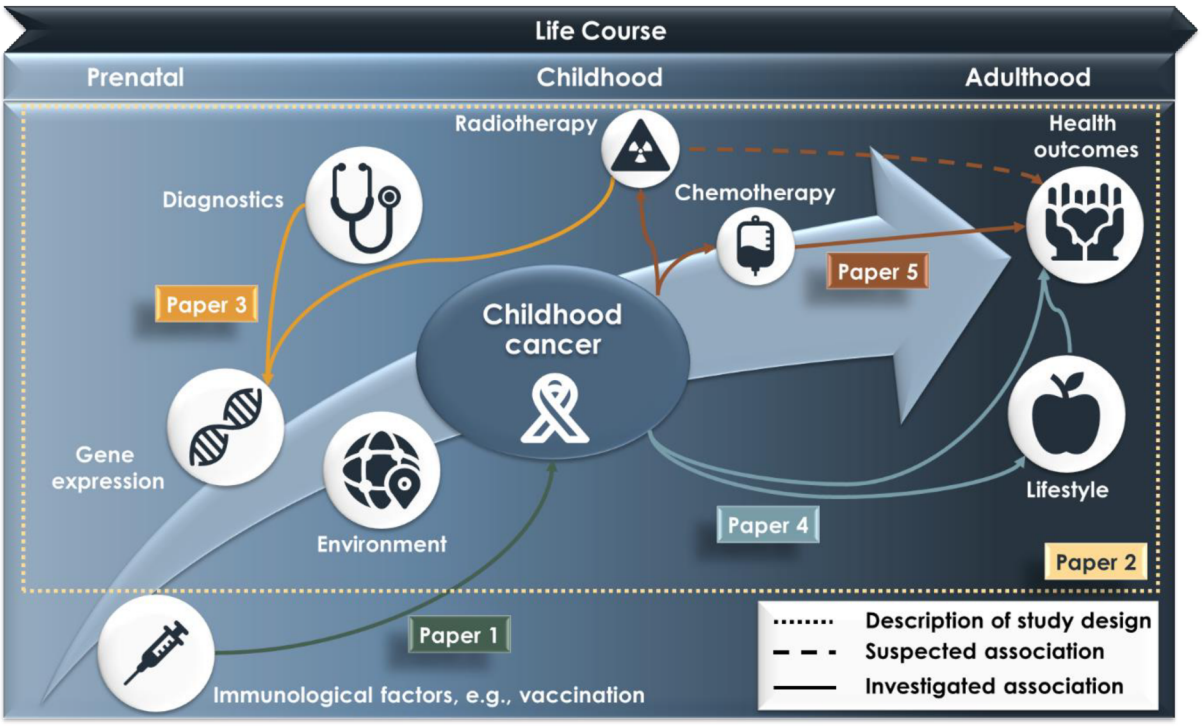

Figure 6: Conceptual framework of the dissertation, including a graphical display of all investigated associations within the included five publications.

5.1.1 Vaccination and the risk of childhood cancer

Within the conducted systematic literature review and the subsequent meta-analysis, evidence for an inverse association between Haemophilus influenzae type b vaccination and ALL was observed. This risk reduction was present in almost all of the pooled studies, except for a recent Danish study, which reported no association between Haemophilus influenzae type b vaccination and ALL [118]. This study was a large cohort study with the highest score in the quality assessment, including valid exposure and outcome assessment as well as proper confounder control. The same study did not report an association between a number of vaccinations and risk of ALL [118], unless other studies [32, 119-121] as well as the pooled results of our meta-analysis support this association. A further association between Bacillus Calmette-Guérin vaccination and leukemia death was mainly driven by two cohorts [122, 123] that presented themselves again with valid assessments for vaccination exposure and cancer outcomes. However, both studies did not control for any confounders, which may have caused uncontrolled confounding.

Taken together, the results of the meta-analysis are consistent with the hypothesis that vaccinations protect against leukemia in childhood. However, studies with good exposure assessment and proper control for confounding factors are rare in the literature. Since all studies included in the systematic literature review and the subsequent meta-analysis had at least one substantial methodological limitation, the obtained results should be interpreted with caution.

5.1.2 Recruitment of childhood cancer survivors and cancer-free controls

Overall, the recruitment of childhood cancer survivors and cancer-free controls within the molecular epidemiological study KiKme and the collection of questionnaires and biosamples was very successful. Even if participation in the study was accompanied by a high time expenditure, in particular for childhood cancer survivors, and it was feared in advance that participation might be difficult due to a skin biopsy in otherwise healthy subjects, relatively high participation proportions were achieved (41% for survivors of at least one SPN, 20% for survivors of FPN, and 61% for cancer-free controls). Due to the successful recruitment, a worldwide unique large repository with hundreds of experimentally usable fibroblast cell lines and other biospecimens for in-depth projects in radiation research in pediatric cancer could be established.

5.1.3 Comparison of time and dose dependent gene expression

The time point with the largest number of differentially expressed genes that was reported by other studies differs for different radiation doses and in different cell types [86, 93, 96]. The primary fibroblasts of the KiKme study participants showed the greatest response to ionizing radiation four hours after irradiation. The reviewed times under four hours after irradiation showed few differentially expressed genes overall, suggesting that cells had little or no response to the radiation stimulus during this time period. At 24 hours after irradiation, the

response was already complete in many cells and differential expression was also low.

The differential expression after four hours already showed differentially expressed genes and pathways in response to the low dose of 0.05Gy. This response was also seen after exposure to the high dose of 2Gy, but then with significantly higher expression. The differences in gene expression on both time points were mainly related to signal transduction pathways of the DNA damage response after two hours and to metabolic pathways, possibly related to cellular senescence, after four hours. Similar to our results, previously conducted studies reported more differentially expressed genes in fibroblasts after exposure to a high than to a low dose of ionizing radiation when comparing different radiation doses and analysis time points [96]. Moreover, they reported only little overlap of differentially expressed genes for low and high radiation doses [93, 94], which is also true for the gene expression in fibroblasts of the KiKme participants. With the largest number of differentially expressed genes in both low and high doses of ionizing radiation, the time point four hours was identified as the best for analyzing differential gene expression in irradiated human fibroblasts. This identified time point will therefore be used to conduct further irradiation experiments in the whole study sample of the KiKme study to identify differences in gene expression between patient groups (survivors of at least one SPN, survivors of an FPN, and cancer-free controls).

5.1.4 Late health effects and lifestyle after surviving childhood cancer

Overall, survivors were more affected by diseases and may consequently took more medication, particularly the survivors with at least one SPN. In detail, this study showed that thyroid diseases without thyroid cancer and disorders of lipid metabolism are more common in survivors regardless of exposure to cancer therapies. As described before, disorders of the thyroid gland are known to be attributable to exposure to cancer therapies and especially to cancer therapies in childhood [124]. In addition, disorders of lipid metabolism could be observed in childhood cancer survivors more often than in cancer-free controls. These disorders

might be associated with the development of cardiovascular diseases in the further course of survival [67, 125]. Since most of the observed associations were only present when comparing survivors with cancer-free controls and disappeared when survivors of at least one SPN were compared to survivors of FPN, we hypothesized that the observed higher disease burden is likely related to the cancer therapy received.

Besides the potential therapy-associated late effects, survivors were found to have a healthier lifestyle including a lower BMI, less soft drink and alcohol consumption, as well as less tobacco smoking. The consumption of harmful substances appears to be avoided by survivors. In particular, the reduced intake of alcohol could be a factor associated with the identified higher intake of regular medication in the survivor group, due to a possible interaction with prescribed medications [126]. Despite a healthier overall lifestyle, survivors seem to be less physically active than controls, which was previously reported by others. Data from a Swiss study showed that only about 50% of the survivors of childhood cancer reached the recommended time of physical activity per day [81]. This overall reduced time of physical activity, however, might be explained by the higher disease burden of survivors.

5.1.5 Exposure to ionizing radiation and cancer therapies in childhood

Within the framework of the KiKme study, a newly developed questionnaire for the retrospective assessment of past cancer therapies could be established. Previous studies on the agreement between self-reported cancer therapy and data from medical records mainly included adult survivors of breast cancer [127-133]. This far, only one other validation study on a retrospective assessment of cancer therapies in childhood was conducted using telephone interviews [134]. Just like similar validation studies in adult cancer patients [127-131], the results obtained in the KiKme study showed very good agreements for the exposure to chemotherapy, which might be caused by the drastic effect of the therapy [135]. The agreement for exposure to radiotherapy was found to be lower in our study as well as in studies conducted before [132, 134]. Since radiotherapy is often not used

as first-line therapy and therefore might be applied a while after diagnosis it might not be remembered as good as chemotherapy by study participants [132]. Nevertheless, the survivors of at least one SPN in our study sample remembered exposure to radiation therapy well at least and reported it with substantial accuracy. Through the successful validation of the questionnaire, an instrument is now available that allows a simple and cost-effective assessment of exposure to cancer therapies even many years after childhood cancer therapy, especially regarding chemotherapies.

The data from the newly validated questionnaire were subsequently used to identify differences in late adverse health effects between survivors with and without exposure to chemotherapeutics within the whole study population of the KiKme study. Previously, as described in *Chapter 4.4* and *Chapter 5.1.4*, we investigated associations between cancer in childhood and the occurrence health effects. In these analyses, however, cancer therapies were only considered as adjustment variable. Since associations were only observed when comparing childhood cancer survivors to cancer-free controls and disappeared when comparing survivors of at least one SPN to survivors of FPN, we hypothesized that the effect may be driven by cancer therapies and conducted the further analyses on associations between therapy exposure and late adverse health outcomes. The results indicate an association between exposure to chemotherapeutic agents in the course of cancer therapies and diseases of the thyroid gland as well as disorders of lipid metabolism. Disorders of the thyroid gland are well-known as adverse late health effects of cancer, which are attributable to exposure to cancer therapies such as radiopharmaceutical agents and tyrosine kinase as well as immune checkpoint inhibitors [124]. The effect is even more pronounced when exposure to cancer therapies occurred in childhood [124]. In addition, survivors were found to have more disorders of lipid metabolism, which is one of the main risk factors for the development of cardiovascular diseases in survivors of childhood cancer [67, 125]. Contrary to previous studies, we did not observe a significant association between cardiovascular diseases as well as occurrence of second primary malignancies with chemotherapy at first diagnosis. However, with regard to the latency of

cardiovascular diseases and second primary malignancies, we expect an increase in these therapy-related sequalae with extended follow-up.

5.2 General discussion and recommendations for prevention

Childhood cancer is a rare disease, accounting for less than 2% of diagnoses in industrialized countries [10]. However, due to the overall good prognosis and the therefore good survival of patients, the number of childhood cancer survivors have increased over the last decades [6, 7]. In light of the overall high disease burden which is caused by the disease itself as well as frequently occurring late adverse health effects [8, 9], effective prevention strategies are required to prevent the occurrence of cancer on one hand, and to prevent secondary diseases, with the development of secondary primary malignancies as the most detrimental consequence, on the other hand.

In order to prevent childhood cancers, there is only a very short window of time in which exposure to potential carcinogens can be prevented [10]. Nevertheless, this short time period is of particular importance because the impact on rapidly dividing cell populations appears to be much greater in this young age group [57]. With greatly improved access to genetic screening technologies in recent years, an increasing number of genetic predispositions to childhood carcinogenesis have been identified [13-15]. In addition to mutations that occur in genetic cancer predisposition syndromes and lead to increased cancer incidence via, for example, dysfunction in ribosomal protein biosynthesis [13, 14] or impaired signal transduction for cell proliferation and differentiation [15], an increasing number of gene variants that are not primarily associated with a familial predisposition or genetic syndromes have recently been identified [16]. Several research groups recommend using these gene variants to prevent the development of SPN after surviving cancer in childhood [17, 18, 136-138]. As a novel approach to identify potential childhood cancer survivors with high-risk profiles, the *McGill Interactive Pediatric OncoGenetic Guidelines* were established in 2017 [137]. These guidelines can be used to identify cancer predispositions in childhood cancer survivors and

refer them to genetic counseling based on their risk profile. A Danish study that investigated predisposition syndromes in childhood cancer patients younger than 18 years applied the *McGill* guidelines to their study sample [138]. Of the 198 participants of their study, 94 (47.5%) had pathogenic variants or showed clinical features that made such variants likely. Of these 94 participants, 29 participants developed cancer later in life (21 in childhood, 8 as adults, and 1 participant both in childhood and as an adult). Of the participants with an SPN in childhood, 18 met the applied *McGill* guidelines. Of the participants that developed an SPN as an adult, only two met the *McGill* criteria [138].

In addition to cancer directly caused by genetic mutations, it would be possible that individual genetic factors cause a special susceptibility to environmental exposures and, in particular, to ionizing radiation. Here, gene expression analyses have found initial differences between sporadic and radiotherapy-induced thyroid carcinomas [139]. Such mutations, which increase susceptibility to exogenous carcinogenic exposures and lead to carcinogenesis in the long term, are particularly important for the development of an SPN after radiotherapeutic treatment for first cancer. Not only is the treatment of FPN with radiotherapy but also with chemotherapy is considered the most important established risk factor for the development of an SPN after childhood cancer [55, 140-145]. In this context and to avoid other therapy-associated late adverse health effects, the development of more targeted therapies should be pursued and, in conjunction with this, a reduction in the therapeutic radiation and chemotherapy doses should be aimed for. Furthermore, it should be clearly stated that the unnecessary use of any kind of ionizing radiation, especially diagnostic radiation in the form of conventional X-rays and computed tomography scans, should be avoided, especially during pregnancy and early childhood [10]. Reduced lifetime radiation exposure could reduce both the incidence of cancer, not only in childhood but also in later life, and the incidence of secondary diseases such as SPNs after cancer in childhood [10]. In the long term, especially in the field of therapy and medical diagnostics, personalized medicine based on molecular biological analyses could be a milestone to reduce the above-mentioned first- and second-line health outcomes.

5.3 Strengths and limitations of the dissertation

The individual studies included in this dissertation each have different strengths and limitations, which will be presented in the following chapter.

5.3.1 Systematic literature review and meta-analysis on vaccinations and risk of childhood cancers

The main strength of the meta-analysis is the comprehensive search strategy that was used to ensure that all relevant publications on this topic were identified. The extensive literature search allowed separate analyses on histological and site-specific childhood cancers, as well as on specific vaccines, age at vaccination, and number of vaccinations. All included studies were assessed for quality, considering important aspects such as latency, quality of statistical methods, interviewer training, exposure assessment in cohort studies, and cancer in controls in case-control studies. Overall, there was no evidence of publication bias, but the power of the test was low in meta-analysis with only a few included studies.

The main limitation of the meta-analysis is the small number of studies for some of the investigated associations between specific exposures and cancers and the relatively large heterogeneity among studies. Random-effects models were used to account for the potential heterogeneity between the studies. In addition, to assess effects on outcomes, analyses were stratified by selected important study characteristics.

5.3.2 Nested case-control study KiKme

Recruitment of childhood cancer survivors and cancer-free controls

In contrast to previous studies investigating the relationship between ionizing radiation and cancer risk, the KiKme study is one of the first to collect detailed molecular biological information before and after exposure to diagnostic and therapeutic doses of ionizing radiation in a large study population. This enables us to study genetic responses to radiation exposure in normal somatic cells of the

study participants. The combination with observational data from questionnaires on medical radiation history and health status allows a comprehensive control of important confounding factors in cancer development. In addition, it has been possible not only to collect information from the study participants themselves, but also on family history of serious diseases, which has allowed consideration of familial predispositions to some extent.

As with all epidemiological studies using self-reports, there is an inherent survivor bias in the KiKme study, since only living patients could be recruited. Severe cases with high mortality (e.g., acute myeloid leukemia following acute lymphoblastic leukemia, or two cancer diagnoses in rapid succession) could not be fully captured in the study. In addition, selection bias cannot be excluded because individuals with severe health problems may have been less motivated to participate in the study and recruitment of cancer-free controls was regionally limited for logistical reasons. In addition, the cancer-free controls were slightly younger than the participating childhood cancer survivors. In addition, the statistical power of the study was limited by the sample size, since the number of available former childhood cancer patients was limited by the number of survivors who met the inclusion criteria and were registered in the German Childhood Cancer Registry.

Comparison of time and dose dependent gene expression

In contrast to previous studies, which often used commercially available cells or only a very limited number of donors, fibroblasts from skin biopsies from a total of 15 donors were used in this study. All samples were cultured for the first time and synchronized in the G_0/G_1 phase of the cell cycle to exclude cell cycle-dependent effects on gene expression. All samples were treated under identical conditions regardless of irradiation profile. Subsequent pathway analysis allowed the analysis of complex RNA data and contributed to the identification of individual expression patterns. However, in the study, only a limited number of radiation doses and analysis time points could be considered. In order to identify two important time points for the analyses, preliminary experiments with smaller sample sizes and a literature search were performed. Regarding the dose, a high

and a low radiation dose with clinical relevance were chosen to mimic the characteristic exposures to ionizing radiation in medical diagnostics and radiotherapy. In addition, samples from all three patient groups of the KiKme study were analyzed, which may have resulted in increased heterogeneity of gene expression levels. To account for factors such as age, sex, and first cancer diagnosis that may have influenced gene expression variability, subjects were matched with respect to these factors.

Analyses of questionnaire data on late health effects and lifestyle after surviving childhood cancer and exposure to ionizing radiation and cancer therapies

Regarding the strengths and limitations of the two publications analyzing questionnaire data from the KiKme study, the study population is the first in which differentiated analyses on cancer and health-related late effects as well as on differences in lifestyle have been performed, also at the level of different numbers of cancer diagnoses. In addition, to our knowledge, only one other validation study has been conducted to retrospectively evaluate childhood cancer treatments. In contrast to their methods, we allowed our participants to obtain as much information as possible about previous cancer treatments before answering our self-administered questionnaire. Valid information on cancer therapies from the participants' treating hospitals and from treatment optimization studies was available to verify self-report.

Because the information for the analyses performed was self-reported by the participants, some recall bias may be present. However, by collecting self-reported information, we were able to obtain information on a large number of variables, allowing us to adjust our models extensively where necessary. In addition, surveillance bias cannot be ruled out in both studies, as former cancer patients may be diagnosed with late effects more frequently due to regular follow-up. However, there are only a small number of subjects with these late adverse health outcomes in the study population, especially for rare diseases such as myocardial infarction, stroke, or severe infectious diseases, because of the sample size and the relatively short follow-up period. However, the number of late health outcomes

could increase over the course of further follow-up of the study collective. Therefore, this unique cohort provides an opportunity to comprehensively analyze the late effects of childhood cancer and its treatment in the future. As the number of outcomes increases, more sophisticated investigations, such as the type, number, and location of therapies received, can be considered.

5.4 Implications for future research

Although the previously described studies, that were included in this dissertation, have contributed to research on risk factors for the development of childhood cancer and its late effects, there is still a lack of large and methodologically well-conducted studies to complete the missing pieces of the mosaic in the underlying mechanisms of childhood carcinogenesis.

In the field of immunologic risk factors for childhood cancer, further studies on vaccination that consider latency periods, collect high-quality data on exposures and outcomes, and adequately adjust for potential confounders are needed. Moreover, there is a lack of comprehensive reviews and high-quality studies on infections and other exposures that affect the human immune system through the same mechanism. Optimally, future studies on immunological risk factors should not only collect data on the exposure under investigation, but also various biosamples that allow analysis at a molecular level. In addition to an investigation of markers of an immune response, such as the presence of immunoglobulins, analyses could then be carried out at the gene level. An example of research in this area is the study on changes in specific DNA methylation patterns that have been shown to contribute to the increased risk of cancer [146]. In their examination of a possible association between lung cancer and DNA methylation patterns in a panel of candidate genes, an association between DNA methylation of *Ras association domain family 1 isoform A (RASSF1A*) and the case/control status of lung cancer was observed.

With the successful establishment of the molecular epidemiological KiKme study, it could be shown that despite an extensive collection of biospecimens including

skin biopsies and subsequent genetic examinations, the willingness of the participants to participate, and especially of the healthy controls, was very high. Based on these promising results, it remains to be seen whether the KiKme study will encourage other research groups to collect biosamples, preferably on a larger scale, as part of new studies, but also in existing study collectives. Only in this way, it is possible to understand the underlying mechanisms of carcinogenesis in the long term. Furthermore, these findings can be used to advance general therapy strategies, but also personalized therapy approaches for example in the course of screening for predisposing cancer genes. Thus, in the long term, a central goal of research should be to sustainably improve not only the prognosis of childhood cancers but also the quality of life of long-term survivors and to prevent the occurrence of late adverse health outcomes.

However, further research is needed in this area to prevent or reduce such late effects in the future. With our questionnaire analyses, we have already been able to show that survivors of childhood cancer suffer more frequently from late adverse health effects than cancer-free controls and that a previous cancer also has an influence on lifestyle. However, since data collection by questionnaire is subject to some bias, further studies using more valid methods would be useful. With regard to lifestyle, for example, physical activity could be objectively measured and investigated with regard to a relationship with cancer severity. It is conceivable here that a particularly severe cancer leads to poorer general health and thus to lower physical activity. Furthermore, such an analysis could also include a possible degree of disability, which was not recorded in the study conducted. For a valid recording of late health effects, it would also be useful to link epidemiological study data with secondary data. For this purpose, for example, claims data from statutory health insurance providers, as available in the German Pharmacoepidemiological Research Database (GePaRD) [147], could be used. Such data could be used not only to link with primary data, but also on their own to reassess the study results obtained. The use of such data offers the advantage of a large study population that can be followed up over time. Accurate coding of diagnoses in the database would allow both cancer diagnoses and late effects to be studied in a differentiated manner. In addition, the therapy-associated late effects

observed in the KiKme study could also be studied in more detail using claims data. In addition to a reproduction of the results on late effects after chemotherapy, an investigation of late effects after radiotherapy would be of particular interest, since this could not be realized due to the small number of cases in the KiKme study. Likewise, therapy dose, frequency, and medication should be investigated in a differentiated manner since a dose-response relationship between cancer therapy and late effects is assumed. Furthermore, other sources of ionizing radiation such as computed tomography or X-rays could be studied to complete the overall effect of medically used ionizing radiation on late effects of childhood cancer.

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Appendix

The appendix includes the five original publications that were included in this dissertation. All of them are already published in an international peer-reviewed journal.

The candidate's contributions to all five publications are listed in *Table 4*.

Table 4: Description of the candidates' contribution to the publications included in this dissertation.

	Publication with co-authorship		**Publications with first authorship**		
	Publication 1	**Publication 2**	**Publication 3**	**Publication 4**	**Publication 5**
First author	**Marron, M.**	**Marron, M.**	**Brackmann, L.K. & Poplawski, A.**	**Brackmann, L.K.**	**Brackmann, L.K.**
Working step					
Theoretical framework and research question	Involved	Involved	Complete	Complete	Complete
Literature research	Predominantly	Involved	Complete	Complete	Complete
Data collection	Predominantly	Involved	Involved	Involved	Involved
Data preparation	Predominantly	Complete	Involved	Complete	Complete
Data analysis	Complete	Complete	Predominantly	Complete	Complete
Discussion and interpretation	Involved	Involved	Complete	Complete	Complete
Manuscript preparation	Involved	Involved	Complete	Complete	Complete
Revision	Involved	Involved	Complete	Complete	Complete

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Publication 1

Vaccination and the Risk of Childhood Cancer - A Systematic Review and Meta-Analysis (Co-Author)

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Vaccination and the Risk of Childhood Cancer—A Systematic Review and Meta-Analysis

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Introduction: Infections may play a role in the etiology of childhood cancer and immunizations may be protective because vaccinations stimulate the immune system. Observational studies reported inconsistent associations between vaccination and risk of childhood cancer. Since a synthesis of the evidence is lacking, we conducted a meta-analysis stratified by histological and site-specific cancer.

Methods: We performed a systematic review (CRD42020148579) following PRISMA guidelines and searched for literature in MEDLINE, Embase, and the Science Citation Index databases. We identified in three literature databases 7,594 different articles of which 35 met the inclusion criteria allowing for 27 analyses of 11 cancer outcomes after exposure to nine different types of vaccinations. We calculated summary odds ratios (ORs) and 95% confidence intervals (CIs) using random effects models.

Results: We observed four inverse associations between childhood leukemia and certain vaccines as well as the number of vaccinations: OR 0.49 (95% CI = 0.32 to 0.74) for leukemia death after bacillus Calmette–Guérin vaccination; OR 0.76 (95% CI = 0.65 to 0.90) for acute lymphoblastic leukemia after Haemophilus influenzae type b vaccination; OR 0.57 (95% CI = 0.36 to 0.88) for leukemia; and OR 0.62 (95% CI = 0.46 to 0.85) for acute lymphoblastic leukemia after three or more vaccinations of any type. All other conducted analyses did not show any associations.

Discussion: The results are consistent with the hypothesis that vaccinations reduce the risk of childhood leukemia. However, the robustness and validity of these results is limited due to the small number, substantial heterogeneity, and methodological limitations of available studies.

Keywords: acute lymphoblastic leukemia, childhood leukemia, leukemia death, immunization, immune system

INTRODUCTION

Childhood cancers include a broad spectrum of histological and site-specific cancers occurring before 18 years of age (1–3). An estimated 10% of childhood cancers can be traced back to specific rare genetic syndromes with a high cancer risk (4, 5) or a common genetic susceptibility with a small increased risk for childhood cancer (6–11). The only established environmental risk factors are high doses of ionizing radiation (3) and certain chemicals such as benzene for leukemia (12) and for acute myeloid leukemia (AML) cytostatic drugs (5). However, the evidence to date does not suggest that environmental risk factors alone can explain the majority of childhood cancers. Indeed gene-environment interactions of several pre- and postnatal factors are assumed to be involved in their etiology (3, 13).

The peaks in the incidence of acute leukemia in children aged 2 to 5 years that parallel the peaks in infection rates supports the hypothesis that immunological risk factors are involved in the etiology of leukemia (14–16). However, no single infectious agent has been identified as a risk factor for the development of leukemia to date (2, 17). Instead, Kinlen (18–20) proposed the "population mixing" hypothesis with an increased risk for leukemia and non-Hodgkin lymphoma in isolated areas due to lower herd immunity to infections. In addition, Greaves (21) suggested the "delayed infection" hypothesis with a higher risk of acute lymphoblastic leukemia (ALL) in children who did not experience any strengthening of the immune system by an acquired infection in their first year of life. This theory corresponds to the current state of science according to which the immune system plays an important role in the development of cancer (22). For acute leukemia, the possible protective role of immunization is based on the assumption that vaccines also stimulate a better performance of the immune system by formation of antibodies (23). Moreover, it has been suggested that vaccination regulates the risk of childhood cancer in general by non-specific stimulation of certain macrophages and natural killer cells that target tumors (24). This non-specific effects of vaccines may be related to cross-reactivity of the adaptive immune system with unrelated pathogens and to training of the innate immune system through epigenetic reprogramming (25). However, the beneficial effect may be limited to specific vaccines e.g. live vaccines, may be reversed with other vaccines and thus may depend on the sequence of different vaccinations (25). In line with these theories, epidemiological studies investigated the relationship between various factors (26–31) that could stimulate the immune system such as vaccinations and the occurrence of leukemia (32) and other childhood cancers (3, 33).

To summarize evidence on the association between vaccination and childhood cancer, only two meta-analyses have been conducted so far. One focusing on poliomyelitis vaccines, simian virus 40 and human cancer was published in 2004 (34) and another one focusing on early vaccination and childhood leukemia was published in 2017 (32). However, to our knowledge, there is no meta-analysis on different types of vaccinations and the risk of childhood cancer in general or on histological and site-specific subtypes other than leukemia. We aimed to fill this gap and conducted a systematic literature search and meta-analysis of the association between different types of vaccination and the risk of childhood cancer including stratification by cancer sites.

Abbreviations: Adj, adjustment; AL, acute leukemia; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BCG, bacillus Calmette–Guérin (vole bacillus; tuberculosis); BCP, B-cell precursor; cALL, common acute lymphoblastic leukemia; Chi, chicken pox (varicella zoster); Cho, cholera; CI, confidence interval; CNS, central nervous system; D, diphtheria; DT, diphtheria-tetanus; DTP, diphtheria-tetanus-pertussis/whooping cough; DTPolio, diphtheria-tetanus-poliomyelitis; Exc, exclusion for meta-analysis; Hep, hepatitis; Hib, *Haemophilus influenzae* type b; Inf, influenza; IPV, inactivated poliomyelitis vaccine; HL, Hodgkin lymphoma; m, months; Mat, matching; Mea, measles; Men, meningococcus; MMR, measles-mumps-rubella; MRC, Medical Research Council; Mum, mumps; NHL, non-Hodgkin lymphoma; OR, odds ratio; P, points; Pne, pneumococcus; Polio, poliomyelitis; SE, standard error; Sma, smallpox; Typ, typhoid; y, years; Yel, yellow fever.

MATERIALS AND METHODS

Following the meta-analysis of observational studies in epidemiology (MOOSE) guidelines (35) and the preferred reporting items for systematic reviews and meta-analyses (PRISMA) (36), we conducted a comprehensive review of the literature to identify all available risk estimates on the association between vaccination and childhood cancer (PROSPERO registration: CRD42020148579).

Search Strategy

To identify studies on the association between vaccination and childhood cancer, we systematically searched the literature databases MEDLINE, Embase, and the Science Citation Index for relevant articles published before November 2020. We used subject headings and keywords in English depending on the search structure of the literature database to combine the references related to the population, the exposure, and the disease. The search was not restricted by language filters and no date limits or other filters were used. A detailed description of the search strategy is provided in **Figure 1**. Included articles were also manually searched for potentially relevant citations not detected by the electronic search.

Study Selection

Duplicates found by the three literature searches were deleted using EndNote. Two independent researchers performed the screening of titles and abstracts for relevant publications and conducted the full-text review of selected articles. Studies were considered for inclusion in the review if they met the following three criteria: They were 1) an original epidemiological study that examined the influence of vaccination on the risk of childhood cancer or cancer death; 2) a proper reference group without cancer or without the same cancer as the investigated outcome; and 3) the recommended first application of the studied vaccine should be before age 10 (e.g. exclusion of human papillomaviruses vaccination). Detailed exclusion

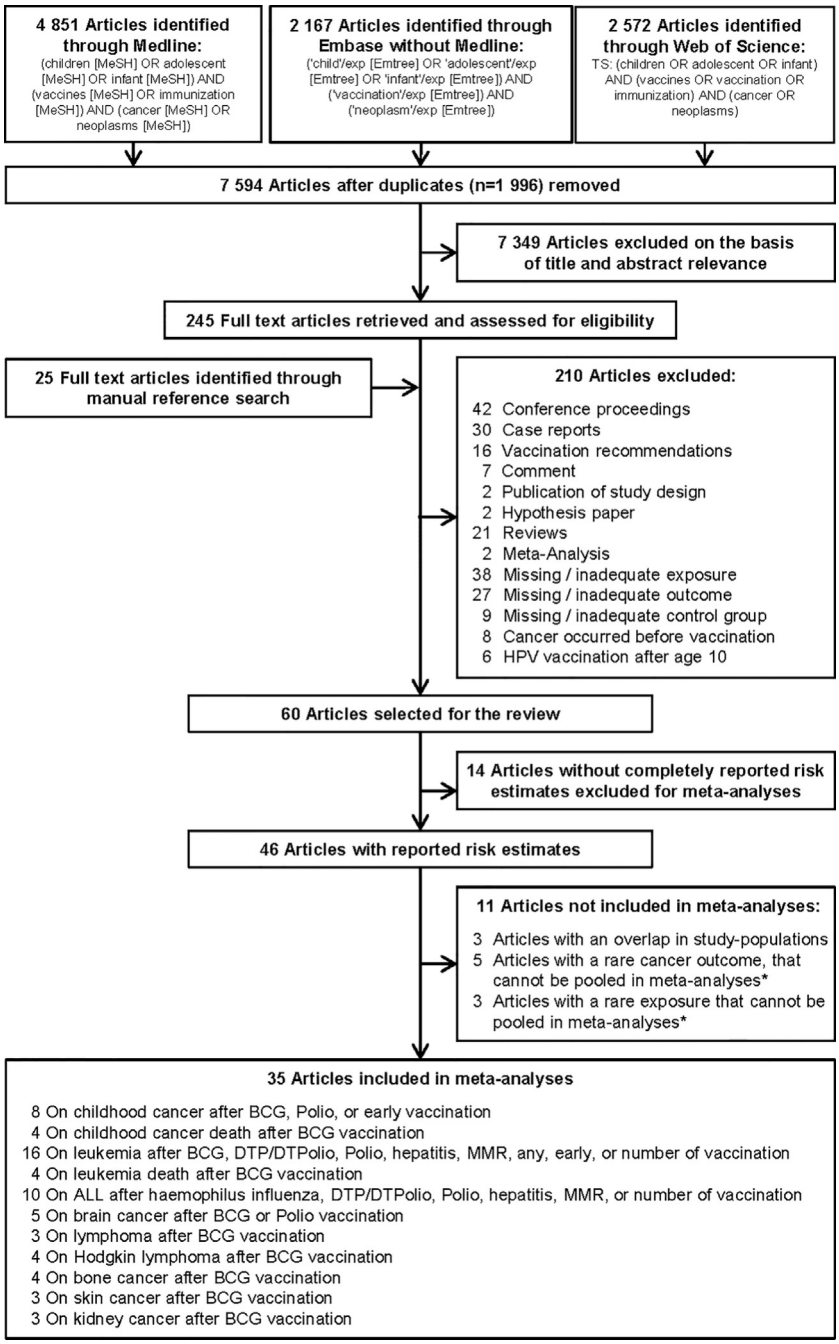

FIGURE 1 | Selection of studies included in the systematic review and meta-analysis on vaccination and risk of childhood cancer. * Studies could not be pooled in the meta-analysis due to an insufficient number of estimates (only one or two) on the same association. ALL, acute lymphoblastic leukemia; BCG, Bacillus Calmette-Guérin; DTP/DTPolio, diphtheria-tetanus-pertussis/-poliomyelitis; MMR, mumps-measles-rubella; Polio, poliomyelitis.

criteria are shown in **Figure 1**. For inclusion in the meta-analysis, studies had to report quantitative risk estimates [odds ratio (OR), relative risk (RR), or hazard ratio (HR)], and their variability [variance, standard error (SE), standard deviation (SD), or confidence interval (CI)], or provide the numbers of cases and controls so that crude risk estimates with CI could be calculated. When multiple articles reported on the same population, the most recent article or the most informative publication was included. Since most studies used non-vaccinated children as a reference, we excluded four studies that compared vaccinated

children in one time period with vaccinated children in another time period (33, 37–39). Disagreements between the two reviewers were resolved by discussion with a third reviewer.

Data Extraction

Two reviewers extracted a predetermined set of data for each risk estimate on vaccinations and childhood cancer independently from each publication (**Supplementary Tables 1** and **2A–C**). The extracted data included the following information: name of the first author, year of publication, study location, study period, study design, number of cases (cancers or cancer deaths), number, and type of subjects in the reference group (e.g. population-based or hospital-based), assessment of the outcome, cancer site, age range at diagnosis or death, exposure assessment, vaccine, age at vaccination, matching factors, adjustment variables, reason for exclusion from meta-analysis (no or incomplete risk estimates, i.e. a RR without the 95% CI, without the *P* value for measures of association and without the number of cases and controls, overlap with other study, less than three studies on an outcome or an exposure), statistical model, risk estimate [OR, RR, HR, standard incidence ratio (SIR) along with SE, SD, 95% CI, *P* value for measures of association], and number of subjects per group. A third reviewer adjudicated inconsistencies between the two original reviewers.

Quality Assessment

The quality of each study was assessed by applying the Newcastle-Ottawa Scale (NOS) (40), which has been widely used as recommended by the Cochrane Collaboration (41). The NOS comprises nine items categorized into three sections. Depending on the study design, NOS items differ. For case-control studies, the quality was assessed by case definition and representativeness, selection and definition of controls, comparability between cases and controls, ascertainment of exposure, same method of ascertainment for cases and controls, and response proportion. The cohort studies were evaluated for representativeness of the exposure cohort, selection of the non-exposed cohort, ascertainment of exposure, demonstration that outcome of interest was not present at start of study, comparability of cohorts on the basis of the design or analysis, validity of the outcome assessment, duration of follow-up long enough for outcomes to occur, and adequacy of the follow-up cohort (complete follow-up or follow-up >50%). Since the NOS is very general (42) and no other tool for quality assessment is established for observational studies (43), we applied a self-developed scoring system in addition. This score (maximum 45 points) was composed of the following eight criteria, each of which consists of 4 to 15 items: study design (up to six points), study size (up to six points), outcome assessment (up to six points), exposure assessment (up to six points), controlling for confounders (up to six points), statistical methods (up to six points), other methods (up to six points), and reported important characteristics of the study population (up to three points). At the end of the evaluation, there was the possibility to decrease the score by up to six points for selection problems, confounding, bias, and other limitations not described in the eight items before. Quality criteria are detailed in **Supplementary Tables 3A, B**. Two reviewers extracted the quality items of the included studies independently, discussed the results, and solved disagreements with a third investigator.

Data Analysis

Pooled ORs with 95% CI were calculated using random effects models if three or more studies on a specific research question were available (44). To assess the association of cancer with increasing number of vaccinations (dose-response), trend analyses were conducted based on the method of Greenland and Longnecker (45) where possible. To conduct the trend analyses required for these analyses, at least two estimates with different numbers of vaccination and the same cancer outcome had to be available. The I^2 statistic was calculated to quantify between-study heterogeneity. We considered values of 50% or less, more than 50 to 75%, and more than 75% to indicate low, moderate, and substantial heterogeneity, respectively (46). Statistical significance of I^2 was analyzed with the Q statistic [*P* value for heterogeneity (*P*)]. We explored whether heterogeneity could be reduced by omitting each study in turn from the meta-analysis (47). Potential sources of heterogeneity were investigated by conducting subgroup analyses by time period before and after contamination of the poliomyelitis vaccine by carcinogenic simian virus 40 (<1964; 1964+), study design (case-control or ecological study; cohort or case-cohort study), quality of exposure assessment (low: self-report or vaccination card; high: trial, registry, or medical documentation), quality of confounder control (low: basic or no adjustment/matching; high: adjustment or matching for other vaccines or immunological factors), consideration of a latency period (no; yes), Newcastle-Ottawa Scale below and above the fourth quintile (low: quality scale <6; high: quality scale 6+), quality score below and above the fourth quintile (low: detailed quality score <24.7; high: detailed quality score 24.7+), and assessment of outcome *via* registries (yes: *via* registries; no: other sources). For analyses with five or more included studies, publication bias was evaluated using funnel plots and the tests described by Egger et al. (48). All *P* values are two-sided. All calculations were performed using STATA version 14 (StataCorp LP, College Station, TX, USA) (49) or Excel version 2013 (Microsoft Cooperation, Redmond, WA, USA).

RESULTS

Literature Search

Of the 9,590 identified articles, 1,996 were duplicate search results from the three literature databases and 7,349 articles were excluded on the basis of title and abstract screening, leaving 245 articles for full-text evaluation (**Figure 1**). We identified additional 25 potentially relevant articles by evaluating cross-references. Finally, 210 full-text articles were discarded according to the exclusion criteria, leaving 60 studies (50–109) in the systematic review (**Supplementary Table 1**). These studies

reported 709 risk estimates for different childhood cancers after diverse types of vaccination (**Supplementary Tables 2A–C**). Overall, 85 effect estimates showed a decreased risk of different childhood cancers, 48 showed an increased risk, and 576 revealed no significant association. For the analyses, 25 of the 60 studies were excluded because (a) they reported no or incomplete risk estimates [number of studies (N) = 14], (b) the study sample overlapped with another included study (N = 3), or (c) the outcome (N = 5) or specific type of vaccination (N = 3) was reported by less than three studies. This left 35 remaining studies for inclusion in 27 specific analyses on 11 different childhood cancer outcomes after exposure to nine different types of vaccination (**Figure 1**).

Study Characteristics and Quality

Characteristics of the 35 studies included in the meta-analysis are provided in **Tables 1–4**. Studies were published between 1968 and 2019 and covered a study period of 65 years (1943 to 2008). They were conducted in Europe (57%), North America (26%), South America (8%), Australia or New Zealand (6%), and Asia (3%) with sample sizes ranging from 148 to 1,224,914 participants. Most studies examined only children under 18 years of age, with some exceptions (60, 63, 64, 66, 73, 74, 77, 79, 98, 99, 104). The distribution of selected quality-related factors is summarized in **Supplementary Tables 3A, B**. The minority of the studies included in the meta-analysis were retrospective cohort or case cohort studies (31%), the majority were case-control studies (69%), of which 18 included population-based (63, 65, 70, 74, 76, 81, 82, 92–95, 97–101, 103, 109) and four hospital-based controls (53, 83, 86, 91). There was only one study with an ecological design that used aggregated data (105). Most studies used laboratory, trial, accounting, registry, or medical documentation to assess the outcome (86%). Only three studies (62, 66, 76) used death certificates, and two studies (77, 94) did not report on this issue. To assess the type and date of vaccination, 40% of the studies used trial, accounting, registry, or medical data, and 40% used parental reports (74, 76, 81–83, 86, 91, 92, 94, 95, 97–99, 109). Further 17% used vaccination cards (63, 93, 100, 101, 103, 104) and 3% used aggregated, external data (105). The majority of studies controlled only for basic confounders (57%), mainly age and sex, while 20% did not take any confounding into account. Ten studies (29%) accounted for a latency period of at least 1 month between the vaccination and the onset of the childhood cancer and verified by this the correct temporal sequence of exposure and outcome. None of the studies reported on the inclusion of secondary cancers and 15 studies (43%) limited their cancers to incidence cases (**Supplementary Table 3B**). Overall, the methodological quality assessments of the 35 studies included in the meta-analysis yielded an average score of 4.7 out of 9.0 for the NOS and 22.0 out of 45.0 for our own detailed quality score (**Supplementary Tables 3A, B**). The quality of the 25 studies that were excluded from the meta-analysis was low (mean: 3.8 out of 9.0 points for the NOS and 13.5 out of 45.0 points for the detailed quality score, **Supplementary Tables 3A, B**). Their characteristics and main results are briefly described in **Supplementary Table 1**.

Results of the Meta-Analysis

Among 27 specific analyses on 11 different childhood cancer outcomes after exposure to nine different types of vaccinations (**Figures 2–4**), we observed four inverse associations between childhood leukemia and certain vaccines as well as after three or more vaccinations of any type.

The summary OR of leukemia death was 0.49 (95% CI 0.32 to 0.74; I^2 = 36%; N = 4; *P* value = 0.20) for bacillus Calmette–Guérin (BCG) vaccination compared to children without this vaccination (**Figure 3**). The four included studies were conducted between 1970 and 1982 and none of the studies accounted for a latency period. Three of the four studies (two cohort and one case-control study) reported a risk estimate below 1.0. Two obvious outlier studies were detected by omitting each study in turn from the meta-analysis (**Table 5B**). The observed risk reduction of the summary OR disappeared after the exclusion of Davignon et al. (54) or of Crispen et al. (66), which are two large and old cohort studies with valid exposure assessment *via* registry and medical documentation. Only the study of Neumann et al. (76) matched by age and sex, whereas the other three studies did not control for any confounders (54, 66, 79). Stratification by study period, study design, exposure assessment, and outcome assessment did not reveal any heterogeneity (I^2 = 0%; **Supplementary Figures 1A–D**).

The association between ALL and Haemophilus influenzae type b (Hib) vaccination was assessed based on five studies, the OR was 0.76 (0.65 to 0.90; I^2 = 20%; N = 5, *P* = 0.29; **Figure 4**). Four of the five studies (one cohort, two case-control, one ecological study) reported a risk estimate below 1.0 (**Figure 4**). Omitting each study in turn from the meta-analysis did not reveal an obvious outlier among the five studies and all summary ORs still showed a risk reduction after any one study was excluded (**Table 5C**). The included studies were conducted between 1999 and 2017. All studies had a good assessment of the outcome and controlled for other vaccinations or other exposures in the immunological pathway e.g. infections. The stratification by study design, exposure assessment, inclusion of a latency period, and study quality did not show any heterogeneity (I^2 = 0%; **Supplementary Figures 1B, C, E, F**).

Of two analyses focusing on the number of vaccine injections, one showed a risk reduction for leukemia (OR = 0.57; 0.36 to 0.88; N = 4; I^2 = 74%; *P* value = 0.01) and one for ALL (OR = 0.62; 0.46 to 0.85; N = 5; I^2 = 55%; *P* value = 0.06) after three or more vaccinations of any type. For both associations, all studies reported a risk estimate smaller than 1.0. Omitting each study in turn from the two analyses revealed obvious outlier studies for both associations. The heterogeneity across studies disappeared after exclusion of the German studies (92, 95) or the French study (101) and the summary OR was no longer significant after exclusion of the US study (100) (**Tables 5B, C**). The included studies covered together a study period of 29 years (1980 to 2008) for ALL and of 15 years (1990 to 2004) for leukemia. Most investigations were case-control studies (92, 93, 95, 100, 101, 109) and only one cohort study (108) was included in the meta-analysis for ALL. This Danish study was also the only study with

TABLE 1 | Characteristics of studies included in meta-analysis of the associations between vaccination and childhood cancer, 1963–1978.

First Author, Year (Ref No.)	Location	Study Years	No. of Cases	No. of Controls	Age Range	Cancer Sites	Vaccines [Early Age]	Results	Outcome	Exposure	Study Design	Comment	Study Quality[d]
Innis, 1968 (53)[a]	Australia (Sydney, Brisbane)	1958–1967	816	816	children	Cancer	D, T, P, Polio, Sma, BCG, Typ, Cho [<1 y]	↑ risk after Polio vaccination >1year; others: no association	Hospital	Record	Case-control	[Mat: age, sex]; hospital- based without cancer; update of Innis 1965	15.8; 3
Davignon, 1970 (54)[a]	Canada (Quebec)	1960–1963	96	191	<15	Leukemia	BCG	↓ leukemia mortality rates in vaccinated group	Registry	Registry	Retrospective cohort	Mortality rate; irrelevant errors in table 1 corrected by Davignon 1971	23.9; 4
MRC, 1972 (60)[a]	England	1950–1952	65	54,174	15–30	Cancer, leukemia, lymphoma	BCG	No association	Follow-Up	Trial	Retrospective cohort	Mortality rate; outcome incidence and cancer deaths; trial-based; original study of Sutherland 1982	21.4; 5
Heinonen, 1973 (62)[a]	USA	1959–1965	24	50,873	0–4	Cancer, neural tumors, leukemia	Polio, Inf [prenatal]	↑ risk after prenatal killed polio; others: no association	Record	Self-report	Cohort	[Adj: race]; prenatal vaccination	19.6; 7
Mathé, 1974 (63)[a]	France	1965	130	130	<20	Leukemia	BCG	No association	Hospital	Vaccination card	Case-control	[Mat: age]; population-based without cancer; socioeconomical status not considered	11.2; 3
Comstock, 1975 (64)[a]	Puerto Rico	1949–1951	135	77,877	1–18	Cancer, leukemia, lymphoma, HL, brain, bone, skin, kidney, ...[b]	BCG	No association	Registry	Trial	Retrospective cohort	Trial based, trial arm according to birth year; original study of Snider 1978	25.1; 5
Salonen, 1975 (65)	Finland	1959–1968	972	972	<15	Cancer, leukemia, brain, eye, kidney, bone, other	Polio, BCG	No association	Registry	Record	Case-control	Mat: age, area, birth season; population-based without cancer; original study of Salonen 1976	25.8; 6
Crispen, 1976 (66)	USA (Chicago)	1957–1969	319	619,907	<20	Cancer, leukemia	BCG [newborns]	↓ risk for cancer death in vaccinated group	Death certificate	Record	Retrospective cohort	Mortality rate; update of Rosenthal 1972	21.5; 5
Salonen, 1976 (67)[a]	Finland	1959–1968	972	972	<15	Cancer, leukemia, brain, eye, kidney, bone, other tumors	Any, BCG	No association	Registry	Record	Case-control	[Mat: age, area, birth season]; population-based without cancer; update of Salonen 1975	22.4; 5
Andersen, 1978 (70)[a]	Denmark (Copenhagen)	1943–1970	63	182	school children	HL	BCG	No association	Registry	Record	Case-control	[Mat: age, sex, socioeconomical status]; 1:3; population-based without cancer; Fisher's exact test	19.3; 3
Snider, 1978 (73)[a]	Puerto Rico	1949–1973	227	77,745	1–18	Cancer, leukemia, lymphoma, HL, brain, bone, skin, kidney, ...[c]	BCG	No association	Registry	Trial	Retrospective cohort	Trial based, trial arm according to birth year; update von Comstock, 1975	24.5; 4

Adj, Adjustment; BCG, Bacillus Calmette–Guérin (vole bacillus; tuberculosis); Cho, Cholera; D, Diphtheria; Inf, Influenza; HL, Hodgkin lymphoma; Mat, Matching; MRC, Medical Research Council; Ref No., Reference number; Sma, Smallpox; Typ, Typhoid; y, years.

[a]Calculation of crude ORs.

[b]Cancer, leukemia, lymphoma, HL, nervous system, bone, kidney, ovary, male genitalia, skin, bladder, salivary glands, mouth, esophagus, stomach, colon, liver, larynx, lungs, breast, cervix, uterus, other endocrine organs, connective tissue.

[c]Cancer, leukemia, multiple myeloma, lymphatic tissue, HL, brain, other nervous system, bone, kidney, bladder, other urinary organs, ovary, prostate, other female/male genital organs, eye, skin, other skin, breast, bronchus and lung, cervix, connective tissue, esophagus, large intestine, larynx, liver, mouth, nose, other digestive organs, other endocrine glands, pancreas, peritoneum, rectum, salivary gland, stomach, thyroid, tonsils, uterus.

[d]Study quality with detailed quality score (–6 to 45 points) and Newcastle-Ottawa Scale (0 to 9 points).

TABLE 2 | Characteristics of studies included in meta-analysis of the associations between vaccination and childhood cancer, 1979–1997.

First Author, Year (Ref No.)	Location	Study Years	No. of Cases	No. of Controls	Age Range	Cancer Sites	Vaccines [Early Age]	Results	Outcome	Exposure	Study Design	Comment	Study Quality[e]
Farwell, 1979 (74)[a]	USA (Connecticut)	1956–1962	120	240	≤19	Central nervous system, glioma, medulloblastoma	Polio [prenatal]	↑ risk for medullablastoma; others: no association	Registry	Self-report	Case-control	[Mat: age, sex, area of residence]; original study of Farwell 1984	15.8; 3
Neumann, 1980 (76)[a]	Germany	1972–1976	74	74	≤14	Cancer, leukemia	D, T, Polio, BCG, Pox	No association	Death certificate	Self-report	Case-control	Cancer death; [Mat: age, sex]; population-based; article in German	13.8; 3
Kendrick, 1981 (77)[a]	USA (Georgia, Alabama)	1950–1977	852	33,915	>5–<20cancer; >5 sub-sites	Cancer, leukemia, multiple myeloma, lymphoma, HL, bone, brain, skin, kidney, ...[d]	BCG	No association	–	Trial	Retrospective cohort	Trial-based; update of Comstock 1971	21.2; 3
Sutherland, 1982 (79)[a]	England	1950–1979	28	54,211	15–30	Leukemia	BCG	No association	Registry	Trial	Retrospective cohort	Mortality rate; trial-based; update/external validation of trial follow-up using registry data of MRC 1972	23.8; 5
Van Steensel-Moll, 1985 (81)	Netherlands	1973–1982	625	615	<15	Leukemia	Any [prenatal]	No association	Registry	Self-report	Case-control	Mat: age, sex, area; Adj: age, sex; population-based	21.8; 5
Kneale, 1986 (82)[b]	England (Oxford)	1953–1977	12,281	12,281	0–15	Cancer, leukemia, lymphoma, cerebral tumor, neuroblastoma, osteosarcoma, Wilms tumor, other solid tumors	Any [0–1 y], Sma, DT, P, Mea, Rub, Polio, BCG	↓ death risk for leukemia, Wilms tumor, neuroblastoma, cerebral tumor and other solid tumors; ↓ death risk for cancer onset age 0–1 after vaccination age 0–1, onset age 2–4 after vaccination age 0–1 and 2–4, onset age 10–15 after vaccination age 10–15 and all ages; others no association	Hospital	Self-report	Case-control	Cancer death; Mat: sex, area, birth date (birth year, season);% risk; population-based child alive; Update of Stewart 1965	19.6; 4
McKinney, 1987 (83)	England (West Midlands, North West, Yorkshire)	1980–1983	234	468	1–15	Leukemia, ML, lymphoma	Any (T, D, P, Polio, Mea, triple, Sma)	↓ risk for leukemia in general; no association for myeloid leukemia, leukemia/lymphoma, lymphoma	Registry	Self-report	Case-control	Mat: age, sex; hospital-based without cancer; original study of Hartley 1988	22.6; 4

(Continued)

TABLE 2 | Continued

First Author, Year (Ref No.)	Location	Study Years	No. of Cases	No. of Controls	Age Range	Cancer Sites	Vaccines [Early Age]	Results	Outcome	Exposure	Study Design	Comment	Study Quality[e]
Nishi, 1989 (86)	Japan (Hokkaido)	1986–1987	63	126	0–14	Non-T cell ALL	BCG, Mea [<2 y]	↓ risk	Hospital	Self-report	Case-control	Mat: age, sex, area; hospital-based	15.6; 2
Petridou, 1997 (91)[c]	Greece	1993–1994	153	300	0–14	Leukemia	DTP, BCG, viral (R, Mum, Mea, Hep)	No association	Hospital	Self-report	Case-control	Mat: age, sex, area; hospital-based without cancer	19.0; 4

Adj, Adjustment; ALL, Acute lymphoblastic leukemia; BCG, Bacillus Calmette–Guérin (vole bacillus; tuberculosis); D, Diphtheria; DT, Diphtheria-Tetanus; DTP, Diphtheria-Tetanus-PertussisWhooping cough; Hep, Hepatitis; HL, Hodgkin lymphoma; Mat, Matching; Mea, Measles; MRC, Medical Research Council; Mum, Mumps; Ref No., Reference number; Sma, Smallpox; y, years.

[a]*Calculation of crude ORs.*

[b]*Calculation of crude ORs taking individual matching into account.*

[c]*Partly calculation of crude ORs not included in meta-analysis.*

[d]*Cancer, leukemia, multiple myeloma, lymphatic tissue, HL, brain, other nervous system, bone, kidney, bladder, other urinary organs, ovary, prostate, other female/male genital organs, eye, skin, other skin, breast, bronchus and lung, cervix, connective tissue, esophagus, large intestine, larynx, liver, mouth, nose, other digestive organs, other endocrine glands, pancreas, peritoneum, rectum, salivary gland, stomach, thyroid, tonsils, uterus.*

[e]*Study quality with detailed quality score (–6 to 45 points) and Newcastle-Ottawa Scale (0 to 9 points).*

exposure assessment based on a medical registry. The stratification by study design, exposure assessment, and consideration of a latency period did not show any heterogeneity (I^2 = 0%; **Supplementary Figures 1B, C, E**). After stratification by study quality and adjustment, substantial heterogeneity was observed with a stronger risk reduction of ALL and leukemia for basic adjustment (ALL: OR = 0.48; 0.36 to 0.64; N = 2; leukemia: OR = 0.41; 0.27 to 0.61; N = 2) as compared to advanced adjustment (ALL: OR = 0.80; 0.65 to 0.99; N = 3; leukemia: OR = 0.73; 0.50 to 1.06; N = 2; **Supplementary Figure 1G**) and a significant risk reduction of ALL and leukemia for low quality below 24.7 points (ALL: OR = 0.50; 0.39 to 0.65; N = 3; leukemia: OR = 0.45; 0.33 to 0.91; N = 3) compared to a non-significant risk reduction for high quality equal or above 24.7 points (ALL: OR = 0.88; 0.66 to 1.04; N = 2; leukemia: OR = 0.83, 0.66 to 1.05; N = 1; **Supplementary Figures 1F, H**). In addition, a dose-response analysis was conducted to assess the risk of leukemia for an increasing number of vaccine injections (**Supplementary Figure 2**). The observed risk reduction was also observed in this analysis, even though not significant (OR = 0.94; 0.89 to 1.00; N = 2; I^2 = 0%; *P* value = 0.54). However, trends required for the dose-response analysis could only be calculated for the studies of Kaatsch (92) and Dockerty (93). The other two studies had to be excluded due to an insufficient number of reported estimates (100) and significant deviations between the reported estimates of the different vaccine injections (101). For the same reasons, it was not possible to conduct a dose-response analysis for number of vaccine injections and ALL.

The remaining 22 specific analyses did not show any association between different types of vaccination (any, early, BCG, poliomyelitis, hepatitis, diphtheria-tetanus-pertussis/-poliomyelitis, measles-mumps-rubella) and overall childhood cancer risk, cancer death, or site-specific cancers (lymphoma, Hodgkin lymphoma, bone cancer, brain cancer, kidney cancer, skin cancer, leukemia, ALL; **Figures 2–4**). We observed substantial heterogeneity for cancer death after BCG vaccination (OR = 0.65; 0.34 to 1.22; N = 4; I^2 = 82%; *P* value < 0.01), for cancer after poliomyelitis vaccination (OR = 1.18; 0.73 to 1.91; N = 3; I^2 = 85%; *P* value <0.01), and for lymphoma after BCG vaccination (OR = 1.55; 0.34 to 7.13; N = 3; I^2 = 77%; *P* value = 0.01). In each of these analyses, heterogeneity disappeared (I^2 = 0%) after exclusion of one specific outlier study. However, the outlier studies differ in different analyses (**Tables 5A–C**). The stratified results are shown in **Supplementary Figures 1A–H**. Overall, no evidence of publication bias was seen in analyses including five or more studies either when using the funnel plot or when using the test by Egger et al. (48) (**Supplementary Figures 3-8A–C**).

DISCUSSION

We observed an inverse association between BCG vaccination and leukemia death, between Hib vaccination and ALL, and

TABLE 3 | Characteristics of studies included in meta-analysis of the associations between vaccination and childhood cancer, 1998–2004.

First Author, Year (Ref No.)	Location	Study Years	No. of Cases	No. of Controls	Age Range	Cancer Sites	Vaccines [Early Age]	Results	Outcome	Exposure	Study Design	Comment	Study Quality[a]
Kaatsch, 1998 (92)	Germany (West Germany)	1992–1994	2358	2588	0–14	Leukemia	Number	↓ risk for leukemia for 0–3and 4–6 *versus* >6 shots; other cancer (NHL, CNS, neuro- and nephroblastoma, bone, soft-tissue sarcoma) not indicated	Registry	Self-report	Case-control	Adj: socioeconomic status, urban-rural status; Mat: age, sex, area; population-based; update Kaatsch 1996 and original study Schüz 1999 and von Kries 2000	21.6; 3
Dockerty, 1999 (93)	New Zealand	1990–1993	121	121	0–14	Leukemia	Any, number, routine, DTP, DT, BCG, Hep and other [>3 m]; MMR and Mea [>9 m]; Polio [>6 m]; R [>15 m]	↓ risk for leukemia after 1–4 different vaccinations (adj. only for age and sex); others no association	Registry	Record (parent held)	Case-control	Adj: age, sex; Mat: age, sex; latency considered; population-based	24.2; 5
Groves, 1999 (94)	USA (IL, IN, IA, MI,MN, NJ, OH, PA, WI)	1989–1993	439	439	0–14	ALL	DTP, D, T, Polio, MMR, Hib	↓ risk for ALL after Hib (conjug.); others no association	–	Record	Case-control	Adj: age, sex, race, birth year, day care attendance, parental education, family income; Mat: age, race, telephone number; population-based	18.0; 4
Schüz, 1999 (95)	Germany	1980–1994	1,010	1,010	0–14	AL, ALL	Number (D, T, P, Polio, Mum, Mea, R, Sma, Men, routine)	↑ risk for leukemia for0–3 and 4–6 *versus* >6 vaccinations	Registry	Self-report	Case-control	Adj: socioeconomic status; Mat: sex, birth year; population-based non-diseased; update Kaatsch 1996 and 1998	22.2; 4
Auvinen, 2000 (96)	Finland	1985–1987	77	113,923	0–14	Leukemia, ALL	Hib (PRP-D) [3, 4,6, and 14/18 m]	No association	Registry	Trial	Retrospective cohort	Adj: other vaccinations; Trial-based	35.4; 6
Von Kries, 2000 (97)	Germany (Lower Saxony)	1988–1993	420	613	0–15	Cancer, leukemia, tumors	BCG [newborns]	No association	Registry	Self-report	Case-control	Adj: age, sex; Mat: age, sex; population-based without cancer; power only 50%; update Kaatsch 1996 and 1998and Schüz 1999	22.2; 5
Krone, 2003 (98)	UK, Bulgaria, Italy, Germany, Estonia, Israel, Austria, France	1994–1997	603	627	0+	Malignant melanoma	BCG, Sma, Inf	↓ risk for melanoma after BCG, smallpox, or both in total and in several single countries	Hospital	Self-report (some cards)	Case-control	Adj: age, sex, race, study center, skin type, pigmented naevi, sunburns, freckling index; population-based	24.4; 6

(Continued)

TABLE 3 | Continued

First Author, Year (Ref No.)	Location	Study Years	No. of Cases	No. of Controls	Age Range	Cancer Sites	Vaccines [Early Age]	Results	Outcome	Exposure	Study Design	Comment	Study Quality[a]
Frentzel-Beyme, 2004 (99)	Austria	1978–1988	88	208	8–25	Osteo- and Ewing-sarcoma, other bone tumors	D, T, P, Polio, BCG, Chi, vaccination reaction	↓ risk after repeated pertussis vaccination in girls univariate; others no association	Registry	Self-report	Case-control	Mat: age, sex; population-based, hospital-based	21.0; 5

Adj, Adjustment; AL, Acute leukemia; ALL, Acute lymphoblastic leukemia; BCG, Bacillus Calmette–Guérin (vole bacillus; tuberculosis); Chi, Chicken pox (varicella zoster); D, Diphtheria; DT, Diphtheria- Tetanus; DTP, Diphtheria-Tetanus-Pertussis/Whooping cough; Hep, Hepatitis; Hib, Haemophilus influenzae type b; Inf, Influenza; m, months; Mat, Matching; Mea, Measles; Men, Meningococcus; MMR, Mumps-Measles-Rubella; Mum, Mumps; NHL, Non-Hodgkin lymphoma; Ref No., Reference number; Sma, Smallpox; y, years.

[a]*Study quality with detailed quality score (–6 to 45 points) and Newcastle-Ottawa Scale (0 to 9 points).*

between a high number of unspecified vaccinations and ALL or leukemia. The other 23 conducted analyses did show any associations. Despite the fact that we included a large number of publications over a long time period, the question of a possible risk reduction of childhood cancer after vaccination has not yet been finally clarified in our review and meta-analysis, since the exposure assessment of many studies has limited validity. This might be one of the reasons why published results are inconsistent. Some of these studies also have insufficient statistical power. In addition, most studies suffer from further methodological limitations, especially regarding the consideration of confounder control and latency periods. For most specific associations of interest, only few studies were available for pooling.

The risk reduction of ALL after Hib vaccination by 24% was fairly consistent across studies and by study characteristics. Only a recent Danish cohort study (108), that had the highest quality score (37.0), showed no association. However, we observed no heterogeneity after the stratification by study quality. A potential explanation for the link between Hib vaccination and ALL risk might be activation of the immune system early in life (21). ALL can frequently be traced back to a pre-leukemic clone carrying a prenatal genetic lesion (13, 110, 111). Postnatal acquired mutations then drive clonal evolution towards overt ALL. The protective role of vaccination in the development of ALL is based on the hypothesis that vaccines like Hib stimulate early formation of antibodies, prevent other infections, and modulate future responses to common childhood infections (23, 112). In line with this, mechanistic studies with mice that were repeatedly exposed to inflammatory stimuli, paralleling chronic infections in childhood, demonstrated that two enzymes, AID and RAG1-RAG2, drive clonal evolution of the most common subtype of ALL, B-cell precursor ALL (113). In addition, *in vivo* genetic studies connected inherited susceptibility to B-cell precursor ALL with postnatal infections by showing that B-cell precursor ALL was initiated in Pax5 heterozygous mice only when they were exposed to common pathogens (114). Moreover, among children in a large population-based birth cohort study, associations were observed between seven investigated serum immunoglobulin G titers and 10 exposures, either administered vaccines (e.g. BCG vaccination) or infections (115). These results indicate the existence of associations between immunogenic exposures and unrelated antibody titers, which may be responsible for non-specific effects of vaccinations on all-cause morbidity and mortality among children. Thus, early exposure to Hib vaccination may be responsible for the observed inverse association regarding ALL risk in our meta-analysis.

Our meta-analysis also showed a risk reduction for leukemia death after BCG vaccination in childhood, but not for the development of leukemia itself. The analyses on leukemia death and cancer death were limited to studies on relative risks or odds ratios of death among vaccinated and unvaccinated children. The study populations consisted of vaccination cohorts with vaccinated and unvaccinated children (60, 79), cohort

TABLE 4 | Characteristics of studies included in meta-analysis of the associations between vaccination and childhood cancer, 2005–2019.

First Author, Year (Ref No.)	Location	Study Years	No. of Cases	No. of Controls	Age Range	Cancer Sites	Vaccines [Early Age]	Results	Outcome	Exposure	Study Design	Comment	Study Quality[b]
Ma, 2005 (100)	USA (California)	1995–2002	323	409	0–14	Leukemia, ALL	DPT, Polio, MMR,Hep [<1 y], Hib	↓ risk for leukemia and ALL after Hib vaccination; others no association	Registry	Vaccination card	Case-control	Adj: birth weight, day care attendance, family income, maternal education; Mat: age, sex, mother´s race, Hispanic status; population-based	23.2; 5
Mallol-Mesnard, 2007 (101)[a]	France	2003–2004	776	1681	<15	AL, ALL, AML	Number [6m]; BCG [newborns]; D, T, P, Hep, Hib, Pne, Men and Polio [<6 m]; Mum, Mea & R [1y]	↑ risk of AML after 1–2 vaccinations <6 months compared to ≥4 vaccinations; others no association	Registry	Vaccination card	Case-control	Adj: age, sex, birth order, maternal and paternal educational level, degree of urbanization; Mat: age, sex; population-based	27.8; 6
MacArthur, 2008 (103)	Canada	1990–1994	399	399	0–14	Leukemia, ALL	D, T, P, Polio, Mum, Mea, R, BCG, Hep, other	No association	Registry	Vaccination card	Case-control	Adj: race, family income, maternal education & age at birth, number of residences since birth; Mat: age, sex area; population-based	26.6; 5
Villumsen, 2009 (104)	Denmark	1965–1976	71	2,073	5–35	Lymphoma, NHL, HL, leukemia	BCG, Sma	↓ lymphoma risk after BCG; others: no association	Registry	Vaccination card	Retrospective case-cohort	Adj: day care, family social class; register-based; Sub-cohort; update of Danish data in Waaler 1970	27.6; 8
Pagaoa, 2011 (105)	USA (Texas)	1995–2006	2800	11,200	2–17	Cancer, ALL, NHL, medullablastoma	DTP, Polio, MMR,Chi, Hep, Hib, combination	↓ risk for all cancers and ALL after Hib and for ALL after combined vaccination by region; ↓ risk for all cancers and ALL after Hep and for ALL after IPV, Hep and combined vaccination, ↑ risk formedullablastoma after Hiband NHL after MMR by country	Registry	Registry	Ecological	Adj: age, sex, race, birth weight, birth year, birth type, birth order, premature birth, maternal education, maternal marital status, prior births, diabetes, preterm labor, tobacco use, and alcohol use, mother age at birth; Mat: sex, birth year; 1:4; population- based without cancer	13.7; 5

(Continued)

TABLE 4 | Continued

First Author, Year (Ref No.)	Location	Study Years	No. of Cases	No. of Controls	Age Range	Cancer Sites	Vaccines [Early Age]	Results	Outcome	Exposure	Study Design	Comment	Study Quality[b]
Soegaard, 2017 (108)	Denmark	1981–2008	490	1,224,914	0–14	ALL	DTPolio [5, 6,16 m], P (<3,10 m], MMR, Hib [3–16 m], routine vaccination	No association	Registry	Registry	Retrospective cohort	Adj; sex, race, birth weight, year, order and mode, other vaccination, gestational age; down syndrome excluded; latency considered; register-based; hazard ratio	37.0; 8
Figueroa, 2019 (109)	Costa Rica	1995–2003	240	578	1–15	ALL	Routine vaccination	↓ risk for ALL	Registry	Self-report	Case-control	Adj; sex, birth year, socioeconomic status	21.6; 6

Adj, Adjustment; AL, Acute leukemia; ALL, Acute lymphoblastic leukemia; AML, Acute myeloid leukemia; BCG, Bacillus Calmette–Guérin (vole bacillus; tuberculosis); Chi, Chicken pox (varicella zoster); D, Diphtheria; DTP, Diphtheria-Tetanus-Pertussis/Whooping cough; DTPolio, Diphtheria-Tetanus-Poliomyelitis; Hep, Hepatitis; Hib, Haemophilus influenzae type b; HL, Hodgkin lymphoma; m, months; Mat, Matching; Mea, Measles; Men, Meningococcus; MMR, Mumps-Measles-Rubella; Mum, Mumps; NHL, Non-Hodgkin lymphoma; Pne, Pneumococcus; Ref No., Reference number; Sma, Smallpox.

[a] *Partly calculation of crude ORs not included in meta-analysis.*

[b] *Study quality with detailed quality score (–6 to 45 points) and Newcastle-Ottawa Scale (0 to 9 points).*

studies calculating cancer mortality within the vaccinated and unvaccinated population (54, 66), or case-control studies with vaccinated and unvaccinated cancer deaths and healthy controls (76, 82). The observed risk reduction for leukemia death after BCG vaccination was mainly driven by two cohort studies (54, 66) with valid exposure and outcome assessment but without control for any confounders. We did not observe any heterogeneity between the four included studies in the stratified results.

In addition, studies comparing a high *versus* a low number of unspecified vaccinations observed an inverse association with ALL (95, 100, 101, 108, 109) and leukemia (92, 93, 100, 101). While this design of unspecified vaccinations mitigates some sources of confounding and bias, we noticed moderate heterogeneity across the study estimates of number of vaccinations. This heterogeneity disappeared after the exclusion of two large German case-control studies where both exposure assessment (interview of parents) and control of confounders (birth year, sex, socioeconomic status, and area) were suboptimal (92, 95). Assessment of the number of vaccinations was based on objective records in other studies but there were still differences (medical records, vaccination cards, medical claims data) that may affect the degree of misclassification (116). Claims data are assumed to have the highest validity regarding information on vaccination and were used in a large Danish cohort study (108). This high-quality study, which also carefully adjusted for confounders and considered latency periods, did not observed an association between number of vaccinations and ALL. In contrast, most other studies analyzing number of vaccinations (92, 95, 100, 101) and all studies that examined BCG vaccination (54, 66, 76, 79) did not take into account a latency period. However, this would result in non-differential misclassification and bias toward the null.

We did not find any other significant risk reduction of childhood cancer other than leukemia in our meta-analysis. With respect to poliomyelitis vaccination, the results of studies included in our meta-analysis were not consistent. Between 1955 and 1963, some poliomyelitis vaccines have been contaminated by simian virus 40 (34) that has the potential to initiate malignancy in various target tissues. This may explain the increased risk of childhood cancer for poliomyelitis vaccinations before 1963 with good exposure assessment. However in our stratified analyses, the increased risk of cancer after poliomyelitis immunization was only observed without consideration of a latency period, insufficient confounder control, low assessment of outcome, and low overall study quality. Moreover, there were also methodological limitations in the recent study that used an ecological design and did not show a correlation between childhood cancer and poliomyelitis vaccination (105). In such a study, an ecological bias may be introduced since only aggregated data are available. In addition, we observed substantial heterogeneity for the analysis of lymphoma after BCG vaccination. The recent Danish case-cohort study from Villumsen et al. (104) with very reliable vaccination information on an individual level,

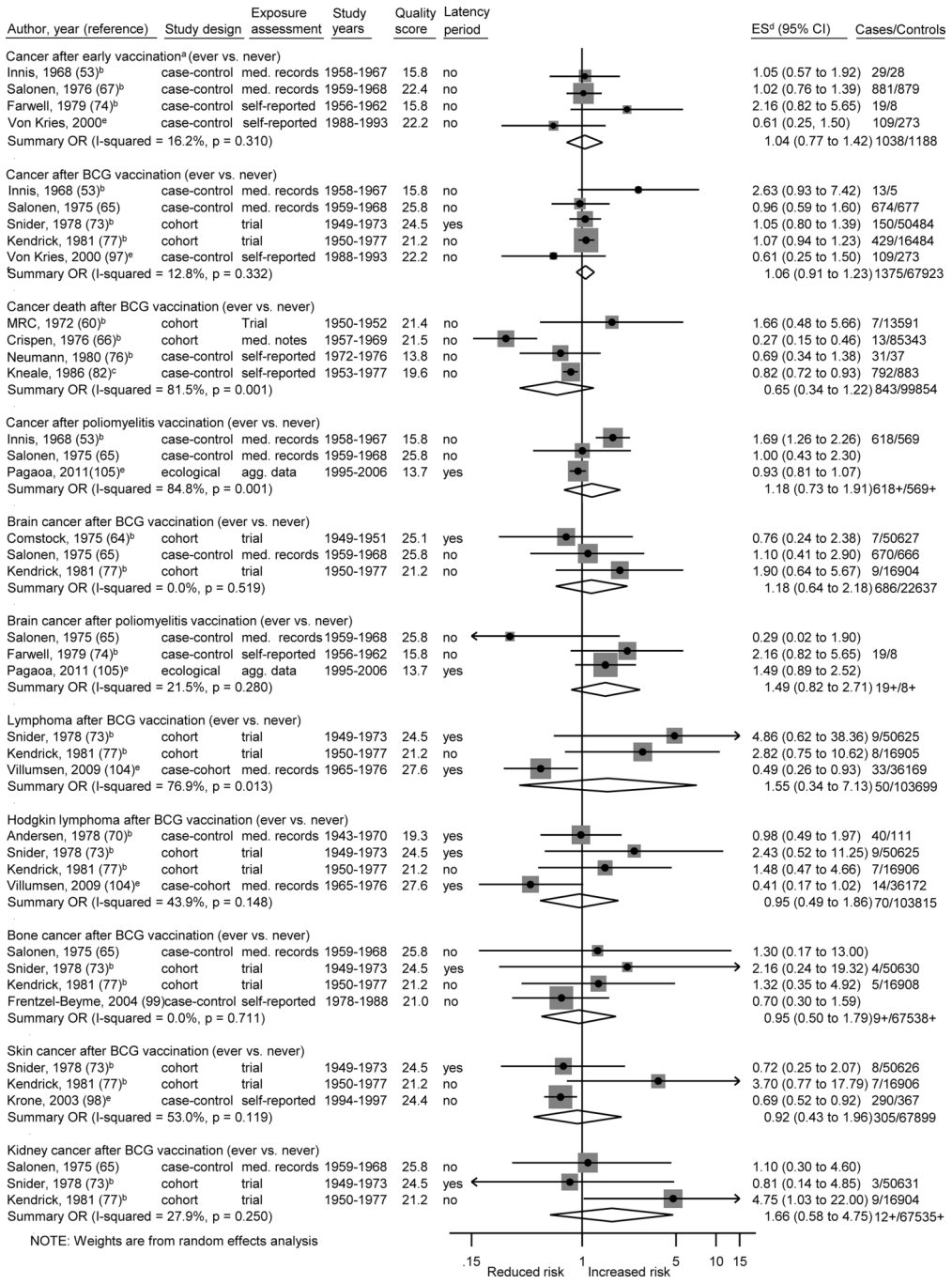

FIGURE 2 | Vaccination and the risk of cancer. agg. data, aggregated data; BCG, Bacillus Calmette-Guérin; med. records, medical records; OR, odds ratio; ES, estimate. (a) Early vaccinations: Innis (poliomyelitis vaccination, age <1), Salonen (any vaccination, perinatal), Farwell (poliomyelitis vaccination, prenatal), von Kries (BCG vaccination, newborns). (b) Calculation of crude ORs. (c) Calculation of crude ORs taking individual matching into account. (d) ES includes single-study odds ratios or hazard ratios and summary odds ratios. (e) Adjusted estimate as indicated by published study.

consideration of a latency period, and good confounder control indicated a beneficial effect of BCG vaccination on the risk of lymphomas. However, the other two old cohort studies in this analysis (73, 77), each with less than 10 cases and low overall quality, did not support this result.

In contrast to the conducted meta-analysis by Morra et al. (32), we did not observed an inverse association between leukemia and early vaccination before the age of 1 year in our synthesis. These different meta-analysis results can be explained by our additional inclusion of the study from Ma et al. (100) from 2005, which did

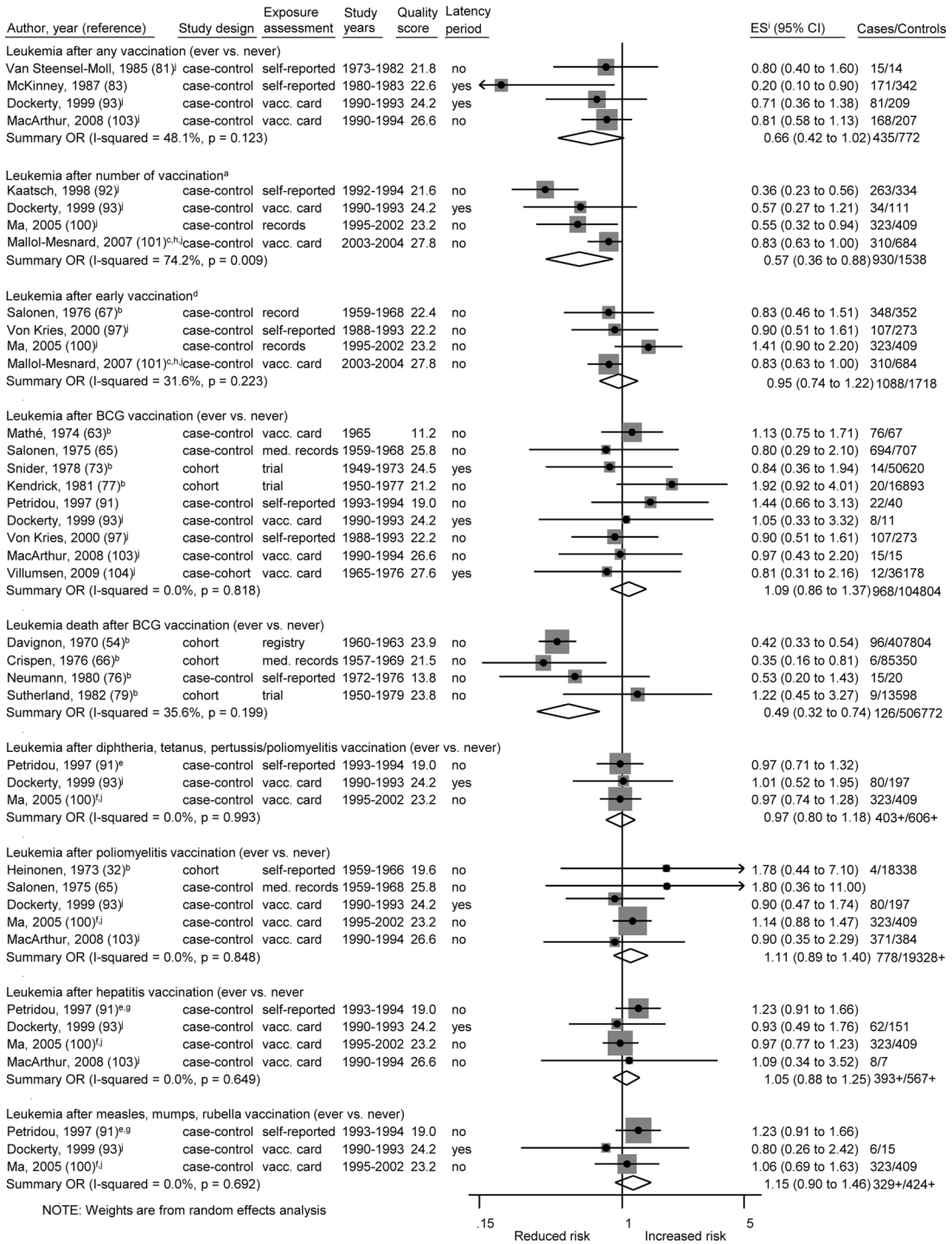

FIGURE 3 | Vaccination and the risk of leukemia. BCG, Bacillus Calmette-Guérin; med. records, medical records; OR, odds ratio; ES, estimate. (a) Number of vaccinations: Kaatsch (any >6 *vs.* 0–3), Dockerty (any 3–4 *vs.* 0), Ma (Haemophilus influenzae type b 3+ *vs.* 1–2), Mallol-Messnard (any >3 *vs.* 3). (b) Calculation of crude ORs. (c) Estimates for acute leukemia. (d) Early vaccinations: Salonen (any ever *vs.* never, perinatal), Ma (hepatitis 3+ *vs.* 1–2, age <1), Mallol-Mesnard (any >3 *vs.* 3, age <0.5) von Kries (BCG vaccination, newborns). (e) Increment by ~3 doses. (f) Each additional dose. (g) Estimate for combination of measles, mumps, rubella, and hepatitis vaccination. (h) Inverted reference category. (i) ES includes single-study odds ratios or hazard ratios and summary odds ratios. (j) Adjusted estimate as indicated by published study.

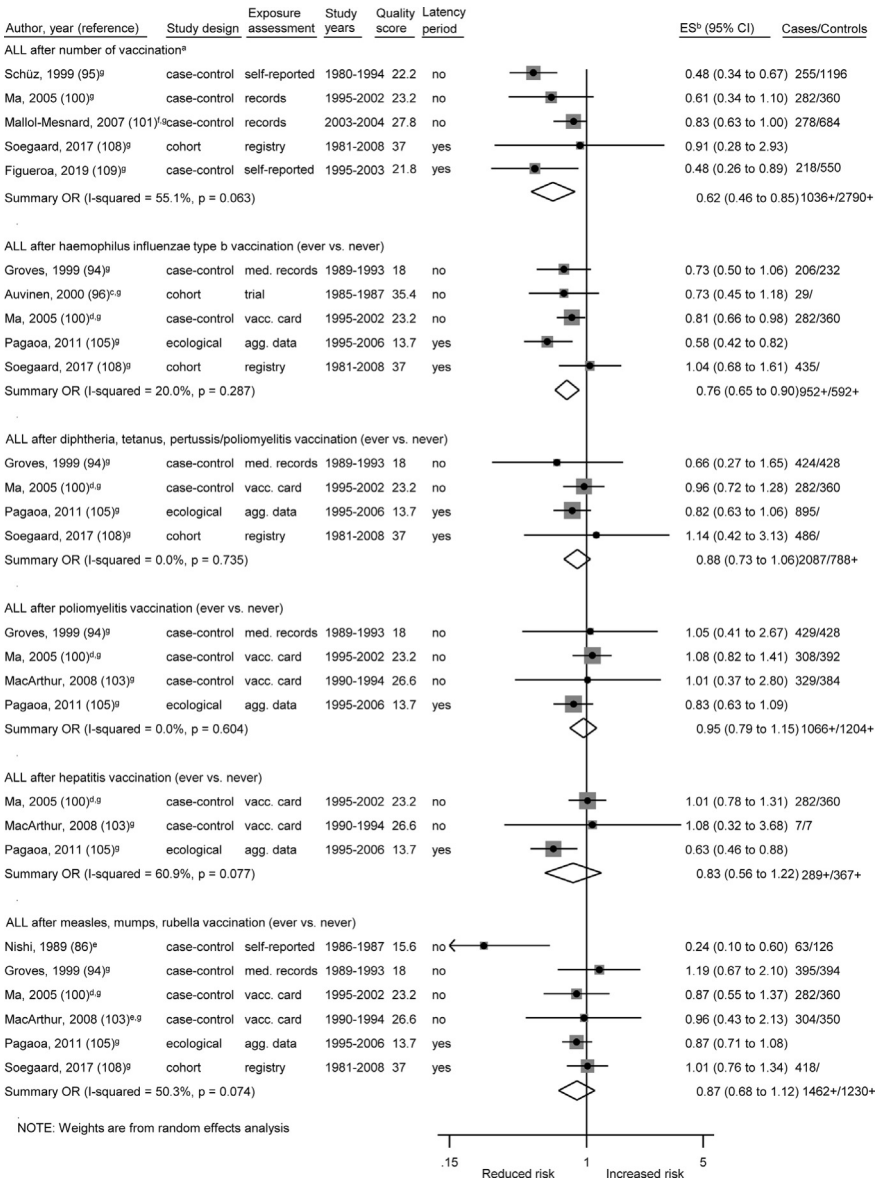

FIGURE 4 | Vaccination and the risk of acute lymphoblastic leukemia (ALL). agg. data, aggregated data; ALL, acute lymphoblastic leukemia; med. records, medical records; OR, odds ratio; ES, estimate. (a) Number of vaccinations: Schüz (any >6 *vs.* 0–3), Ma (Haemophilus influenzae type b 3+ *vs.* 1–2), Mallol-Mesnard (any >3 *vs.* 3), Soegaard (complete *vs.* no/incomplete routine vaccination), Figueroa (complete *vs.* no/incomplete routine vaccination). (b) Calculation of crude ORs. (c) Early *vs.* late vaccination. (d) Each additional dose. (e) Estimate for measles. (f) Inverted reference category. (g) ES includes single-study odds ratios or hazard ratios and summary odds ratios. (h) Adjusted estimate as indicated by published study.

not show any association between early vaccination and leukemia with careful adjustment. In addition contrary to our analysis, the meta-analysis of Morra et al. (32) included two old and large studies (54, 66) on leukemia death after early BCG vaccination, which found a strong risk reduction without control for any confounders. We preferred to analyze these studies on BCG vaccination and early immunization based on the different outcomes, leukemia death, and leukemia incidence, separately.

Our meta-analysis has specific strengths including the extensive search strategy we used to ensure that all relevant publications on this topic were identified. This enabled us to conduct separate analyses on histological and site-specific childhood cancers as well as on certain vaccines, age at vaccination, and number of vaccinations. To consider the overall study quality, we used the established NOS as well as our own more detailed quality assessment scale. The latter additionally considered important issues such as latency periods, quality of statistical methods, training of interviewers, exposure assessment in cohort studies, and cancer among controls of case-control studies. There was no conclusive evidence of publication bias, but the power of the test is poor in a meta-analysis with only a few

TABLE 5 | Exclusion of single studies A) for **Figure 2**, B) for **Figure 3**, and C) for **Figure 4**.

Model description	OR (95% CI)	*I*-squared	*P* value
A) OMITTED STUDY Figure 2			
Cancer after early vaccination[a] (ever *vs.* never)			
Innis, 1968 (53)[b]	1.06 (0.63 to 1.79)	44.1%	0.167
Salonen, 1976 (67)[b]	1.09 (0.58 to 2.02)	43.7%	0.169
Farwell, 1979 (74)[b]	0.98 (0.76 to 1.27)	0.0%	0.551
Von Kries, 2000 (97)	1.09 (0.82 to 1.45)	6.0%	0.345
Cancer after BCG vaccination (ever *vs.* never)			
Innis, 1968 (53)[b]	1.05 (0.93 to 1.18)	0.0%	0.657
Salonen, 1975 (65)	1.07 (0.87 to 1.32)	32.1%	0.220
Snider, 1978 (73)[b]	1.06 (0.78 to 1.43)	34.5%	0.205
Kendrick, 1981 (77)[b]	1.05 (0.74 to 1.47)	34.0%	0.209
Von Kries, 2000 (97)	1.07 (0.95 to 1.22)	2.9%	0.378
Cancer death after BCG vaccination (ever *vs.* never)			
MRC, 1972 (60)[b]	0.55 (0.27 to 1.11)	86.4%	0.001
Crispen, 1976 (66)[b]	0.82 (0.72 to 0.93)	0.0%	0.472
Neumann, 1980 (76)[b]	0.65 (0.27 to 1.58)	87.6%	0.000
Kneale, 1986 (82)[c]	0.60 (0.23 to 1.56)	77.8%	0.011
Cancer after poliomyelitis vaccination (ever *vs.* never)			
Innis, 1968 (53)[b]	0.93 (0.81 to 1.07)	0.0%	0.867
Salonen, 1975 (65)	1.24 (0.69 to 2.22)	92.4%	0.000
Pagaoa, 2011 (105)	1.52 (1.00 to 2.30)	25.5%	0.247
Brain cancer after BCG vaccination (ever *vs.* never)			
Comstock, 1975 (64)[b]	1.40 (0.68 to 2.91)	0.0%	0.465
Salonen, 1975 (65)	1.22 (0.50 to 3.00)	22.1%	0.257
Kendrick, 1981 (77)[b]	0.94 (0.45 to 1.98)	0.0%	0.631
Brain cancer after poliomyelitis vaccination (ever *vs.* never)			
Salonen, 1975 (65)	1.62 (1.02 to 2.56)	0.0%	0.507
Farwell, 1979 (74)[b]	0.97 (0.24 to 3.98)	47.0%	0.170
Pagaoa, 2011 (105)	1.04 (0.16 to 6.92)	60.5%	0.112
Lymphoma after BCG vaccination (ever *vs.* never)			
Snider, 1978 (73)[b]	1.06 (0.19 to 5.84)	81.6%	0.020
Kendrick, 1981 (77)[b]	1.24 (0.14 to 11.27)	76.9%	0.038
Villumsen, 2009 (104)	3.31 (1.08 to 10.10)	0.0%	0.664
Hodgkin lymphoma after BCG vaccination (ever *vs.* never)			
Andersen, 1978 (70)[b]	1.01 (0.34 to 3.01)	61.9%	0.073
Snider, 1978 (73)[b]	0.81 (0.41 to 1.62)	43.9%	0.168
Kendrick, 1981 (77)[b]	0.86 (0.37 to 2.00)	55.4%	0.106
Villumsen, 2009 (104)	1.22 (0.70 to 2.12)	0.0%	0.531
Bone cancer after BCG vaccination (ever *vs.* never)			
Salonen, 1975 (65)	0.92 (0.47 to 1.79)	0.0%	0.526
Snider, 1978 (73)[b]	0.88 (0.45 to 1.71)	0.0%	0.677
Kendrick, 1981 (77)[b]	0.85 (0.41 to 1.77)	0.0%	0.591
Frentzel-Beyme, 2004 (99)	1.46 (0.54 to 3.96)	0.0%	0.925
Skin cancer after BCG vaccination (ever *vs.* never)			
Snider, 1978 (73)[b]	1.33 (0.27 to 6.62)	76.5%	0.039
Kendrick, 1981 (77)[b]	0.69 (0.53 to 0.91)	0.0%	0.939
Krone, 2003 (98)	1.47 (0.30 to 7.19)	65.2%	0.090
Kidney cancer after BCG vaccination (ever *vs.* never)			
Salonen, 1975 (65)	2.09 (0.37 to 11.77)	53.8%	0.141
Snider, 1978 (73)[b]	2.19 (0.52 to 9.16)	48.8%	0.162
Kendrick, 1981 (77)[b]	0.98 (0.33 to 2.91)	0.0%	0.790
B) OMITTED STUDY Figure 3			
Leukemia after any vaccination (ever *vs.* never)			
Steensel-Moll, 1985 (81)	0.58 (0.31 to 1.10)	64.9%	0.058
McKinney, 1987 (83)	0.79 (0.60 to 1.04)	0.0%	0.942
Dockerty, 1999 (93)	0.60 (0.31 to 1.15)	65.4%	0.056
MacArthur, 2008 (103)	0.55 (0.27 to 1.11)	57.8%	0.093
Leukemia after number of vaccination[d]			
Kaatsch, 1998 (92)[e]	0.73 (0.55 to 0.95)	20.3%	0.285
Dockerty, 1999 (93)	0.56 (0.32 to 0.97)	82.7%	0.003
Ma, 2005 (100)	0.56 (0.31 to 1.03)	81.9%	0.004
Mallol-Mesnard, 2007 (101)[e,f]	0.45 (0.33 to 0.61)	0.0%	0.382
Leukemia after BCG vaccination (ever *vs.* never)			
Mathé, 1974 (63)[b]	1.07 (0.80 to 1.42)	0.0%	0.738
Salonen, 1975 (65)	1.11 (0.87 to 1.41)	0.0%	0.777
Snider, 1978 (73)[b]	1.11 (0.87 to 1.42)	0.0%	0.778
Kendrick, 1981 (77)[b]	1.02 (0.80 to 1.30)	0.0%	0.968
Petridou, 1997 (91)	1.06 (0.83 to 1.35)	0.0%	0.796
Dockerty, 1999 (93)	1.09 (0.86 to 1.38)	0.0%	0.732
Von Kries, 2000 (97)	1.13 (0.87 to 1.46)	0.0%	0.789
MacArthur, 2008 (103)	1.10 (0.86 to 1.40)	0.0%	0.741
Villumsen, 2009 (104)	1.11 (0.87 to 1.41)	0.0%	0.776
Leukemia death after BCG vaccination (ever *vs.* never)			
Davignon, 1970 (54)[b]	0.59 (0.28 to 1.22)	45.2%	0.161
Crispen, 1976 (66)[b]	0.57 (0.31 to 1.04)	53.7%	0.115
Neumann, 1980 (76)[b]	0.50 (0.28 to 0.89)	55.8%	0.104
Sutherland, 1982 (79)[b]	0.42 (0.33 to 0.53)	0.0%	0.820
Leukemia after diphtheria, tetanus, pertussis/poliomyelitis vaccination (ever *vs.* never)			
Petridou, 1997 (91)[h]	0.98 (0.76 to 1.26)	0.0%	0.912
Dockerty, 1999 (93)	0.97 (0.79 to 1.19)	0.0%	0.909
Ma, 2005 (100)[i]	0.98 (0.74 to 1.29)	0.0%	0.914
Leukemia after poliomyelitis vaccination (ever *vs.* never)			
Heinonen, 1973 (62)[b]	1.10 (0.88 to 1.38)	0.0%	0.818
Salonen, 1975 (65)	1.11 (0.88 to 1.39)	0.0%	0.784
Dockerty, 1999 (93)	1.15 (0.90 to 1.46)	0.0%	0.823
Ma, 2005 (100)[i]	1.03 (0.64 to 1.67)	0.0%	0.742
MacArthur, 2008 (103)	1.13 (0.89 to 1.43)	0.0%	0.762
Leukemia after hepatitis vaccination (ever *vs.* never)			
Petridou, 1997 (91)[h,j]	0.97 (0.78 to 1.20)	0.0%	0.973
Dockerty, 1999 (93)	1.06 (0.88 to 1.27)	0.0%	0.474
Ma, 2005 (100)[i]	1.17 (0.89 to 1.52)	0.0%	0.735
MacArthur, 2008 (103)	1.05 (0.88 to 1.25)	0.0%	0.440
Leukemia after mumps, measles, rubella vaccination (ever *vs.* never)			
Petridou, 1997 (91)[h,j]	1.02 (0.68 to 1.53)	0.0%	0.645
Dockerty, 1999 (93)	1.17 (0.92 to 1.50)	0.0%	0.578
Ma, 2005 (100)[i]	1.19 (0.89 to 1.60)	0.0%	0.465
C) OMITTED STUDY Figure 4			
ALL after number of vaccination[k]			
Schüz, 1999 (95)[e]	0.74 (0.58 to 0.94)	11.3%	0.336
Ma, 2005 (100)	0.67 (0.42 to 1.07)	73.8%	0.022
Mallol-Mesnard, 2007 (101)[e]	0.52 (0.40 to 0.66)	0.0%	0.685
Soegaard, 2017 (108)	0.61 (0.43 to 0.85)	65.8%	0.033
Figueroa, 2019 (109)	0.65 (0.46 to 0.94)	61.6%	0.050
ALL after Haemophilus influenzae type b vaccination (ever *vs.* never)			
Groves, 1999 (94)	0.77 (0.62 to 0.95)	39.1%	0.178
Auvinen, 2000 (96)[l]	0.76 (0.62 to 0.94)	39.5%	0.175
Ma, 2005 (100)[i]	0.74 (0.58 to 0.94)	31.9%	0.221
Pagaoa, 2011 (105)	0.81 (0.70 to 0.95)	0.0%	0.623
Soegaard, 2017 (108)	0.74 (0.64 to 0.86)	0.0%	0.416
ALL after diphtheria, tetanus, pertussis/poliomyelitis vaccination (ever *vs.* never)			
Groves, 1999 (94)	0.89 (0.74 to 1.07)	0.0%	0.644
Ma, 2005 (100)	0.82 (0.65 to 1.05)	0.0%	0.728
Pagaoa, 2011 (105)	0.94 (0.72 to 1.23)	0.0%	0.688
Soegaard, 2017 (108)	0.87 (0.72 to 1.05)	0.0%	0.605
ALL after poliomyelitis vaccination (ever *vs.* never)			
Groves, 1999 (94)	0.95 (0.79 to 1.15)	0.0%	0.405
Ma, 2005 (100)[i]	0.86 (0.66 to 1.10)	0.0%	0.846
MacArthur, 2008 (103)	0.95 (0.79 to 1.15)	0.0%	0.399
Pagaoa, 2011 (105)	1.07 (0.83 to 1.38)	0.0%	0.991
ALL after hepatitis vaccination (ever *vs.* never)			
Ma, 2005 (100)[i]	0.65 (0.48 to 0.89)	0.0%	0.403
MacArthur, 2008 (103)	0.81 (0.51 to 1.28)	79.9%	0.026
Pagaoa, 2011 (105)	1.01 (0.79 to 1.31)	0.0%	0.916

(Continued)

TABLE 5 | Continued

Model description	OR (95% CI)	*I*-squared	*P* value	Model description	OR (95% CI)	*I*-squared	*P* value
Leukemia after early vaccination[g]				**ALL after mumps, measles, rubella vaccination (ever *vs.* never)**			
Salonen, 1976 (67)[b]	0.99 (0.71 to 1.39)	53.2%	0.118	Nishi, 1989 (86)	0.93 (0.80 to 1.08)	0.0%	0.824
Von Kries, 2000 (97)	0.97 (0.69 to 1.38)	54.4%	0.112	Groves, 1999 (94)	0.83 (0.63 to 1.10)	55.8%	0.060
Ma, 2005 (100)	0.84 (0.69 to 1.03)	0.0%	0.967	Ma, 2005 (100)[i]	0.86 (0.63 to 1.18)	60.2%	0.040
Mallol-Mesnard, 2007 (101)[e,f]	1.07 (0.76 to 1.51)	20.2%	0.285	MacArthur, 2008 (103)	0.86 (0.65 to 1.14)	60.1%	0.040
				Pagaoa, 2011 (105)	0.84 (0.58 to 1.23)	59.6%	0.042
				Soegaard, 2017 (108)	0.81 (0.58 to 1.15)	56.2%	0.058

ALL, Acute lymphoblastic leukemia; BCG, Bacillus Calmette–Guérin (vole bacillus, tuberculosis); CI, confidence interval; OR, odds ratio.

[a] *Early vaccinations: Innis (poliomyelitis vaccination ever vs. never, age <1), Salonen (any vaccination, newborns), Farwell (poliomyelitis vaccination ever vs. never, prenatal).*

[b] *Calculation of crude ORs.*

[c] *Calculation of crude ORs taking individual matching into account.*

[d] *Number of vaccinations: Kaatsch (any >6 vs. 0–3), Dockerty (any 5+ vs. 0), Ma (Haemophilus influenzae type b 3+ vs. 1–2), Mallol-Messnard (any >3 vs. 3).*

[e] *Inverted reference category.*

[f] *Estimates for Acute Leukemia.*

[g] *Early vaccinations: Salonen (any ever vs. never, perinatal), Ma (hepatitis 3+ vs. 1–2, age <1), Mallol-Mesnard (any >3 vs. 3, age <0.5).*

[h] *Increment by ~3 doses.*

[i] *Each additional dose.*

[j] *Estimate for combination of hepatitis and MMR vaccine.*

[k] *Number of vaccinations: Schüz (any >6 vs. 0–3), Ma (Haemophilus influenzae type b 3+ vs. 1–2), Mallol-Mesnard (any >3 vs. 3), Soegaard (complete vs. no/incomplete routine*

[l] *Early vs. late vaccination.*

included studies. However, a graphical examination of the plots also did not suggest a publication bias.

The small number of studies for each exposure-disease association and the relatively high level of heterogeneity across studies in some of our analyses is the main limitation of this meta-analysis. When the number of studies and the true fraction of heterogeneity is small, there appears a substantial positive bias for I^2 but this bias is typically negative when the true fraction of heterogeneity is large and the number of studies is small (117). To account for potential heterogeneity we used random effects models and to assess its effect on our results, we stratified our analyses by study characteristics.

In conclusion, we found evidence of an inverse association between BCG vaccination and leukemia death, Hib vaccination and ALL, and a high number of unspecified vaccinations and ALL as well as leukemia. However, these results should be interpreted with caution given the small number of studies, no consideration of latency, and limited exposure assessment in some studies as well as insufficient confounder adjustment, in particular for infections. All studies included in this review and meta-analysis had at least one of these substantial limitations. Finally, although risk reductions after vaccination appear biologically plausible in leukemia, studies on dose effect and on age at vaccination with good exposure assessment and advanced confounder controlling are rare. Large cohort studies with valid assessment of immunizations, adequate consideration of the latency period, and detailed information on possible confounders (e.g. infections and other vaccines) are needed to assess the association between different types of vaccinations and specific childhood cancers.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

MM designed the meta-analysis, the analytic strategies and the detailed quality score, and directed its implementation, including quality assurance and control. She was in charge of creating the manuscript and conducting the literature review and the meta-analysis. LB and PK assisted in conducting the literature review and meta-analysis including acquisition of data, quality assessment, and preparation of figures and tables. LC assisted conducting the literature review including database searches, selections, and assessment of publications. IL, UH, and WA contributed to the analyses and interpretation of the results. WA assumes an overall scientific responsibility during the creation of the publication and thus assumes a particular responsibility for the quality and integrity of the publication. All authors revised the manuscript critically for important intellectual content and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.610843/full#supplementary-material

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Publication 2

Identification of Genetic Predispositions Related to Ionizing Radiation in Primary Human Skin Fibroblasts From Survivors of Childhood and Second Primary Cancer as Well as Cancer-Free Controls: Protocol for the Nested Case-Control Study KiKme (Co-Author)

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Protocol

Identification of Genetic Predispositions Related to Ionizing Radiation in Primary Human Skin Fibroblasts From Survivors of Childhood and Second Primary Cancer as Well as Cancer-Free Controls: Protocol for the Nested Case-Control Study KiKme

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Abstract

Background: Therapy for a first primary neoplasm (FPN) in childhood with high doses of ionizing radiation is an established risk factor for second primary neoplasms (SPN). An association between exposure to low doses and childhood cancer is also suggested; however, results are inconsistent. As only subgroups of children with FPNs develop SPNs, an interaction between radiation, genetic, and other risk factors is presumed to influence cancer development.

Objective: Therefore, the population-based, nested case-control study KiKme aims to identify differences in genetic predisposition and radiation response between childhood cancer survivors with and without SPNs as well as cancer-free controls.

Methods: We conducted a population-based, nested case-control study KiKme. Besides questionnaire information, skin biopsies and saliva samples are available. By measuring individual reactions to different exposures to radiation (eg, 0.05 and 2 Gray) in normal somatic cells of the same person, our design enables us to create several exposure scenarios for the same person simultaneously and measure several different molecular markers (eg, DNA, messenger RNA, long noncoding RNA, copy number variation).

Results: Since 2013, 101 of 247 invited SPN patients, 340 of 1729 invited FPN patients, and 150 of 246 invited cancer-free controls were recruited and matched by age and sex. Childhood cancer patients were additionally matched by tumor morphology, year of diagnosis, and age at diagnosis. Participants reported on lifestyle, socioeconomical, and anthropometric factors, as well as on medical radiation history, health, and family history of diseases (n=556). Primary human fibroblasts from skin biopsies of

the participants were cultivated (n=499) and cryopreserved (n=3886). DNA was extracted from fibroblasts (n=488) and saliva (n=510).

Conclusions: This molecular-epidemiological study is the first to combine observational epidemiological research with standardized experimental components in primary human skin fibroblasts to identify genetic predispositions related to ionizing radiation in childhood and SPNs. In the future, fibroblasts of the participants will be used for standardized irradiation experiments, which will inform analysis of the case-control study and vice versa. Differences between participants will be identified using several molecular markers. With its innovative combination of experimental and observational components, this new study will provide valuable data to forward research on radiation-related risk factors in childhood cancer and SPNs.

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KEYWORDS

fibroblast; irradiation; childhood cancer; neoplasm; second primary neoplasm; second cancer; study design; participation; feasibility; cell line

Introduction

Childhood cancer is defined as a malignant neoplasm or any neoplasm in the central nervous system occurring in children and adolescents before the age of 20 years [1]. Worldwide, the age-standardized incidence rate (world standard) is 152.8 per million person-years in those aged 0 to 19 years, is slightly higher in boys than in girls, and varies for different diagnostic groups dependent on age and region [2]. Risk factors for most childhood cancers remain largely unknown [1]. Common genetic susceptibility with low risk and rare genetic disorders with high risk explain less than 10% of the cases [3-15]. Corresponding with the current state of science, the immune system also plays an important role in the development of cancer [16], and several environmental factors [17-26], such as early infections [27] and vaccination [28], have been suggested but not established to be protective by modulating immunological pathways, in particular for childhood leukemia. In contrast, specific chemical substances such as benzene are established risk factors for the development of leukemia and antineoplastic agents (eg, DNA alkylating agents, topoisomerase II inhibitors, doxorubicin) for the development of acute myeloid leukemia and sarcomas in childhood [29]. However, these substances do not constitute the major part in the development of childhood cancer, since only a minority of children is exposed to such chemical carcinogens [30].

Exposure to high doses of ionizing radiation, either due to nuclear disasters [31] or in cancer therapies [32-35], is a rare and known environmental risk factor for acute myeloid leukemia in childhood [1] and second primary neoplasms (SPNs) [1,29,36-39,41]. Indeed gene-radiation interactions are assumed to be involved in the etiology of childhood cancer [1,42] and SPNs [43-46] as well. Besides high-dose ionizing radiation, the magnitude of the risk for first primary neoplasms (FPNs) in childhood from very low doses (≤0.05 Gray [Gy]) is still uncertain and difficult to resolve via conventional epidemiological studies [1]. Low doses of ionizing radiation are commonly used in medical diagnostics, like computed tomography examinations [47], and regarded as a risk factor in addition to the directly exposed treatment volume, where high doses of ionizing radiation are applied during radiation therapy [48]. Exposure to low doses also occurs during the staging procedure of neoplasms via computed tomography examinations and follow-up after treatment. In utero exposure to ionizing radiation during abdominal X-rays of pregnant women was consistently observed to be a risk factor for acute leukemia in many epidemiological studies conducted in the 1950s and 1960s [49-56]. Today, X-ray examinations during pregnancy are conducted using lower radiation doses [57], and recent studies were not able to identify any increased risk anymore [58]. Similarly, a recent study on cancer incidence after exposure to postnatal diagnostic X-rays did not find an increased risk for leukemia, lymphoma, central nervous system tumors, blastomas, or sarcomas [59]. However, data on the effect of low doses are still scarce and inconsistent due to missing direct biological human evidence [60,61]. Additionally, observational studies are often small and may not show proper confounder control [62-69].

To address these open questions and challenges with a more powerful approach, we designed a nested, molecular-epidemiological, case-control study that combines observational epidemiological research with standardized experimental components in primary human fibroblasts. We want to identify genetic predispositions related to the cellular response to high and low doses of ionizing radiation in SPN cases compared with FPN controls first and in childhood cancer cases compared with cancer-free controls second. This publication focuses on the description of the innovative study design and its potential use in research as well as on procedures of sampling and proportions of participation.

Methods

Aim and Study Design

The population-based, nested case-control study KiKme (German: “Krebserkrankungen im Kindesalter und molekulare Epidemiologie”; English: “Cancer in childhood and molecular-epidemiology”) was designed to analyze genetic predispositions and other molecular-biological factors associated with ionizing radiation in primary human fibroblasts from former childhood cancer patients (SPNs and FPNs) and cancer-free controls. Applying a molecular-epidemiological, case-control study design, using primary human skin fibroblasts as a model of normal human somatic tissue enables us to

measure individual changes in reaction to different radiation exposures on a cellular level and to conduct an informed search for genomic causes in fibroblasts from the same person simultaneously [70]. The combination with observational data from questionnaires and the linkage of therapy data on chemo- and radiotherapy from treating hospitals complete the study and allow us to control for known confounding factors.

Study Population

More than 70,000 former childhood cancer patients are registered in the German Childhood Cancer Registry [71]. This large cohort provides the basis for the nested case-control study KiKme. Since 1980, this registry has recorded population-based childhood cancer cases occurring in children younger than 15 years old in former Western Germany with almost complete coverage. Since 1991, cases from former Eastern Germany are recorded as well. In 2009, the age limit for recorded childhood cancer was raised from under 15 years old to under 18 years old [32]. Diagnoses of childhood cancer are validated in cooperation with treating hospitals and an open-end follow-up is conducted with an emphasis on obtaining information on SPNs [72]. The cohort in which our case-control study KiKme was nested includes children with only 1 cancer diagnosis (FPN) as well as with multiple cancer diagnoses over time (SPN). Subjects were eligible if they were diagnosed with an FPN in childhood, were at least 18 years old (as of June 2012), showed survival after cancer diagnosis for 1 year or more, and were still alive when the study was performed. Additionally, an address and an agreement for data storage in the German Childhood Cancer Registry had to be available. The inclusion criteria resulted in a maximum of 1976 available former childhood cancer patients (247 SPNs with 1729 matching FPNs). All these former childhood cancer patients were initially contacted by the German Childhood Cancer Registry in consideration with the guidelines of the Association for Pediatric Oncology and Hematology in Germany.

For the pilot study of this project, 48 former childhood cancer patients with any morphology of FPN and SPN were included. Within the main study period, only participants (n=392) with an FPN of the most common childhood cancers of the International Classification of Childhood Cancer - third edition (ICCC-3) [73] were recruited: leukemia ICCC-3 I(a), I(b), I(c), I(d); lymphoma ICCC-3 II(a), II(b), II(c); and tumors of the central nervous system ICCC-3 III(a), III(b), III(c), III(d), IV(a). Cancer sites of the second primary diagnosis had to be at a potentially radiation-related site: thyroid carcinoma ICCC-3 XI(b); skin carcinoma ICCC-3 XI(e); leukemia ICCC-3 I(a), I(b), I(d) (all causally related to radiation [41]); or malignant melanoma ICCC-3 XI(d) (potentially related to radiation [41]). The number of possible SPN cases meeting the inclusion criteria was limited by the quantity of potential SPN participants who were still alive (n=247). Potential FPN controls (n=1729) were matched by age at recruitment (maximal age range of 5 years), sex, cancer morphology (ICCC-3), year of diagnosis (maximal range of 7 calendar years), and age at diagnosis (maximal age range of 4 years) to available SPN cases using a risk set sampling approach. Taking the year of diagnosis into account enables us to control for changes in therapy procedures. To be included as a possible FPN control, no SPN diagnosis had to exist at the date of the second diagnosis of the corresponding SPN case, and the FPN control had to be alive.

In order to not only be able to compare genetic predispositions related to ionizing radiation in SPN cases and FPN controls, we also recruited cancer-free controls for each matching group in an additional hospital-based study arm in the Department of Orthopedics and Traumatology of the University Medical Center Mainz. They were matched by sex and within a maximal 10-year age range at the time of the recruitment to participating SPN cases and FPN controls. Cancer-free controls were mainly recruited from patients who were hospitalized for elective orthopedic surgery after an accident. Cancer-free controls with severe or chronic diseases (eg, cancer, Alzheimer's disease, multiple sclerosis, cardiovascular disease, diabetes) were excluded from participation due to a possible association with shared genetic predispositions and cancer development [74].

Procedures and Survey Modules

The study combines information from questionnaires and molecular-biological experiments including investigations on radiation-induced effects using primary human skin fibroblasts derived from skin biopsies of the participants. In addition, saliva samples were collected as a second, independent source for DNA. Participants who reported being infected with severe infectious diseases (eg, hepatitis or AIDS) were excluded from a skin biopsy and saliva collection to avoid any transmission in the laboratory. Also, skin biopsies were not conducted if participants suffered from other severe diseases (eg, hemophilia) to prevent them from suffering adverse health consequences.

Questionnaires

Most study participants (SPN, FPN, cancer-free control) answered a self-completed questionnaire to assess socioeconomic and anthropometric factors, as well as information on lifestyle, medical history, and health. The general questionnaire contained questions on birth characteristics, ethnic origin, anthropometric factors, education, current life circumstances, smoking, drinking, diseases, and medications, as well as medical therapies and lifelong exposure to medically applied radiation (medical radiation history) of the participant. Data on cancer therapies were validated by comparing questionnaire data with information on type and dose of medication as well as dose and number of radiotherapy fractions from therapy protocols of treating hospitals [75]. All therapy data will be used to develop an individual exposure matrix for each participant. Furthermore, there were questions on family history of severe diseases. The complex information on family history of cancer was additionally requested in a personal interview in the clinic or through a telephone interview for all participants not attending the clinic in Mainz. The interview included information about cancer type and age at diagnosis within their relatives (children, siblings, nephews and nieces, parents, grandparents, aunts, uncles, and cousins).

Saliva Collection, Processing, and Storage

Saliva collection took place using the Oragene DNA Kit (DNA Genotek Inc, Ottawa, Ontario, Canada). The participant was asked not to drink, eat, smoke, or chew chewing gum 30 minutes before collection. Five minutes before the start, the participant

rinsed his or her mouth and filled the saliva tube of the kit with saliva without air bubbles. The saliva was mixed with the DNA stabilizing fluid and immediately forwarded to the laboratory within the recruitment center. For persons participating near their residence, saliva samples were sent to the laboratory in Mainz in a provided cardboard box by standard mail. After receiving the collected samples, half of each saliva sample was lysed and incubated at 56 °C in the laboratory. After incubation, samples were mixed with ethanol, and the lysate was loaded in a NucleoSpin Blood L Column and centrifuged. After washing the silica membrane, the DNA was eluted with DNA buffer. The DNA sample was then stored at –80 °C. The remaining half of saliva from each participant was stored at –20 °C for later use.

Skin Biopsy Collection, Processing, and Storage

Skin samples were taken by punch biopsy under local anesthesia with a diameter of 3 mm at the cubital region for cancer patients and during surgery in the scar region for cancer-free controls. The resulting wounds were sewn with a single stitch. After successful extraction, biopsied skin was transferred to a vial with rich cell culture medium (Amniogrow, CytoGen GmbH, Wetzlar, Germany), stored at room temperature, and immediately taken to the laboratory or by courier service within 24 hours. Subcutaneous tissue was removed, and the biopsy was dissected in rich cell culture medium (Amniogrow, CytoGen GmbH, Wetzlar, Germany) and cultured in a humidified incubator at 37 °C with 5% CO_2 (Heracell Vios 160i, Thermo Fisher Scientific, Waltham, MA) to allow the outgrowth and expansion of fibroblasts. Culture medium (Amniogrow, CytoGen GmbH, Wetzlar, Germany) was changed every 3-4 days. Passaging of fibroblasts was done using 0.05% trypsin with 0.1% ethylenediaminetetraacetate when reaching approximately 70% confluence. After the first passage, cells were cultured in low glucose Dulbecco's minimal essential medium (Sigma-Aldrich, St. Louis, MO) containing 1% nonessential amino acids, 15% fetal bovine serum, and 1% penicillin/streptomycin (all supplements from Biochrom GmbH, Berlin Germany). Cultures were grown for 2-4 weeks to reach sufficient cell numbers for cryopreservation in liquid nitrogen or nitrogen gas.

Sampling

All applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. Approval by the Ethics Committee of the Medical Association of Rhineland-Palatinate was obtained (no. 837.262.12 (8363-F), no. 837.103.04 (4261), and no. 837.440.03 (4102)). Study participants who voluntarily gave consent for examinations, collection of samples, subsequent analysis, time-limited storage of personal data, and collected samples were included. Participants could consent to single components of the study while abstaining from others at any time. After confirmation to participate in the KiKme study, an appointment for the discussion of the informed consent was made. A date for skin biopsy, saliva sampling, and telephone or personal interview was obtained. Cases participating at the University Medical Center Mainz were offered the possibility of medical consultation. These consultations were not documented for this report. Participants were reimbursed and compensated for travel costs. To further increase participation despite potential long travel to Mainz, all cancer patients were also given the option to participate near their residence. If available, participants could name their attending dermatologist. Otherwise, the study team contacted a dermatologist near the residence of the participant. The attending dermatologists were asked to act as a cooperating partner, were trained for the study, and took the skin biopsy with the signed informed consent.

Potential cancer-free control participants were identified in the surgery schedules of the department for orthopedic surgery. They were contacted and informed about the content of the study during their stay in the hospital. Participation could be refused at any time during the procedure. To increase the study participation of cancer-free controls, the biopsy was taken from excess material during their surgical procedure.

Analysis Plan

From all participants, cultured human fibroblasts from 156 participants with the best matching results based on our criteria (52 triplets each with 1 SPN, 1 FPN, and 1 cancer-free control participant) will be selected for the radiation experiments (mean age of participants at sampling: SPN 33 years, range 20-51 years; FPN 33 years, range 21-49 years; controls 33 years, range 19-48 years; median age of participants at first neoplasm: SPN 8 years, range 0-14 years and FPN 8 years, range 1-14 years; mean calendar year of the first neoplasm: SPN 1991, range 1980-2011 and FPN 1991, range 1980-2009). During radiation experiments, cultured human fibroblasts from each of the 156 selected and carefully matched participants will be exposed to a low (eg, 0.05 Gy) as well as a high dose (2 Gy) of X-rays and will be sham-irradiated (0 Gy). The low dose of radiation will be applied to mimic an exposure scenario during medical diagnostics (eg, computed tomography), and the high dose represents an average single tumor dose applied to the target volume of conventional fractionated radiation therapy. The fibroblast of each triplet will be treated simultaneously to avoid batch effects within groups. In a preliminary analysis, we identified the time point after radiation with the highest amount of differentially expressed genes for our chosen radiation doses [76]. The identified time point will be used to analyze differences in gene expression patterns between patient groups. The high number of samples from different participants in irradiation experiments (around one-third of the participants) allows us to distinguish possible gene expression patterns with candidate genes and underlying cellular pathways between groups and to identify differences between SPN cases and FPN controls as well as differences between former childhood cancer patients (SPNs and FPNs) and cancer-free controls. To be able to compare gene expression before and after exposure to ionizing radiation, RNA from 468 dishes with cultured human fibroblasts of the irradiation experiments (156 exposed to 0.05 Gy, 156 exposed to 2 Gy, and 156 sham-irradiated; 3 dishes for each participant) will be extracted and Illumina-sequenced. RNA sequencing data will be processed and cleaned as well as normalized using the Voom method [77]. Gene expression of irradiated cells will be compared with the expression of sham-irradiated cells after the same time interval for each participant. Differentially expressed genes dependent on

radiation dose will be detected using linear models and empirical Bayesian statistics. The differential gene expression after irradiation will be computed by comparing measurements of fibroblasts from each participant with measurements after sham-irradiation (eg, counts of transcripts in cells of each individual after 0 Gy versus counts after 2 Gy). *P* values will be computed for the interaction between the effect of radiation and group and for the effect of radiation alone using the R package limma (lmFit, eBayes, makeContrasts) with *patient ID* as a block variable and the factors *patient group* and *radiation doses* [78]. The analyses will be performed without adjustment, with adjustment for age only, and with adjustment for age and gender. For the comparison between former childhood cancer patients with and without SPNs, the analyses will additionally be adjusted for age at first primary neoplasm diagnosis and for tumor subtype. Furthermore, sensitivity analyses will be performed separately for male participants and female participants with age adjustment. Differentially expressed genes will then be selected at a false discovery rate (FDR) level of 0.05 (Benjamini-Hochberg procedure). In addition, differentially expressed genes and their $\log_2$ fold change will be examined using Ingenuity Pathway Analysis (IPA; Version 1.13, QIAGEN Inc, 2018) with a right-tailed Fisher exact test examining pathway enrichment and z-score (≥|2|) indicating (in-) activation of pathways [79]. In addition, IPA will be employed to predict upstream regulators as well as downstream diseases and functions. We will choose promising marker genes to validate the RNA sequencing experiments via real-time quantitative polymerase chain reaction. Thus, RNA sequencing data intend to identify differentially expressed candidate genes, which finally enables a weighted analysis of DNA single-nucleotide variants (SNVs) in these genes and related regions by selecting the smallest *P* value from all comparisons. To filter SNVs, a gene list will be created that contains all genes that were identified as differentially expressed in the messenger RNA and long noncoding RNA analyses after Bonferroni correction (with adjustment for age and gender as well as with adjustment for age at first tumor diagnosis and for tumor type). Furthermore, the list could be supplemented with genes from the associated pathways of the Ingenuity Pathway Database and known radiation-associated genes (RadAtlas) [80] as well as genes associated with childhood cancer (International Cancer Genome Consortium [ICGC], Pediatric Cancer Genomic Data Portal [PeCan], PedcBio portal, Pediatric cancer gene database [Pedican], Xena browser) [81,82]. SNVs will be assigned to the genes if they are located in an area that includes the gene body, consisting of exons and introns, and 500 kilobases upstream and downstream of the gene body. In addition, SNVs will be assigned to the genes that were identified in the Genotype-Tissue Expression (GTEx) project [83] as expression quantitative trait loci (eQTLs) for the gene [84]. The analysis will be carried out using forest tests (RVTEST) [85,86] applying a single-variant Wald test at the SNV level. The burden test (combined multivariate and collapsing [CMC] method) [87], sequence kernel association test (SKAT) [88], and variable threshold method [89] will be used for the gene-based examination of the DNA sequencing data at RVTEST. Association studies will be performed based on the generated gene list using FDR as correction for multiple testing with a significance level of 5% and genome wide without FDR adjustment. Simulation studies assuming our sample size and different SNV effect sizes (odds ratio [OR] 1.3, 1.5, 2, 3, and 4) for genome-wide association studies resulted in the significance level selection of 5% at the gene level and 0.005% at the SNV level. In addition, a weighted analysis of SNVs will be performed genome wide by using likelihood-based boosting [85] and gene list *P* values as weights. Both tumor groups (former SPN and FPN patients) will be compared against the cancer-free controls, and, additionally, the tumor groups will be compared against each other. Results of the SNV analysis will be verified in a 2-stage procedure: First, identified genetic group differences in fibroblasts from about one-half of the participants (n=286) will be replicated in DNA sequenced from the saliva of the same participants. In the second stage, validated results will be replicated in the saliva DNA of an independent confirmation collective consisting of the remaining half of the participants (n=275). This 2-stage approach enables us to ameliorate problems of false discovery. Possible confounding or effect modification (eg, by sex, age at diagnosis of first or second primary neoplasm, type of first or second primary neoplasm, or batch effects) will be taken into account in this analysis. In addition, sensitivity analysis for other possible confounding factors like family history of cancer or received therapies will be conducted.

To identify possible risk associations with cancer treatment, participants were asked whether they had received cancer therapies. Used medications and affected body regions will be additionally inquired (n=556). For validation, self-reports will be compared with data from cancer therapies of the patients from hospitals and clinical studies [75]. By measuring sensitivity and specificity, the quality of binary variables will be analyzed. Receiver operatic characteristic curves will be used for a graphical comparison. Positive and negative predictive values will be used to analyze the validity of the questionnaire. Cohen kappa will be used to measure the concordance between the information from questionnaires and from treating hospitals. Influencing factors (eg, number of neoplasms, sex, sociodemographic factors, comorbidities, time since cancer treatment) on the dichotomous outcome variable *degree of agreement* will be analyzed using logistic regression [75]. If the questionnaire is reliable, conditional logistic regression and mixed models will be used to estimate possible risk associations with cancer therapies.

Differences in family history between childhood cancer patients with FPNs and SPNs as well as cancer-free controls could also be a confounder or effect modifier and will be investigated concerning family history of cancer, degree of family relatedness, age of diagnosis, and family history of chronic disease (n=556). Our interest here is to identify whether an increased number of cancer cases in families is associated with childhood cancer incidence. A family history of cancer was recorded as dichotomous variables for each degree of kinship, for maternal and paternal kinship, and for sex of family members in the questionnaires. The number of cases within families will be related to family size. Clustering of cancer within families will be estimated by the genealogical index of familiarity [90] and stratified by groups (SPN, FPN, cancer-free controls) to ascertain whether the average kinship among affected

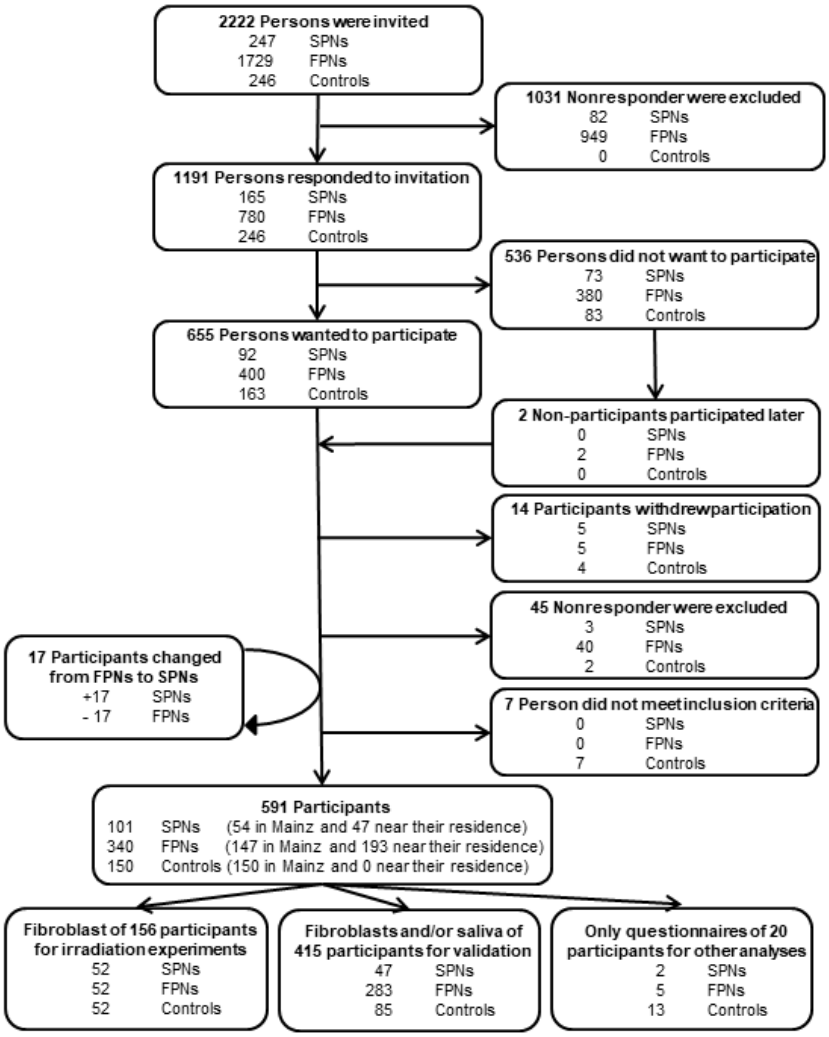
individuals in a pedigree differed from a randomly drawn control set of that pedigree. The kinship sum test [91] will be applied to identify affected individuals exhibiting a closer relationship to other affected individuals than would be expected by chance. Conditional logistic regression will be applied to investigate the association between family history of cancer and the risk of primary childhood cancer (SPN and FPN). Analyses will be adjusted for sex and age at recruitment and stratified for kinship and sex. Cox proportional hazard models will be calculated adjusted for age, sex, family history of cancer, and primary childhood tumor entity to estimate standard incidence rates for SPNs among the cohort of childhood cancer patients. Further, conditional logistic regressions will be used to explore the associations between childhood cancer (SPN and FPN) and other diseases in the family (eg, diabetes, hypertension, elevated blood cholesterol).

The available biosamples of the study will further be used to forward research on other biological markers (eg, hyper- and hypovariability of gene expression, noncoding RNA, copy number variations, epigenetic changes like methylation pattern of genes, proteins associated with double-strand breaks, chromosomal aberrations) and to investigate their possible association with radiation-related cancer development in other KiKme research projects.

Results

The recruitment started in 2013, and the result is shown in Figure 1. Originally, we invited 247 SPNs and 1729 FPNs to participate in the study, of which 92 SPNs (92/247, 37.3%) and 399 FPNs (399/1729, 23.1%) were willing to participate. During the recruiting process, some participants refused their participation while others accepted. Thus, some rematching was needed. To gain complete matching groups in the radiation experiments, we allowed 17 FPN patients that developed an SPN later in life to migrate to the SPN group. However, taking the risk set sampling approach into account, their questionnaire data could be used both as an SPN case and as an FPN control in the questionnaire-based analyses (eg, on the risk of family history of cancer). Overall, 54.4% of the participants (47 SPN and 193 FPN of 441 total participants) participated in the study near their residence in a medical practice of 1 of the 182 cooperating dermatologists.

Figure 1. Enrollment of participants (SPNs, FPNs, and controls) in the population-based, nested case-control study KiKme. FPN: first primary neoplasm; SPN: second primary neoplasm.



A total of 591 former childhood cancer patients and cancer-free controls aged 19 to 53 years (mean age 32 years, 51% women and 49% men) participated in the study (Table 1). The age distribution of participants with SPNs compared with FPNs was very similar (χ^2 test: P=.28), whereas participating cancer-free controls were slightly younger than participants with childhood cancer (χ^2 test: P<.001). Similar differences were found for nonparticipating childhood cancer survivors and nonparticipating cancer-free controls (χ^2 test nonparticipants with SPNs versus FPNs: P=.11; χ^2 test nonparticipating cancer-free controls versus nonparticipating childhood cancer survivors: P<.001). Further characteristics of participants and nonparticipants like age at diagnosis and tumor morphology are summarized in Table 1 and Table 2.

Table 1. Characteristics of included study participants and nonparticipants.

Characteristics	Participants				Nonparticipants[a]			
	SPNs[b] (n=101)	FPNs[c] (n=340)	Controls (n=150)	Total (n=591)	SPNs (n=146)	FPNs (n=1389)	Controls (n=96)	Total (n=1631)
Female, n (%)	50 (49.5)	189 (55.6)	62 (41.3)	301 (50.9)	71 (48.6)	606 (43.6)	42 (43.8)	719 (44.1)
Male, n (%)	51 (50.5)	151 (44.4)	88 (58.7)	290 (49.1)	65 (44.5)	657 (47.3)	54 (56.2)	776 (47.6)
Sex missing, n (%)	N/A[d]	N/A	N/A	N/A	10 (6.8)	126 (9.1)	0 (0)	136 (8.3)
Age at recruitment (years), mean (range)	32 (19-51)	34 (19-53)	29 (18-48)	32 (19-53)	34 (18-49)	34 (18-51)	31 (18-51)	33 (18-51)
<25 years old, n (%)	19 (18.8)	44 (12.9)	57 (38.0)	120 (20.3)	18 (12.3)	111 (8.0)	17 (17.7)	146 (9.0)
25-29 years old, n (%)	25 (24.8)	69 (20.3)	40 (26.7)	134 (22.7)	18 (12.3)	234 (16.8)	25 (26.0)	277 (17.0)
30-34 years old, n (%)	19 (18.8)	78 (22.9)	20 (13.2)	117 (19.8)	24 (16.4)	245 (17.6)	19 (29.8)	288 (17.7)
≥35 years old, n (%)	38 (37.6)	149 (43.8)	33 (22.0)	220 (37.2)	75 (51.4)	672 (48.4)	30 (31.3)	777 (47.6)
Age missing, n (%)	N/A	N/A	N/A	N/A	11 (7.5)	127 (9.1)	5 (5.2)	143 (8.8)
Age at 1st diagnosis (years), mean (range)	7 (0-14)	8 (0-16)	N/A	N/A	8 (0-14)	7 (0-15)	N/A	N/A
Year of 1st diagnosis	1980-2011	1980-2012	N/A	N/A	1980-2005	1980-2012	N/A	N/A
Years between 1st and 2nd diagnoses, mean (range)	16 (2-35)	N/A	N/A	N/A	16 (1-30)	N/A	N/A	N/A
Age at 2nd diagnosis (years), mean (range)	23 (5-46)	N/A	N/A	N/A	24 (5-41)	N/A	N/A	N/A
Year of 2nd diagnosis	1986-2018	N/A	N/A	N/A	1989-2014	N/A	N/A	N/a

[a]Information available only for nonparticipants from the main study.

[b]SPNs: second primary neoplasms.

[c]FPNs: first primary neoplasms.

[d]N/A: not applicable.

Table 2. Cancer sites and cancer therapies of the included study participants and nonparticipants.

Cancer site (International Classification of Childhood Cancer 3rd Edition)	Participants		Nonparticipants[a]	
	SPNs[b] (n=101)	FPNs[c] (n=340)	SPNs (n=146)	FPNs (n=1389)
1st neoplasm, n (%)				
Leukemia (I(a), I(b), I(c), I(d))	41 (40.6)	166 (48.8)	66 (45.2)	641 (46.1)
Lymphoma (II(a), II(b), II(c))	41 (40.6)	135 (39.7)	40 (27.4)	485 (34.9)
Central/peripheral nervous system (III(a), III(b), III(c), III(d), IV(a))	15 (14.9)	35 (10.3)	29 (19.9)	138 (9.9)
Other tumors V, VI(a), IX(a), IX(e)	4 (4.0)	4 (1.2)	0 (0.0)	0 (0.0)
2nd neoplasm, n (%)				
Thyroid cancer (XI(b))	30 (29.7)	N/A[d]	55 (37.7)	N/A
Skin carcinoma (XI(e))	32 (31.7)	N/A	53 (36.3)	N/A
Malignant melanoma (XI(d))	4 (4.0)	N/A	11 (7.5)	N/A
Leukemia (I(a), I(b), I(d))	9 (8.9)	N/A	16 (11.0)	N/A
Lymphoma (II(a), II(b))	6 (5.9)	N/A	N/A	N/A
Central nervous system (III(a), III(b), III(e))	9 (8.9)	N/A	N/A	N/A
Breast cancer (XI(f))	3 (3.0)	N/A	N/A	N/A
Other unspecific carcinoma (XI(f))	6 (5.9)	N/A	N/A	N/A
Sarcoma (IX(d), IX(e))	2 (2.0)	N/A	N/A	N/A
3rd neoplasm, n (%)				
Renal carcinomas (VI(b))	1 (1.0)	N/A	_[e]	—
Skin carcinoma (XI(e))	2 (2.0)	N/A	—	—
Breast cancer (XI(f))	1 (1.0)	N/A	—	—
Other and unspecified carcinomas (XI(f))	2 (2.0)	N/A	—	—
Other specified intracranial and intraspinal neoplasms (III(e))	2 (2.0)	N/A	—	—
4th neoplasm, n (%)				
Thyroid cancer (XI(b))	1 (1.0)	N/A	—	—
Cancer therapies for the 1st neoplasm, n (%)				
Chemotherapy	93 (92.1)	312 (91.8)	—	—
Radiation therapy	74 (73.3)	225 (66.2)	—	—
Surgery	25 (24.8)	64 (18.8)	—	—
Cancer therapies for the 2nd neoplasm, n (%)				
Chemotherapy	22 (21.8)	N/A	—	—
Radiation therapy	21 (20.8)	N/A	—	—
Surgery	56 (55.4)	N/A	—	—
Cancer therapies for the 3rd neoplasm, n (%)				
Chemotherapy	1 (1.0)	N/A	—	—
Surgery	2 (2.0)	N/A	—	—
Cancer therapies for the 4th neoplasm, n (%)				
Surgery	1 (1.0)	N/A	—	—

[a]Information available only for nonparticipants from the main study.

[b]SPNs: second primary neoplasms.

[c]FPNs: first primary neoplasms.

[d]N/A: not applicable.

[e]Information on 3rd and 4th diagnoses were obtained only from participants; therefore, this information is not available for nonparticipants.

For 95% (87/91) of participating SPN cases, suitable FPN controls with a maximum difference of 3 calendar years between first diagnoses could be identified (Multimedia Appendix 1). For the remaining 5%, the time difference was increased to 4-7 calendar years. The matching rate was comparable to the age at first diagnosis: 98% of SPN cases and FPN controls were diagnosed within 3 years of age, and 100% were diagnosed within 4 years of age. Matching for age at recruitment was accomplished within a 3-year age range for 93% (85/91) of participating SPN cases and FPN controls. The remaining 7% were matched by a maximum age range of 5 years. For 7 SPN cases (7/101, 6.9%), no suitable FPN cases participated in the study. However, their information from genetic analyses and questionnaires as well as the information from all other incomplete matching groups will also be included in the analyses.

Cancer-free controls (n=150) were recruited during their stay in the orthopedic surgery department and matched by age and sex to participating SPN cases and FPN controls. Participation proportion for cancer-free controls was originally 66.3% (163 participants of 246 directly contacted persons), but 6 cancer-free controls were excluded due to cancer diagnoses, 4 cancer-free controls actively withdrew from participation during the study period, 2 had to be excluded due to nonresponse, and 1 was excluded due to diabetes (Figure 1). An additional cancer-free control took part in both the pilot study and the main study, and therefore, this participant was excluded from the pilot data.

The difference in age at recruitment for participating SPN cases and cancer-free controls was not larger than 3 years for 95% (76/81) of cancer-free controls and not more than 5 years for 98% (79/81; Multimedia Appendix 1). Only 2 cancer-free controls (2/81, 2%) could not be matched within this age range. Included controls had a short hospital stay due to injuries or their consequences (87/150, 58.0%), joint diseases (17/150, 11.3%), osteopathy and chondropathy (14/150, 9.3%), diseases of the soft tissue (9/150, 6.0%), arthrosis (6/150, 4.0%), orthopedic after treatments (2/150, 1.3%), diseases of the skin and subcutaneous tissue (2/150, 1.3%), congenital malformations or deformities of the musculoskeletal system (1/150, 0.7%), diseases of the musculoskeletal system and connective tissue (1/150, 0.7%), or diseases of nerves, nerve roots, and nerve plexus (1/150, 0.7%). For 6.7% (10/150) of controls, no reason for the hospital stay was given.

Taking group changes from FPN to SPN into account, final participation proportions were 40.9% (101 participants out of 247 invited persons) for SPN cases, 19.7% (340 participants out of 1729 invited persons) for FPN controls, and 61.0% (150 participants out of 246 contacted persons) for cancer-free controls (Table 1). Mentioned reasons for refusal to participate were lack of interest or perceived lack of personal benefit (7 SPN, 49 FPN, 34 cancer-free controls), expenditure of time (36 SPN, 130 FPN, 14 cancer-free controls), illnesses (12 SPN, 20 FPN, 5 cancer-free controls), fear of skin biopsy (12 SPN, 50 FPN, 14 cancer-free controls), and unavailability due to insufficient language skills or problems of comprehension or incorrect contact information (1 SPN, 6 FPN, 5 cancer-free controls). All other participants (1235/1631, 75.7%) provided no reason for their refusal to participate.

In summary, this study successfully obtained questionnaire data for 85 SPN cases (84.2% of 101 participating SPN), 325 FPN controls (95.6% of 340 participating FPN), and 146 cancer-free controls (97.3% of 150 participating cancer-free controls). Skin biopsies were available from 92 SPN cases (91.1% of 101 participating SPN), 307 FPN controls (90.3% of 340 participating FPN), and 100 cancer-free controls (66.7% of 150 participating cancer-free controls). Overall, 3886 cryogenic tubes with primary skin fibroblasts were cryopreserved in liquid nitrogen for further experiments with a mean of 6.8 tubes per participant (SD 4.2, range: 0-28). In total, saliva samples were dispensed from 84 SPN cases (83.2% of 101 participating SPN) and 319 FPN controls (93.8% of 340 participating FPN), as well as from 108 cancer-free controls (72.0% of 150 participating cancer-free controls). Only 2 SPN cases, 3 FPN controls, and 13 cancer-free controls were unwilling to provide any biosamples for RNA and DNA analyses. Further, 2 FPN controls were excluded from the extraction of biosamples because of former hepatitis infections. Details on available survey modules and biosamples for participants are shown in Table 3 for each donor group.

Table 3. Actual available survey modules and biosamples for participants in each donor group.

Type of data	SPNs[a] (n=101)	FPNs[b] (n=340)	Controls (n=150)	Total (n=591)
Questionnaire data, n (%)				
Participant information	85 (84.2)	325 (95.6)	144 (96.0)	554 (93.7)
Family history of diseases	85 (84.2)	325 (95.6)	146 (97.3)	556 (94.1)
Both questionnaires	85 (84.2)	325 (95.6)	144 (96.0)	554 (93.7)
Biosamples, n (%)				
Biopsy	92 (91.1)	307 (90.3)	100 (66.7)	499 (84.4)
Saliva	84 (83.2)	319 (93.8)	108 (72.0)	511 (86.5)
Biopsy and saliva	77 (76.2)	291 (85.6)	71 (47.3)	439 (74.3)
Biopsy or saliva	99 (98.0)	335 (98.5)	137 (91.3)	571 (96.6)
No bio-samples	2 (2.0)	5 (1.5)	13 (8.7)	20 (3.4)
Cryopreserved tubes of fibroblasts				
Total, n	757	2179	950	3886
Tubes per participant, mean (SD)	7.7 (4.3)	6.5 (3.1)	6.9 (5.9)	6.8 (4.2)
Tubes per participant, minimum	0	0	0	0
Tubes per participant, maximum	20	16	28	28
DNA extracts, n (%)				
From fibroblasts	90 (89.1)	301 (88.5)	97 (64.7)	488 (82.6)
From saliva	84 (83.2)	319 (93.8)	107 (71.3)	510 (86.3)

[a]SPNs: second primary neoplasms.

[b]FPNs: first primary neoplasms.

Discussion

Principal Findings

Our molecular-epidemiological study is the first attempting to analyze observational data from questionnaires and molecular-biological factors associated with ionizing radiation in primary human fibroblasts of a unique childhood cancer survivor cohort. To study molecular-biological factors, we succeeded in obtaining fibroblasts derived from 499 skin biopsies and 511 saliva samples of former childhood cancer patients (SPNs and FPNs) and cancer-free controls. With this source, we can measure individual reactions to ionizing radiation in primary human skin fibroblasts. We will use these data for an informed analysis of potential genetic predispositions. Predispositions defined through DNA mutations can be identified using the DNA extracted from fibroblasts as well as saliva samples. Combining these results with observational data from questionnaires allows us to control for several confounding factors. During the recruitment process, we invited all former SPN and matched FPN patients from the German Childhood Cancer Registry who met our inclusion criteria. However, the number of eligible former childhood cancer patients was limited to 1990 even in such a large and long-running childhood cancer survivor cohort. While the participation of cancer-free controls was high (61%), the rate of participation among former childhood cancer patients was rather low (SPN 41%, FPN 20%). Different participation proportions can be explained by the nature of this study's sampling strategy. Cancer-free controls were contacted in the hospital before undergoing surgery. Biopsies were then taken during that procedure without further effort for the patient. In contrast, SPN and FPN patients needed to travel or keep set appointments made for the biopsy. In general, the study involved complex logistics and high time expenditure for participants, especially for SPN and FPN participants. By implementing the possibility for former childhood cancer patients to participate near their residence, we reduced their effort and time spent on recruitment to a minimum. Our design required immense efforts in recruitment and data collection for the study centers. These efforts were worthwhile as they increased the rate of participation, even though an invasive procedure, such as skin biopsy, was demanded from more or less healthy individuals, and individual genetic analyses were performed. In summary, our study provides a new way of exploring the interplay between childhood cancer and second primary cancer predisposition and ionization radiation. We hope that this study will set a precedent and encourage others to perform similar projects on the international scale, requiring primary fibroblasts for experiments from large childhood cancer survivor cohorts and to investigate the underlying reasons for childhood cancer. This would help to improve therapeutic strategies, reduce the risk of developing a second primary cancer, and enhance the quality of the patients' lives.

To identify molecular mechanisms potentially related to radiation and the development of childhood cancer, analyses at different levels are required to increase our knowledge. On the genomic level, single nucleotide polymorphisms (SNPs) can

and should be analyzed in a population-based sample as it is common in genome-wide association studies (GWAS). Our sample size is limited by the number of available SPN cases and thus corresponds more to the size of a clinical cohort, which does not allow direct transfer of a GWAS approach. However, such clinical cohorts often consider gene expression and less frequently SNPs, which makes direct transmission difficult [92]. Additionally, the investigation of radiation-induced effects will be carried out experimentally by gene expression measurement before and after irradiation. To investigate the connection between radiation and childhood cancer, statistical techniques from these 3 perspectives — GWAS, clinical cohorts, and experiments — must be combined. With this combination, an increase in statistical power can be achieved. However, sufficient statistical power will still be limited to strong associations.

Strengths and Limitations

In contrast to previously conducted studies that investigated the association between ionizing radiation and cancer risk [35,62-69,93-106], this epidemiological study is one of the first enabling the collection of detailed molecular-biological information before and after exposure of primary fibroblasts from a large number of participants exposed to diagnostic and therapeutic doses of ionizing radiation to investigate innate genetic radiation responses in the patients' normal somatic cells [60,61]. We chose to perform experiments with primary fibroblasts, although lymphocytes used in other studies [107] would have been easier to attain by venipuncture. However, their survival and prolonged cultivation without immortalization by Epstein-Barr virus transformation are very limited [108]. Moreover, as some of our SPN and FPN donors have received bone marrow transplants, blood samples would have contained foreign blood cells of the bone marrow donors [109], which makes it impossible to analyze germline mutations of included cases. By measuring individual reactions to different exposures of radiation in normal somatic cells of the same person, our design enables us to create several exposure scenarios for the same participant simultaneously and therefore to trick the problem of counterfactual thinking and to avoid some confounding and bias [70]. The combination with observational data from questionnaires on medical radiation history, health, and family history of diseases allows comprehensive control for important confounders in the development of cancer. With additional collection of saliva samples from participants, DNA from an independent source is available for the validation and replication of results.

There are also several limitations to our study design. Given that we will analyze primary fibroblasts as monolayer cell cultures in vitro, this approach does not allow consideration of nontargeted radiation responses, such as the intercellular transmission of primarily adverse radiation effects to unirradiated neighboring cells via the so-called bystander effect, and their role in the development of therapy-related SPN [110]. Thus, the complexity of the 3D interaction of the in vivo radiation response and its clinical manifestation cannot be adequately represented by experiments in our study with monolayers of a single cell type. In addition, gene expression and radiation response of the chosen primary fibroblasts might not be representative of cells of various target organs and all cancer subtypes. However, the experiments conducted in this study enable first and very important insights into the etiology of childhood cancer and SPN. Moreover, the biological endpoints of this study might be influenced by the exposure history of the fibroblasts to possible carcinogenic factors (eg, cancer therapy, alcohol, tobacco, medication). To deal with this problem, our questionnaires cover a broad spectrum of possible confounding factors and allow us to control for them. As with all epidemiological studies requiring biological material from patients, our study underlies an inherent survivor bias, as solely living patients could be recruited. Severe cases with high mortality (eg, acute myeloid leukemia after acute lymphoid leukemia or 2 diagnoses in rapid succession) cannot be captured to a full extent by this study. A selection bias cannot be ruled out in this study, as individuals, either without long-term health damages or with severe health problems, might be less motivated to participate. Moreover, a family history of cancer might influence the willingness to participate, and the statistical power might be limited by the sample size of available former childhood cancer cases. However, the invitations to this study included the maximum number of former childhood cancer patients registered in the German Childhood Cancer Registry that met the inclusion criteria. The recruitment of living patients several years after their diagnosis for the study further limited our analysis to particular patients that suffered from first and second malignancies with a good prognosis. The source population of hospital-based, cancer-free controls is regionally limited to the rural and urban areas around the University Medical Center in Mainz, while population-based cases were recruited all over Germany. However, we do not expect any major differences in the source populations since we expect that neither the interplay between hereditary dispositions and radiation nor cancer have any causal effect on hospitalization after an accident in the Mainz area. Thus, restricting the majority to these controls is equivalent to taking a simple random sample of the original population [74]. In addition, it is known that participation decreases in populations with lower education as well as in very high-income groups. Even though there is no information on socioeconomic status for nonparticipants, we were able to compare the available information of the nonparticipants with the obtained information of the participants. The distribution of sex, age, and age at first diagnosis was similar among participants and nonparticipants and is representative for former childhood cancer patients with these diagnoses in Germany [32].

Conclusions

To our knowledge, this is the first molecular-epidemiological study on radiation, childhood cancer, and second primary cancer providing a large number of primary fibroblasts from skin biopsies of well-characterized and carefully matched participants for irradiation experiments. In this study, we were able to successfully recruit 441 former SPN and FPN patients from the large survivor cohort of the German Childhood Cancer Registry long after their diagnosis and 150 cancer-free control patients from the Department of Orthopedics and Traumatology of the University Medical Center Mainz. In future projects, the combination of experimental and observational data with a

unique study sample, including primary normal somatic cells from former childhood cancer patients and cancer-free controls, will forward research on radiation-related risk factors for childhood cancer, SPNs, and its underlying genetics. Using the gained knowledge from irradiation experiments and analyses on different molecular levels (eg, DNA, RNA, epigenetics), we aim to overcome challenges of personalized childhood cancer therapies and gain insight into the detrimental cellular responses and potential mechanisms of low medically applied radiation doses.

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Authors' Contributions

MM is a principal investigator of the KiKme study and developed its design, which was implemented and monitored by MM and LKB. PK supported the development of strategies for the recruitment of former childhood cancer patients. MM, LKB, IS, and DG conducted the recruitment of the participants, which was organized and planned by MM, LKB, and IS. MM, LKB, HS, and PD monitored the recruitment of controls. DG, SZ, and HS established the method of fibroblast sampling. CG, PD, and JH were responsible for biopsy sampling. They were trained and supervised by MM and HS. In the study, HSZ takes care of the project´s biobank and controls for the quality of all biosamples. IS conducts the work in the laboratory, including the processing of saliva samples and skin biopsies. LKB and SZ were responsible for the pseudonymization of all biosamples. MM, HB, MH, and AP developed the analyses pipelines for the project. Analysis data of biosamples are processed by AP and TH. LKB and WHB are responsible for data management. HSZ, SZ, DG, IS, JM, PSK, PK, AP, HB, TH, MB, and HS contributed to the writing process, which was initially prepared by MM and LKB. All authors revised the manuscript and agreed to be accountable for all aspects of the work.

Conflicts of Interest

None declared.

Multimedia Appendix 1

Number of matching groups and time spans for matching between patient groups of participants.
[DOCX File , 19 KB-Multimedia Appendix 1]

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Abbreviations

CMC: combined multivariate and collapsing
eQTLs: expression quantitative trait loci
FDR: false discovery rate
FPN: first primary neoplasm
GTEx: genotype-tissue expression
GWAS: genome-wide association study
Gy: Gray
ICCC-3: International Classification of Childhood Cancer, Third edition
ICGC: International Cancer Genome Consortium

IPA: Ingenuity Pathway Analysis
OR: odds ratio
PeCan: Pediatric Cancer Genomic Data Portal
Pedican: Pediatric cancer gene database
RVTEST: forest tests
SKAT: sequence Kernel association test
SNP: single nucleotide polymorphism
SNV: single-nucleotide variant
SPN: second primary neoplasm

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Comparison of time and dose dependent gene expression and affected pathways in primary human fibroblasts after exposure to ionizing radiation

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Abstract

Background: Exposure to ionizing radiation induces complex stress responses in cells, which can lead to adverse health effects such as cancer. Although a variety of studies investigated gene expression and affected pathways in human fibroblasts after exposure to ionizing radiation, the understanding of underlying mechanisms and biological effects is still incomplete due to different experimental settings and small sample sizes. Therefore, this study aims to identify the time point with the highest number of differentially expressed genes and corresponding pathways in primary human fibroblasts after irradiation at two preselected time points.

Methods: Fibroblasts from skin biopsies of 15 cell donors were exposed to a high (2Gy) and a low (0.05Gy) dose of X-rays. RNA was extracted and sequenced 2 h and 4 h after exposure. Differentially expressed genes with an adjusted p-value < 0.05 were flagged and used for pathway analyses including prediction of upstream and downstream effects. Principal component analyses were used to examine the effect of two different sequencing runs on quality metrics and variation in expression and alignment and for explorative analysis of the radiation dose and time point of analysis.

Results: More genes were differentially expressed 4 h after exposure to low and high doses of radiation than after 2 h. In experiments with high dose irradiation and RNA sequencing after 4 h, inactivation of the *FAT10 cancer signaling pathway* and activation of *gluconeogenesis I*, *glycolysis I,* and *prostanoid biosynthesis* was observed taking p-value (< 0.05) and (in) activating z-score (≥ 2.00 or ≤ -2.00) into account. Two hours after high dose irradiation, inactivation of *small cell lung cancer signaling* was observed. For low dose irradiation experiments, we did not detect any significant ($p < 0.05$ and z-score ≥ 2.00 or ≤ -2.00) activated or inactivated pathways for both time points.

(Continued on next page)

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Conclusions: Compared to 2 h after irradiation, a higher number of differentially expressed genes were found 4 h after exposure to low and high dose ionizing radiation. Differences in gene expression were related to signal transduction pathways of the DNA damage response after 2 h and to metabolic pathways, that might implicate cellular senescence, after 4 h. The time point 4 h will be used to conduct further irradiation experiments in a larger sample.

Keywords: Childhood cancer, Fibroblasts, Gene-radiation interaction, High dose, Ionizing radiation, IPA, Low dose, RNA sequencing, Second primary neoplasm

Background

Exposure to ionizing radiation induces complex stress responses in cells (Albrecht et al. 2012) and can lead to genomic instability (Kadhim and Hill 2015). These effects are not only limited to the irradiated cells but also observed in adjacent, untreated bystander cells (Mavragani et al. 2016). Such radiation-induced changes in human cells can lead to long-term health outcomes such as cancer (Brooks et al. 2016; Hwang et al. 2008; Cardis et al. 2007; Ronckers et al. 2008; Goodhead 2009; Richardson et al. 2015; Leuraud et al. 2015) as well as cardiovascular (Baselet et al. 2016; Stewart 2012; Menezes et al. 2018; Adams et al. 2003; van der Pal et al. 2005), and other chronic diseases (Vrijheid et al. 2007). Several research groups investigated various types of skin cells to identify differences in gene expression after exposure to ionizing radiation (Sokolov and Neumann 2015). Studies comparing different doses of radiation and time points of analyses reported on more differentially expressed genes (DEGs) in fibroblasts after exposure to a high (HDIR) than to a low dose (LDIR) of ionizing radiation (Hou et al. 2015) and only little overlap of expressed genes between LDIR and HDIR (Velegzhaninov et al. 2015; Mezentsev and Amundson 2011). Moreover, the time point with the highest numbers of DEGs differed from 4 h (Ding et al. 2005) over 16 h (Mezentsev and Amundson 2011) and 24 h (Hou et al. 2015; Mezentsev and Amundson 2011) to 30 h (Albrecht et al. 2012) in a dose-dependent manner. Besides these quantitative differences of gene expression in primary human skin fibroblasts, qualitative divergences, like different expression profiles of genes included in *p53*-associated pathways, have been shown 1 h, 2 h, 4 h and 24 h after exposure to LDIR (0.02 Gray (Gy)) and HDIR (4Gy) (Ding et al. 2005).

Despite the available studies on changes in gene expression and affected pathways in human fibroblasts after exposure to ionizing radiation, the understanding of underlying mechanisms and biological effects is still incomplete for this cell type, especially for low doses (Albrecht et al. 2012; Sokolov and Neumann 2015). The results of the conducted studies are difficult to compare since a variety of different experimental setups were used: Gene expression was measured at different time points, after exposure to different radiation doses and in different cell types (Sokolov and Neumann 2015; Ding et al. 2005; Ray et al. 2012; Yunis et al. 2012; Warters et al. 2009; Stecca and Gerber 1998). Most of the studies were conducted with only a small number of cell donors (Albrecht et al. 2012; Warters et al. 2009; Berglund et al. 2008; Goldberg et al. 2004). Others used skin models (Mezentsev and Amundson 2011; Ray et al. 2012; Yunis et al. 2012), which are not an exact copy of the skin in living humans (De Wever et al. 2015) or established cell lines (Hou et al. 2015; Velegzhaninov et al. 2015; Ding et al. 2005; Kalanxhi and Dahle 2012), whose genotype and phenotype might have changed over time (Kaur and Dufour 2012).

In this study we aim to establish the experimental settings and setup the analysis to identify DEGs and corresponding pathways for further irradiation experiments. Primary human fibroblasts from a subsample of 15 selected cell donors will be irradiated with a high and a low radiation dose, and experiments will be ended at two predefined time points from the literature and preliminary experiments. Comparing these time points, we aim to identify the time point with the highest number of DEGs. The identified time point should then be used in a further project to identify differences in gene expression of former childhood cancer patients with and without a second primary neoplasm (SPN) and cancer-free controls in a study sample of 153 participants. In addition to the descriptive analysis of DEGs, gene expression patterns and affected pathways will be analyzed and compared as well as upstream and downstream effects will be predicted.

Design, subjects and methods

Study design and participants

All donors were participants of the ongoing population-based nested case-control study KiKme (Marron et al. 2020). The KiKme project aims to identify differences in genetic predispositions and gene-radiation interactions between former childhood cancer patients and cancer-

free controls ($N = 591$). Since radiation-induced changes in human cells can lead to long-term health outcomes such as cancer (Brooks et al. 2016; Hwang et al. 2008; Cardis et al. 2007; Ronckers et al. 2008; Goodhead 2009; Richardson et al. 2015; Leuraud et al. 2015), the identified time point from this work should be used as guidance in further research projects of the study to analyze differences in gene expression patterns between the different groups of study participants. Since differential gene expression might differ between cancer patients and cancer-free controls, we choose to analyze samples from all three patient groups in this work. The recruitment for the KiKme study started in 2013 and includes 591 participants until now. Recruiting strategies and development as well as information on data collection are described in detail elsewhere (Marron et al. 2020). Briefly, the study population consists of former childhood cancer patients with a first primary neoplasm (FPN) only or a subsequent SPN registered at the German Childhood Cancer Registry (Scholz-Kreisel et al. 2018). FPN patients were matched as cancer controls by age, sex, cancer site, year of diagnosis, and age at diagnosis to available SPN cases using an incidence density sampling approach. Cancer-free controls for each matching group were recruited from the Department of Orthopaedics and Traumatology at the Johannes Gutenberg-University in Mainz (Germany) and matched by sex and age within a maximal 5-year age range to the participating SPN cases and FPN controls. They were included if they were hospitalized for an elective surgery unrelated to cancer. Patients with severe diseases were excluded from participation (e.g. cancer, hemophilia, HIV, hepatitis, diabetes). For this work, skin biopsies were taken from 15 participants by punch biopsy with a diameter of 3 mm on the inside of the cubital region for cases and near the surgery region for cancer-free controls. Fibroblasts were isolated, cultivated, and cryopreserved until further usage. Moreover, saliva collection with subsequent DNA extraction took place, and each study participant answered a self-completion questionnaire to assess socio-economical and anthropometric factors as well as information on lifestyle, medical history, and health.

Irradiation of fibroblasts with subsequent ribonucleic acid (RNA) isolation

For radiation experiments, fibroblasts were cultivated and synchronized in the G_0/G_1 phase of the cell cycle by contact inhibition to exclude cell cycle-dependent effects on gene-expression profiles. To this end, cells were seeded at a density of 9000 cells per cm^2 and cultured for 14 to 15 days. G_0/G_1 arrest was confirmed by flow cytometry when the experiment was performed (Web Figure 1). Radiation experiments were conducted using the D3150 X-ray therapy system (Gulmay Medical Ltd., Byfleet, UK). Fibroblasts were exposed to a HDIR of 2Gy, comparable to an average single tumor-dose of fractionated radiation therapy (Seidlitz et al. 2017), and a LDIR of 0.05Gy, comparable to an organ dose of a computed tomography scan (Pearce et al. 2012) or were sham-irradiated (0Gy). Cells from matched triplets, consisting of an SPN, an FPN, and a corresponding cancer-free donor, were cultivated and treated simultaneously to prevent batch effects within groups. For HDIR with 2Gy, fibroblasts were exposed to 140 kV X-rays at a dose rate of 3.62Gy per minute. To apply LDIR of 0.05Gy with the same X-irradiation system, a dose rate of 0.34Gy per minute was achieved by increasing the distance from the source to target by 30 cm and via reduction of the voltage to 50 kV. Cells were exposed at room temperature and sham-irradiated cells for each time point of analysis were kept at the same conditions in the radiation device control room.

To identify the time points post-radiation with the highest numbers of DEGs, we conducted preliminary experiments with several time points with fibroblasts from 3 cancer-free controls (Web Figure 2). From these experiments the time point 2 h was chosen due to the largest number of DEGs after radiation exposure for both, the LDIR and HDIR. We selected the time point of analysis after 4 h for LDIR from the literature (Ding et al. 2005). Thus, the final experimental settings for fibroblasts from 5 SPN cases, 5 FPN controls and 5 cancer-free controls were defined as follows: irradiation with 2Gy and RNA extraction after 2 h (2Gy–2h), irradiation with 2Gy and RNA extraction after 4 h (2Gy–4h), irradiation with 0.05Gy and RNA extraction after 2 h (0.05Gy–2h), irradiation with 0.05Gy and RNA extraction after 4 h (0.05Gy–4h), no radiation and RNA extraction after 2 h (0Gy–2h), no radiation and RNA extraction after 4 h (0Gy–4h).

RNA was isolated using the NucleoSpin RNA Plus (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). RNA integrity was assessed using a Bioanalyzer 2100 (Agilent RNA 6000 Nano Kit, Agilent Technologies, Santa Clara, California, USA). Sequencing library construction was done using 1 μg of total RNA (as quantified by QuBit, Thermo Fisher Scientific, Waltham, Massachusetts, USA) with an RNA integrity number greater or equal to 8 with the TruSeq RNA Sample Prep Kit v2 (Set A and B, Illumina, San Diego, California, USA) following the manufacturer's instruction. RNA-Sequencing libraries were pooled, cBot-clustered, and sequenced on a HiSeq2500 instrument (Illumina, San Diego, California, USA) in high-output mode. Single-end reads with a length of 50 base pairs were generated using TruSeq Single Read Cluster Kit v3 (Illumina, San Diego, California, USA) and TruSeq SBS Kit v3 (Illumina, San Diego, California, USA). Data was generated by Real Time Analysis Version 1.8.4 and

converted into FASTQ format using bcl2fastq Version 1.8.4 (Illumina, San Diego, California, USA).

We chose *CDKN1A* (*Cyclin-Dependent Kinase Inhibitor 1A*) and *MDM2* (*Mouse double minute 2 homolog*) as marker genes to validate the RNA-sequencing experiments via Real-Time Quantitative Polymerase-Chain-Reaction (qPCR) in 6 participants (2 SPN, 2 FPN, and 2 cancer-free controls). They consist of two matched groups, each including an SPN, an FPN, and a cancer-free control. The first diagnosis of the SPN and FPN was leukemia or lymphoma, respectively. The site of the SPN was chosen to be potentially radiation-associated (thyroid cancer or leukemia). The methods for this validation were described elsewhere (Galetzka et al. 2020).

Bioinformatical and statistical analyses

To identify the time point with the largest number of DEGs after radiation exposure, RNA sequencing data had to be processed first. Raw reads were cleaned for adapter sequences using Trimmomatic (Bolger et al. 2014): Bases with a quality less than 3 were removed and reads were trimmed if the average quality over 4 bases was less than 15. Processed reads were aligned to the human reference genome (GRCh38) using STAR (STAR-2.6.0c) (Dobin et al. 2013). Expression per gene, given as the number of aligned reads per gene, was quantified using FeatureCounts (Rsubread v1.30.9) (Liao et al. 2014). Only genes with a minimum of 10 counts in at least 4 samples were analyzed. Data were normalized for sequencing depth using the DESeq package (v1.28.0) (Anders and Huber 2010). Reads were aggregated (summed) on the level of UCSC gene annotations. To address intra-patient correlation, random effect models fitted with lme4 (Bates et al. 2015) were used to estimate the among-patient variation, and the resulting residuals were further inspected. Afterwards, a principal component analysis was conducted with the standardized residuals using the R package stats (R-3.4.4). Correlation of the first three principal components and RNA quality parameters as well as the number of aligned raw reads and normalized number of aligned reads were inspected visually.

For the analysis of differential expression, data was transformed via the Voom (Law et al. 2014) method implemented in the limma package (v3.34.9) (Ritchie et al. 2015). DEGs dependent on radiation dose were detected for defined time points using linear models implemented in the limma package (Ritchie et al. 2015) with blocking on the patient. For each time point and radiation dose the gene expression was compared to the same time point post-radiation after sham-irradiation not taking the disease status into account. To account for expressional variability, we used variance modeling and borrowing information across genes (Ritchie et al. 2015). Additionally, our limma model included the patient identifiers accounting for a random variance. DEGs with a *p*-value smaller than 0.05 after adjustment for false discovery rate (Benjamini-Hochberg procedure) were flagged as significant and used for pathway analyses. Since also small coordinated changes in gene expression might lead to important physiological changes (Christmann and Kaina 2013), there was no restriction set regarding the log fold change.

Finally, pathway analyses were conducted via Ingenuity Pathway Analysis (IPA, Version 1.13, QIAGEN Inc., 2018). As input, lists of DEGs containing previously generated gene-wise *p*-values for each combination of time point and radiation dose, as well as log2-fold changes were used. Settings for comparison analyses in IPA were selected for experimental data in human fibroblasts or alike cells, molecule types, and data sources. The complete setting list can be found in the Supplement file 1. Negative log (−log10) *p*-values of at least 1.30 (≙ p-value = 0.05) were defined as significant. Activating z-score threshold was chosen as greater or equal than 2 or less than or equal minus 2 (Krämer et al. 2014). The z-score indicates pathway (de-)activation by comparing given expressional directions of pathway components with information from the data set entered for analysis (e.g. log-fold change). In addition, we used the comparison analysis in IPA to display and compare pathways across all experiments. Moreover, we included an overview of predicted downstream outcomes and upstream regulators. Analyses were conducted on March 3, 2020, and based on the IPA December 2019 Update.

Results

A sample of 15 participants was selected from the KiKme study ($N = 591$). They were grouped into 5 matched triplets, each consisting of 1 SPN, 1 FPN, and 1 cancer-free control. Cells originated from 9 male and 6 female participants with a mean age of 28.27 years (age at recruitment: 21–40 years). FPN diagnoses were lymphoma ($n = 6$) or leukemia ($n = 4$) and they were diagnosed at a mean age of 8.10 years (age at FPN diagnosis: 4–14 years). SPN diagnoses were thyroid ($n = 2$) or skin cancer (n = 2) or leukemia ($n = 1$) and occurred at a mean age of 20.00 years (age at SPN diagnosis: 10–36 years).

Primary fibroblasts of the 15 participants were irradiated with a high and a low radiation dose. RNA was isolated 2 h and 4 h after the exposure and used to identify differential gene expression via RNA-sequencing. After normalizing for sequencing depth and removing inter-patient variation, no obvious correlation of RNA quality or sequencing depth with expression variation was observed (Web Figure 3). The validation of the RNA-sequencing experiments was successfully done using *CDKN1A* and *MDM2* as

marker genes (Web Figure 4–5). The qPCR furthermore showed that all cells reacted similarly.

Differential gene expression in reaction to LDIR and HDIR

We compared the gene expression of irradiated and sham-irradiated cells ignoring the tumor status because the sample size of 15 participants is too small to compare different groups of patients. The gene expression 2 h after irradiation differed markedly from the response 4 h after irradiation compared to unirradiated cells. This is indicated by separation of both time points along the first two principal components. The first and fifth principal variance components additionally showed variability of the radiation doses. HDIR samples showed a higher separation from the non-irradiated samples compared to the LDIR samples (Web Figure 6).

Compared to unexposed cells, a larger number of DEGs was found at 4 h after exposure to LDIR (N = 757 genes, Web Table 1B) and to HDIR (N = 4472 genes, Web Table 1D) than after 2 h for both radiation doses (LDIR: N = 202 genes, Web Table 1A; HDIR: N = 2778 genes, Web Table 1C). For the LDIR treatment, differential expression of 9 and 67 genes was found in the 0.05Gy–2h and 0.05Gy–4h sample only, respectively (Fig. 1a, Web Table 1A, 1B, 1C, 1D). An increase in DEGs was also present for the HDIR treatment. Considering genes that were only differentially expressed in the experiment with 2Gy irradiation, about twice as many genes (N = 2906) were found to be differentially expressed exclusively after 4 h compared to 2 h (N = 1505; Fig. 1a, Web Table 1C). Additional 841 DEGs were identified at both time points after exposure to HDIR. Twelve genes were found to be differentially expressed in all 4 experimental settings.

Pathway analysis

Using the Qiagen Knowledge Base in IPA, we identified 5 cellular pathways related to the DEGs. In these pathways, differential expression of genes exceeded a significant p-value in at least one experimental setting and the activating z-score threshold was surpassed to determine an activation or inhibition of pathways (Fig. 2, Web Figure 7–8, Web Table 2). For each pathway, a ratio of DEGs divided by the number of total genes in the pathway (k/K) is given as an indication of the enrichment.

For the 2Gy-2h samples, *small cell lung cancer signaling* pathway (z = – 2.12, k/K = 15/56) was predicted to be inactivated. For 2Gy-4h samples, 4 pathways with significant p-values and z-score were detected (*FAT10 cancer signaling pathway, gluconeogenesis I, glycolysis I,* and *prostanoid biosynthesis*). Three of them were predicted to be activated, indicated by a positive z-score (*gluconeogenesis I*: z = 2.00, k/K = 4/4; *glycolysis I*: z = 2.24, k/K = 5/5; *prostanoid biosynthesis*: z = 2.00, k/K = 4/6). *FAT10 cancer signaling pathway* was predicted to be inactivated (z = – 2.07, k/K = 19/39). In addition, 2 pathways based on liver and T-cells (*hepatic fibrosis signaling* (z = – 2.29, k/K = 70/214) and *T-cell exhaustion* (z = – 2.68, k/K = 26/72)) were predicted to be inactivated. However, these 2 pathways were excluded concerning content for discussion. None of the mentioned pathways for HDIR were significantly altered at both time points (Web Table 2). Based on the applied criteria, no pathways were significantly altered in any of the LDIR samples.

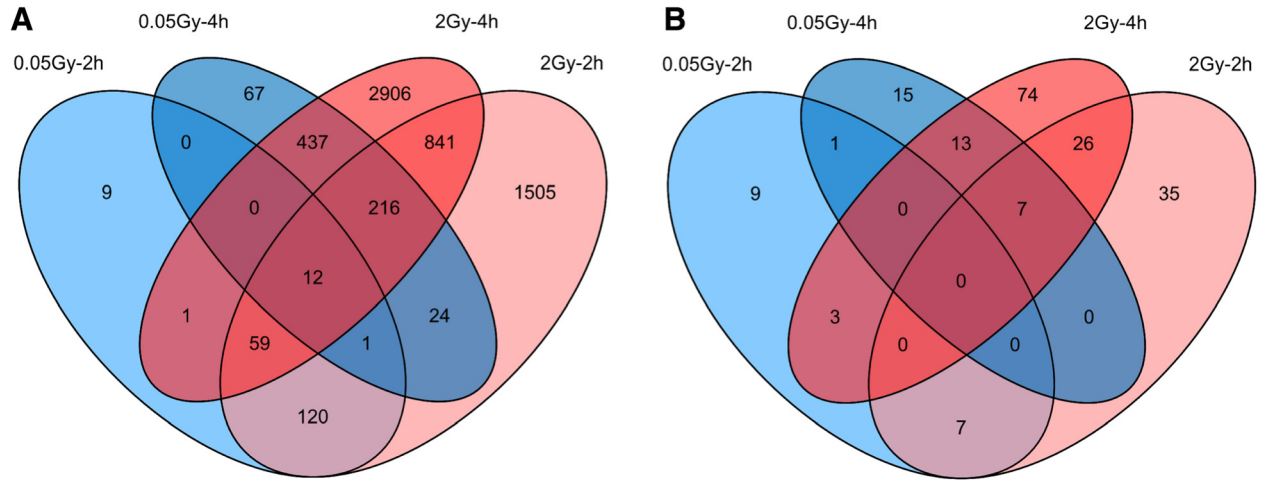

Fig. 1 Differentially expressed genes (DEGs) (**a**) and pathways affected by DEGs (fisher's exact test, $p \leq 0.05$) (**b**) in human fibroblasts after exposure to ionizing radiation. **a** DEGs 2 and 4 h after low (0.05 Gray (Gy)) and high dose (2Gy) radiation exposure (adjusted for false discovery rate (< 0.05)). **b** Number of identified pathways in Ingenuity Pathway analysis, where fisher's exact test showed a significant overlap of genes in pathway subset and DEGs (–log(p-value) ≤1.3) but not significant activational prediction (z-score: - 2 ≤ z ≤ 2)

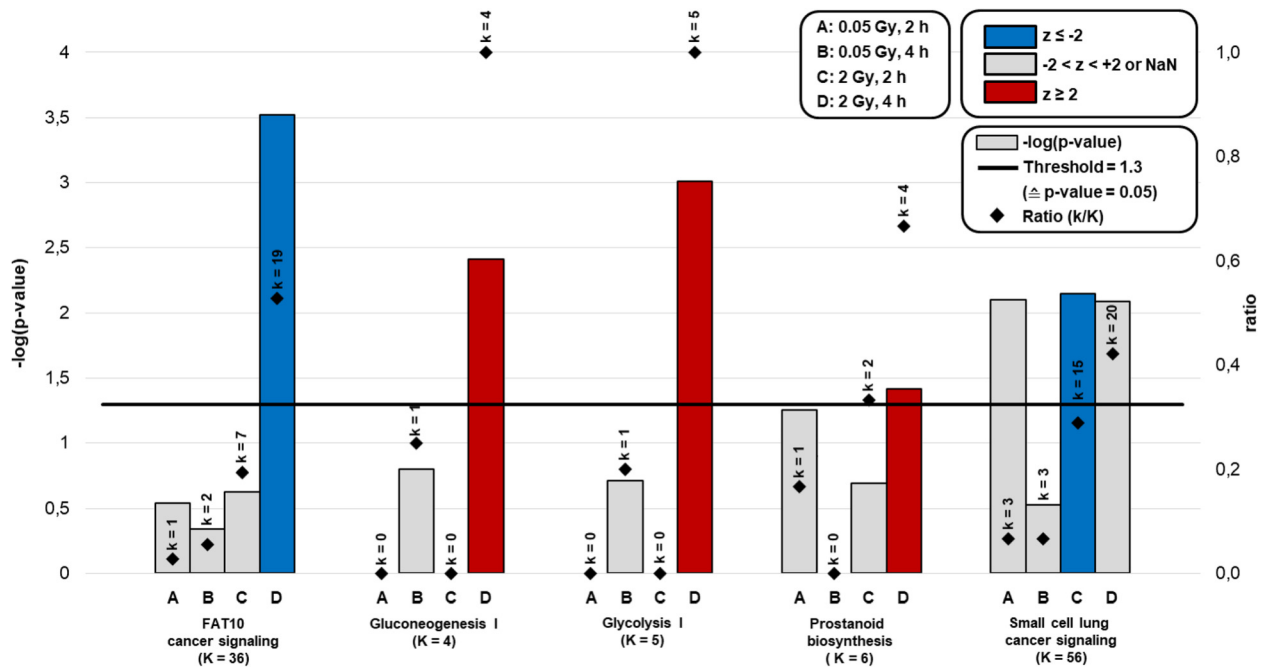

Fig. 2 Pathways affected by differentially expressed genes after high or low dose irradiation of primary human fibroblasts. Abbreviations: not a number (NaN), Gray (Gy), hours (h), *Gq protein alpha subunit* (*Gaq*), *phosphoinositide 3-kinase* (*PI3K*), *v-akt murine thymoma viral oncogene* (*AKT*), z-score (z), number of differential expressed genes (k), number of total genes in pathway (K)

We further identified 190 additional cellular pathways, where differential expression activity in genes reached a significant *p*-value but were not predicted to be activated or inactivated via z-score (Fig. 1b, Web Figure 7–8, Web Table 2). However, none of the pathways were found to be activated or inactivated in more than one experimental setting (Web Figure 7–8). In 0.05Gy-4h samples, mainly metabolic pathways exceeded a –log(p-value) of 1.30. Signaling pathways with only significant p-value were identified for both time points after exposure to LDIR. A stronger time-dependent increment of significant pathways (only in p-value, Fig. 1b) was observed after LDIR (20 pathways after 2 h compared to 36 pathways after 4 h, an increase of 80%) than after HDIR (75 pathways after 2 h compared to 123 pathways after 4 h, increase of 64%). Two hours after exposure to HDIR, differences in gene expression were related to signal transduction pathways of the DNA damage response. Four hours after exposure to HDIR, the pattern had changed to metabolic pathways (Fig. 2, Web Table 2, Web Figure 7–8).

When considering resulting diseases and functions in a downstream prediction, LDIR experiments again showed only a few results for activity patterns (Web Figure 9). Two hours after exposure to LDIR, only *cell proliferation of fibroblasts,* which can be grouped as a function of cell cycle progression, was predicted to be inactivated (z = – 0.07). Likewise, only functions of cell cycle progression were found to be inactivated after 4 h after LDIR. However, cell cycle progression was indicated as activated at this time point and radiation dose. After exposure to HDIR, processes of cell cycle progression were found to be inactivated at both time points (Web Figure 9). While 2 h after exposure to HDIR additionally functions related to senescence and cell transformation were predicted to be inactivated, functions of senescence, apoptosis, metabolism, and repair mechanisms were mainly predicted to be activated in IPA. In the prediction of upstream regulators especially *p53* was found to be activated after exposure to HDIR after 2 h (z = 1.77) and after 4 h (z = 1.72, Web Figure 10). Moreover, Interleukins and *mechanistic Target of Rapamycin* (*mTOR*) were predicted to be downregulated after 2 h with a significant z-score > |2| (Web Figure 10).

Discussion

To identify the time point with the highest number of DEGs in primary human fibroblasts after exposure to LDIR or HDIR for the usage in later study projects, we compare gene expression profiles and associated cellular pathways at 2 h and 4 h post radiation. More DEGs were detected 4 h after exposure to both LDIR and HDIR. In 2Gy-2h samples, *small cell lung cancer signaling* was predicted to be inactivated. In 2Gy-4h samples, we observed inactivation of *FAT10 cancer signaling,* and activation of *gluconeogenesis I*, *glycolysis I,* and *prostanoid*

biosynthesis. Exposure to LDIR did not cause a significant difference in pathway activation prediction via z-score for both time points of analysis.

Differentially expressed genes after irradiation

As reported by previous studies (Albrecht et al. 2012; Hou et al. 2015; Mezentsev and Amundson 2011), the number of DEGs differed largely across our 4 experimental settings. In total, more genes were differentially expressed after exposure to HDIR than to LDIR at both time points. The increase of DEGs from 2 h to 4 h was much more pronounced in LDIR compared to HDIR. Following HDIR, a fast cellular response is expected according to the strong genotoxic impact inducing a high count of DEGs already after 2 h. Therefore, the increase of DEGs from 2 h to 4 h after exposure to HDIR might be rather minor compared to LDIR since the stimuli of the lower energetic nature in LDIR may cause a more delayed response and rise of DEGs. In line with our assumptions, Ding et al. (Ding et al. 2005) reported on a maximum of DEGs 2 h after exposure to 4Gy and 4 h after exposure to 0.02Gy. In our study, also the number of significant pathways (only in *p*-value) showed a time-dependent increase for low and high doses, corresponding with this hypothesis of delayed gene expression patterns post-radiation. Only 12 genes were found to be differentially expressed under all experimental conditions. This finding is in line with results from several other groups indicating only a little overlap of DEGs and activated pathways for different time points and radiation doses (Sokolov and Neumann 2015; Velegzhaninov et al. 2015; Mezentsev and Amundson 2011). In addition, we compared the DEGs of our experiments with genes listed in the RadAtlas (Xu et al. 2020), which is a recently published database for radiation-associated genes. In the 2Gy-4h experiment, 244 (29%) of our DEGs were found in the 844 genes described in the database. In the other experiments, 15% (2Gy-2h), 5% (0.05Gy-4h) and 1% (0.05Gy-2h) of our DEGs were listed in the RadAtlas, respectively (data not shown).

We furthermore compared our results on affected pathways to this database (Xu et al. 2020) and other existing datasets (Ghandhi et al. 2015). Therefore, we choose all available single-fraction datasets with existing sham-irradiated (0Gy) control cells, manually calculated their log-fold changes, and included them to our IPA analysis. We identified similar patterns of activation and inactivation of pathways (Web Figure 11). Likewise, our results on downstream diseases and functions (Web Figure 12) and on upstream regulators (Web Figure 13) were also comparable to those from available datasets (Xu et al. 2020; Ghandhi et al. 2015), especially when considering other human samples. However, predicted downstream effects from gene expression in mouse blood cells tend to differ from available human samples. In particular, cell death of lymphocytes was predicted to be inactivated in mice, whereas lymphocytes in human samples are known to activate processes of cell death after radiation exposure (Miszczyk et al. 2018). This was also observed in human samples in our comparison analysis (Web Figure 11). Interestingly, *Interleukins 1A, 1B*, and *17A* were predicted to be inactivated as upstream regulators in our 2Gy-2h experiments, whereas they were predicted to be activated in human blood samples 4 h after exposure to 1.25Gy of ionizing radiation (Web Figure 13). Interleukins are important factors for cell signaling and cancer progression (Mantovani et al. 2018), and usually described to increase after exposure to ionizing radiation (Liu et al. 2006; Liao et al. 2017; Li et al. 2015). However, we observed inactivation of *mTOR* in the same experiment, which was previously described to suppress the translation of *Interleukin 1A* (Laberge et al. 2015).

Affected pathways following HDIR

Corresponding to the identified genes from RNA sequencing and subsequent processing, the *small cell lung cancer signaling* pathway was found to be inhibited in 2Gy-2h samples compared to sham-irradiation. The *small cell lung cancer signaling* pathway includes the two key players *Phosphoinositide 3-kinase* (*PI3K*) and *nuclear factor kappa-light-chain-enhancer of activated B cells* (*NF-κB*). *PI3K* showed decreased gene expression in our 2Gy-2h experiments. Lack of *PI3K* leads to activation of *NF-κB*, which is usually linked to stress response (e.g. exposure to ionizing radiation) (QIAGEN 2018) and has been previously reported as a potential radiation biomarker (Stecca and Gerber 1998; Park et al. 2002) as well as a key player in inducing transcription of anti-apoptotic genes after exposure to ionizing radiation (QIAGEN 2018; Maier et al. 2016). *PI3K* and *NF-κB* also play important roles in other pathways, that were found to be significant in the 2Gy-2h experiment, but failed to exceed a z-score > |2| (Web Figure 7). As an example, the *lymphotoxin-β receptor signaling* pathway ($p = 0.01$; $z = -1.89$) activates several signaling pathways, including *NF-κB* and cell death. In addition, *PI3K* is closely associated with the *prolactin signaling* pathway, which was also significant via *p*-value in our analysis ($p = 0.01$; $z = -1.94$). When comparing our analysis data to available datasets from other study groups (Xu et al. 2020; Ghandhi et al. 2015), the *small cell lung cancer signaling* pathway was also be found as significantly affected via p-value in human blood cells 4 h after exposure to 1.25Gy irradiation (Xu et al. 2020) and to all available datasets from human coronary artery endothelium cells and mouse tissues (Xu et al. 2020) (Web Figure 11).

However, for none of these samples, a significant activity prediction could be calculated.

In addition, in 2Gy-2h samples, the *p53 signaling* pathway was found to be significant in *p*-value ($p = 0.02$; z = 1.94). *P53* is a very well-known mediator of the response to genotoxic stress and several other studies reported on *p53* stabilization and activation of its downstream signaling pathways as a response to HDIR (Albrecht et al. 2012; Hou et al. 2015; Mezentsev and Amundson 2011; Warters et al. 2009; Jen and Cheung 2005). We furthermore found *p53* as predicted to be activated as an upstream regulator in our IPA analysis 2 h after exposure to HDIR (Web Figure 10). This finding was also pronounced in 2Gy-4h samples, but with a smaller −log(p-value).

While we observed changes in the activity of pathways associated with intracellular signaling at 2 h after irradiation, cellular metabolic pathways were affected after 4 h. This shows a chronological trend in response to ionizing radiation. Immediately after irradiation, a complex signaling network of the DNA damage and cell cycle response is activated (2Gy-2h) causing a transient cell cycle arrest or its manifestation as premature senescence (2Gy-4h, Web Figure 9). The frequent induction of premature differentiation and senescence in fibroblasts after irradiation is in line with the significant activation of the *glycolysis I* pathway in 2Gy-4h samples since senescent fibroblasts show an increased rate of glucose metabolism through glycolysis (James et al. 2015). Likewise, the *gluconeogenesis I* pathway shows a significant activation in the 2Gy-4h samples. Since gluconeogenesis represents the reverse process of glycolysis, there is a large redundancy regarding the involved processes and enzymatic reactions and a concurrent activity of both pathways seems likely. Neither *glycolysis I* nor *gluconeogenesis I* was found to be affected in available data from other studies (Xu et al. 2020; Ghandhi et al. 2015) (Web Figure 11).

The activation of the *prostanoid biosynthesis* pathway comprising only 6 genes is driven by activation of 4 prostaglandin-E Synthase genes (Web Table 2). Their expression can be induced by *p53* and may be involved in *p53* mediated apoptosis (Polyak et al. 1997). Since the *p53 signaling pathway* in the 2Gy-4h samples also shows a significant activation via *p*-value ($p < 0.01$), the activation of the pathway seems plausible, although the z-score with 0.82 was not significant. The *prostanoid biosynthesis* pathway was also affected, when analyzing available data from radiation experiments with human blood cells (1.25Gy-4h) (Xu et al. 2020). However, the activity prediction showed no significant results for these samples (Web Figure 11).

Furthermore, we observed an enhanced expression of the *FAT10 cancer signaling pathway* in our 2Gy-4h experiment. The enhanced expression of this pathway was expected as a reaction to DNA damage according to a recent study (Chen et al. 2018) and can lead to prolonged survival and proliferation (Aichem and Groettrup 2016). When comparing our analysis data to available datasets from other studies (Xu et al. 2020; Ghandhi et al. 2015), the *FAT10 cancer signaling pathway* was also be found as significantly affected via *p*-value in human blood samples 4 h after exposure to a radiation dose of 1.25Gy (Web Figure 11). Likewise, samples from mouse blood showed this pathway to be affected 24 h after exposure to 1Gy irradiation (Web Figure 11). However, for both of these samples, the activity prediction did not exceed a z-score > |2|.

Some pathways were significant in p-value but received a z-score of "Not a Number". For these pathways activity prediction is not possible, as data in the IPA-database was not sufficient for calculation of the z-score at the time of analysis. Hence, there is not enough information to date to predict the effect of our DEGs and calculate a reliable z-score. Nevertheless, results with this informational gap are also important, as some known radiation- and stress response-related pathways can be observed in this category. Significant pathways that had z = "Not a Number" were examined concerning content (Web Table 2, Web Figure 14–28).

In 2Gy-4h samples, the *base excision repair (BER) system* pathway was given as "Not a Number" via activating z-score ($p = 0.04$, Web Table 2, Web Figure 14). *BER* is one of the most prominent DNA repair pathways which is activated after exposure to genotoxic stressors including ionizing radiation (QIAGEN 2018; Chaudhry 2007; Krokan and Bjørås 2013). The gene expression of several members of *BER* repair was affected including proliferation cell nuclear antigen, *DNA polymerase beta (POLB), DNA ligase I (LIG1),* and *DNA-(apurinic or apyrimidinic site) lyase (APEX1)*, highlighting the important role of this DNA repair pathway to maintain genomic integrity.

Furthermore, in both of our HDIR experiments, the *molecular mechanisms of cancer* pathway was flagged as *p*-value significant (2Gy-2h: $p = 0.03$; 2Gy-4h: $p < 0.01$, Web Table 2, Web Figure 15). This pathway fosters tumor progression and generation of mutations in onco- or tumor suppressor-genes as well as activation of related signaling pathways (QIAGEN 2018). Our data suggest a high radiation-related expression of key players of cell cycle regulation and death, e.g. of *CDKN1A*, *PUMA*, and *MDM2* as well as of the proto-oncogene *c-Fos*.

Comparable to our results, published data from other studies (Hou et al. 2015; Mezentsev and Amundson 2011; Ding et al. 2005; Warters et al. 2009; Kalanxhi and Dahle 2012) identified pathways related to signal

transduction of the DNA damage response and senescence in a time-dependent manner: In one of the first conducted studies by Ding and colleagues (Ding et al. 2005), exposure to HDIR (4 Gy) resulted in apoptosis and cell proliferation in the human skin fibroblast cell line HSF42. Similar results for HDIR were found by a recent study using another human skin fibroblast cell line (AG01522) (Hou et al. 2015). In this study, 6 h after exposure to a high dose of 2Gy, cells responded to DNA damage by activation of the *p53* signaling network, apoptosis, and control of cell cycle. At the earlier time point (3 h) DEGs were mostly involved in *G-protein-coupled receptor downstream signaling*. They stated that cellular response started at 3 h to 6 h after irradiation, which was also reported by another study (Kalanxhi and Dahle 2012), and that cellular defense mechanisms occurred earlier after exposure to HDIR than to LDIR. Activation of *p53*-related pathways (Mezentsev and Amundson 2011; Warters et al. 2009) and cell cycle control (Mezentsev and Amundson 2011) after exposure to different high doses of ionizing radiation was also reported by other studies for the time points 4 h (Mezentsev and Amundson 2011; Warters et al. 2009), 16 h (Mezentsev and Amundson 2011) and 24 h (Mezentsev and Amundson 2011).

The time dependency of pathways related to different processes in the cell could be found in our data in the prediction of downstream diseases and functions in IPA (Web Figure 9). Comparable to the results from the study groups mentioned above (Hou et al. 2015; Mezentsev and Amundson 2011; Ding et al. 2005; Warters et al. 2009; Kalanxhi and Dahle 2012), functions related to senescence, apoptosis, metabolism, and repair mechanisms were predicted to be affected 4 h after exposure to HDIR in our experiments. None of them were found to be predicted as activated 2 h after exposure.

Affected pathways following LDIR

For LDIR, no pathways surpassed our thresholds for *p*-value and activating z-score thresholds. This observation can either correspond to the hypothesis of delayed gene expression patterns in LDIR or can be caused by a high inter-individual variation in the response to LDIR (Wilson et al. 2010), which hinders the detection of significant differences. However, we identified several pathways that are related to DEGs after LDIR and were significant only in *p*-value, but not in activating z-score. Like after HDIR, the *molecular mechanisms of cancer* pathway was also found to be p-value significant in the 0.05Gy-4h experiment ($p < 0.01$, Web Table 2, Web Figure 15). However, given the result "Not a Number", activity prediction for this pathway is not possible.

Similar to our LDIR pathway analysis, a study investigating the transcriptional response to LDIR in skin biopsies was also not able to identify a significant activation or inactivation of pathways previously identified after in vitro LDIR of normal human skin fibroblasts (AG01522) (Berglund et al. 2008). They conducted their experiments with skin biopsies obtained from five prostate cancer patients after in vivo exposure during radiation therapy. Even if we could not identify significant pathways via p-value and z-score in our LDIR experiments, other studies reported on changes in gene expression related to several mechanisms in the cell after exposure to LDIR. A recent study in normal human skin fibroblasts (AG01522) identified biological processes responding to stress induced by ionizing radiation shortly after exposure (Hou et al. 2015). Amongst others, these processes included activation and signaling amplification of G proteins, apoptotic pathways, DNA and RNA metabolic processes, kinase activity, DNA repair, and replication as well as cell cycle arrest (Hou et al. 2015). Another study from Ding et al. (Ding et al. 2005) identified 16 genes responding only to a low dose of 0.02Gy in normal human skin fibroblasts (HSF42). These genes were found to be involved in cell-cell signaling, cell proliferation, signal transduction, and transcriptional regulations.

When not only considering affected pathways but also predicted downstream diseases and functions in our data, we were also able to identify pathways related to functions of cell cycle progression (Web Figure 9), likewise the study groups from Ding (Ding et al. 2005) and Hou (Hou et al. 2015). Two hours after exposure to LDIR, cell proliferation of fibroblasts was predicted to be inactivated in our results. However, the amount of inactivation was only minor ($z = -0.07$). Similar results were found 4 h after exposure to LDIR. Here, DNA synthesis and cell proliferation were predicted to be inactivated. Cell cycle progression was indicated as activated at this time point. However, with a z-score of 0.56, this predicted activation is also not significant.

Due to the low number of DEGs after LDIR and therefore only limited information input, prediction of upstream regulators only showed inactivation of the tumor necrosis factor (TNF) as a predictable result (Web Figure 10). Despite that the threshold of $z > |2|$ could not be reached here either, it appears to be a reaction that occurs shortly after the stimulus in a dose-dependent manner.

To sum up, previously conducted studies comparing different doses of radiation and time points of analyses reported on more DEGs in fibroblasts after exposure to a high than to a low dose of ionizing radiation (Hou et al. 2015) and only little overlap of expressed genes between low and high dose (Velegzhaninov et al. 2015; Mezentsev and Amundson 2011). This also applies to our study. Since the time point with the largest number

of DEGs differs in published studies from 4 h (Ding et al. 2005) over 16 h (Mezentsev and Amundson 2011) to 24 h (Hou et al. 2015; Mezentsev and Amundson 2011) for different radiation doses and in different cell types, we identified 4 h after irradiation as the best point for our analysis in primary human fibroblasts. At this time point, the largest number of DEGs could be observed for both LDIR and HDIR.

Despite the conducted studies on changes in gene expression and triggered pathways in human fibroblasts after exposure to ionizing radiation, the understanding of underlying mechanisms and biological effects is still incomplete for this cell type, especially for low doses (Albrecht et al. 2012; Sokolov and Neumann 2015). Using RNA sequencing data of 15 participants to analyze underlying pathways, we were able to guide further research on radiation-related changes in gene expression. Gained results can be used to conduct radiation experiments in a larger extend and to differentiate between patient groups.

Strengths and limitations

The present study has several strengths: Unlike previous studies using commercialy available cells (Hou et al. 2015; Velegzhaninov et al. 2015; Mezentsev and Amundson 2011; Ding et al. 2005; Jen and Cheung 2005; Ghandhi et al. 2008) or only a limited number of donors (Albrecht et al. 2012; Warters et al. 2009; Berglund et al. 2008; Goldberg et al. 2004), we used fibroblasts from skin biopsies from a total of 15 donors. All samples were cultivated for the first time and synchronized in the G_0/G_1 phase of the cell cycle by contact inhibition to exclude cell cycle-dependent effects on gene-expression profiles. G_0/G_1 arrest was confirmed by flow cytometry for all samples. To guarantee the same conditions for all of our samples, non-irradiated samples were kept and analyzed under identical conditions as irradiated ones. Pathway analysis via IPA allows analyses of complex RNA data and gives insight beyond single expressional patterns. This expands the investigational frame and adds knowledge to the overall picture of radiation biology.

Besides the mentioned strengths, the main constrains of our study are a limited number of radiation doses and time points of analysis. To identify two potent time points for our analysis, we conducted preliminary experiments with smaller sample sizes and literature research. Longer post-irradiation time points might also be interesting for subsequent pathological changes such as cancer. However, genes and pathways affected directly after exposure to ionizing radiation (immediate early genes) are also assumed to affect long term radiation-induced outcomes (Averbeck et al. 2020). Regarding dose, a high and a low radiation dose with clinical relevance (Seidlitz et al. 2017; Pearce et al. 2012; Averbeck et al. 2020) were chosen to mimic characteristic exposures to ionizing radiation used in medical diagnostics and radiation therapy. In addition, we choose to analyze samples from all 3 patient groups (SPN, FPN, cancer-free controls) of the KiKme study. This might increase the heterogeneity of gene expression levels. However, expressional variability that may be introduced to the analysis by gender, age, and FPN diagnosis was accounted for in matching for these factors. Moreover, regarding the long-term goal of the KiKme study, it was important to include samples of all 3 patient groups into the analysis of this work, since differential gene expression might differ between the groups. A comparison between groups will be conducted in a subsequent study with an increased sample size and therefore more statistical power. Here, the preliminary analysis indicated no relevant differences between unadjusted and adjusted models.

Conclusions and outlook

In this work, we detected different patterns of DEGs after exposure to LDIR and HDIR in radiation experiments with primary human fibroblasts from 15 participants from the KiKme study. Besides changes in expression patterns of single genes, expression patterns of related pathways were altered as well. We observed a shift from DNA damage-associated towards metabolism-related genes and associated pathways. The choice of the time point with the best fit for the expressional analysis of irradiation was a key task of this study. While several time points have been used in the literature our results suggest that measurement of gene expression is best done at 4 h after irradiation. At this time point, the largest effect on differential gene expression has been observed. Therefore, all subsequent experiments of the large molecular-epidemiological study KiKme will use the time point 4 h to identify differences in genetic predispositions and gene-radiation interactions between former childhood cancer patients and cancer-free controls.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s10020-020-00203-0 .

Additional file 1: Web Figure 1. Representative measurements of the cell cycle distribution of HOECHST33258-stained fibroblasts by flow cytometry during (A) log-phase growth or (B) after G0/1 synchronization over 14 days for radiation experiments. **Web Figure 2.** Total number of differentially expressed genes in human fibroblasts from cancer free-controls at 0.25 h, 2 h and 24 h after exposure to low (0.05 Gray (Gy)) or high dose (2Gy) of X-rays compared to unirradiated fibroblasts ($N = 3$). **Web Figure 3.** Correlation of RNA quality metrics (RIN, Qbit RNA-concentration), expression variation (PC1–3) and number of aligned reads (aligned reads, aligned reads normalized) for all experiments. The color indicates the sequencing run (red = run 1, blue = run 2). **Web Figure 4.** Relative expression of *Cyclin-Dependent Kinase Inhibitor 1A* (*CDKN1A*) in Real-Time Quantitative Polymerase-Chain-Reaction (qPCR) analyzing the

expression of *CDKN1A* in fibroblasts of 6 participants 2 h and 4 h after exposure to 0.05 Gray (Gy) or 2Gy ionizing radiation compared to sham-irradiated samples (0Gy, reference). *** $p < 0.001$. **Web Figure 5.** Relative expression of *Mouse double minute 2 homolog* (*MDM2*) in Real-Time Quantitative Polymerase-Chain-Reaction (qPCR) analyzing the expression of *MDM2* in fibroblasts of 6 participants 2 h and 4 h after exposure to 0.05 Gray (Gy) or 2Gy ionizing radiation compared to sham-irradiated samples (0Gy, reference). *** $p < 0.001$. **Web Figure 6.** Expression variation in fibroblasts summarized for all experiments and attributed to time point post irradiation (circle = 2 h, cross = 4 h) and dose (orange = 0 Gray (Gy), blue = 0.05Gy, green = 2Gy).

Additional file 2: Web Figure 7. Shared pathways from low and high dose ionizing radiation experiments. Gy = Gray. **Web Figure 8.** Pathways only affected in high dose ionizing radiation experiments. Gy = Gray.

Additional file 3: Web Figure 9. Predicted downsteam diseases and functions. **Web Figure 10.** Predicted upstream regulators. LDIR = Low dose of ionizing radiation (0.05 Gray), HDIR = High dose of ionizing radiation (2 Gray).

Additional file 4: Web Figure 11. Comparison of affected pathways in different data sets.

Additional file 5: Web Figure 12. Comparison of predicted downstream diseases and functions in different data sets.

Additional file 6: Web Figure 13. Comparison of predicted upstream regulators in different data sets.

Additional file 7: Gene expression in the "Not a Number" pathways (blue = downregulation, red = upregulation). **Web Figure 14.** *Base excision repair (*BER*) system*. **Web Fig. 15.** *Molecular mechanisms of cancer*. **Web Fig. 16.** *Assembly of RNA polymerase III complex*. **Web Fig. 17.** *DNA double-strand break repair by homologous recombination*. **Web Fig. 18.** *Interleukin 4 (IL-4) signaling*. **Web Fig. 19.** *Interleukin 17 (IL-17) signaling*. **Web Fig. 20.** *Interleukin 17A (IL-17A) signaling in fibroblasts*. **Web Fig. 21.** *Mitochondrial dysfunction*. **Web Fig. 22.** *Myc mediated apoptosis signaling*. **Web Fig. 23.***Nucleotide excision repair*. **Web Fig. 24.** *Protein ubiquitination*. **Web Fig. 25.** *Retinoic acid receptor (RAR) activation*. **Web Fig. 26.** *Role of Janus kinase 2 (JAK2) in hormone-like cytokine signaling*. **Web Fig. 27.** *Role of Janus kinase (JAK) family kinases in Interleukin 6 (IL-6) type cytokine signaling*. **Web Fig. 28.** *Tight junction signaling*.

Additional file 8: Web Table 1A. Differentially expressed genes 2 h after exposure to low dose ionizing radiation (0.05 Gray).

Additional file 9: Web Table 1B. Differentially expressed genes 4 h after exposure to low dose ionizing radiation (0.05 Gray).

Additional file 10: Web Table 1C. Differentially expressed genes 2 h after exposure to high dose ionizing radiation (2 Gray).

Additional file 11: Web Table 1D. Differentially expressed genes 4 h after exposure to high dose ionizing radiation (2 Gray).

Additional file 12: Web Table 2. Differential expression activity in cellular pathways and involved molecules

Additional file 13: Supplement file 1. Settings for comparison analyses in IPA.

Abbreviations

BER: *Base excision repair*; *CDKN1A*: *Cyclin-Dependent Kinase Inhibitor 1A*; DEG: Differentially expressed gene; DNA: Deoxyribonucleic acid; FPN: First primary neoplasm; *Gaq*: *Gq protein alpha subunit*; Gy: Gray; H: Hour; HDIR: High dose ionizing radiation; IPA: Ingenuity Pathway Analysis; LDIR: Low dose ionizing radiation; *MDM2*: *Mouse double minute 2 homolog*; *mTOR*: *Mechanistic Target of Rapamycin*; *NF-κB*: *Nuclear factor kappa-light-chain-enhancer of activated B cells*; qPCR: Real-Time Quantitative Polymerase-Chain-Reaction; *PI3K*: *Phosphoinositide 3-kinase*; *POLB*: *DNA polymerase beta*; *PUMA*: *P53 upregulated modulator of apoptosis*; RIN: RNA integrity number; RNA: Ribonucleic acid; SPN: Second primary neoplasm

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Authors' contributions

LKB and AP contributed equally to this work. LKB and MM implemented the KiKme study, which was developed by MM. LKB, IS, DG, and MM conducted participant recruitment, which was organized and planned by LKB, IS, and MM. CG and LE were responsible for biopsy sampling. They were trained and supervised by MM and HS. The method of fibroblast sampling was established by SZ, DG, and HS. The validation of RNA expression data via qPCR was conducted by DG. HSZ takes care of the project's biobank and controls for quality of all biosamples. MM conceptualized the research idea on differential gene expression at different time points after exposure to high and low doses of ionizing radiation and designed the experiments. IS conducted the work in the laboratory, including the processing of skin biopsies and the performance of radiation experiments. LKB and SZ were responsible for the pseudonymization of all biosamples. The analysis pipeline for the project was developed by MM, AP, MH, and HB. Analysis data of biosamples was processed by AP, MH, SR, and TH. LKB, AP, and CLG conducted the statistical analysis. All authors contributed to the writing process, which was initially drafted by LKB, AP, CLG, HSZ, and MM. The authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and analyzed during the current study are not publicly available due to ethic and data protection reasons but are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. Approval by the Ethics Committee of the Medical Association of Rhineland-Palatinate was obtained (no. 837.262.12 (8363-F), no. 837.103.04 (4261) and no. 837.440.03 (4102)). Study participants will not undergo any procedures unless they give consent for examinations, collection of samples, subsequent analysis and storage of personal data and collected samples. Study subjects can consent to single components of the study while abstaining from others.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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Late health effects and changes in lifestyle factors after cancer in childhood with and without subsequent second primary cancers – The KiKme case-control study (First Author)

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Late health effects and changes in lifestyle factors after cancer in childhood with and without subsequent second primary cancers – the KiKme case-control study

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Background: Improved treatments for childhood cancer result in a growing number of long-term childhood cancer survivors (CCS). The diagnosis and the prevalence of comorbidities may, however, influence their lifestyle later in life. Nonetheless, little is known about differences in late effects between CCS of a first primary neoplasm (FPN) in childhood and subsequent second primary neoplasms (SPN) and their impact on lifestyle. Therefore, we aim to investigate associations between the occurrence of FPN or SPN and various diseases and lifestyle in the later life of CCS.

Methods: CCS of SPN (n=101) or FPN (n=340) and cancer-free controls (n=150) were matched by age and sex, and CCS additionally by year and entity of FPN. All participants completed a self-administered questionnaire on anthropometric and socio-economic factors, medical history, health status, and lifestyle. Mean time between FPN diagnosis and interview was 27.3 years for SPN and 26.2 years for FPN CCS. To confirm results from others and to generate new hypotheses on late effects of childhood cancer as well as CCS´ lifestyles, generalized linear mixed models were applied.

Results: CCS were found to suffer more likely from diseases compared to cancer-free controls. In detail, associations with cancer status were observed for hypercholesterinemia and thyroid diseases. Moreover, CCS were more likely to take regular medication compared to controls. A similar association was observed for CCS of SPN compared to CCS of FPN. In contrast to controls, CCS rarely exercise more than 5 hours per week, consumed fewer soft and alcoholic drinks, and were less likely to be current, former, or passive smokers. Additionally, they were less likely overweight or obese. All other exploratory analyses performed on cardiovascular, chronic lung, inflammatory bone, allergic, and infectious diseases, as well as on a calculated health-score revealed no association with tumor status.

Conclusion: CCS were more affected by pathologic conditions and may consequently take more medication, particularly among CCS of SPN. The observed higher disease burden is likely related to the received cancer therapy. To reduce the burden of long-term adverse health effects in CCS, improving cancer therapies should therefore be in focus of research in this area.

KEYWORDS

childhood cancer survivors (CCS), body mass index - BMI, physical activity, diet, alcohol, smoking, thyroid disease, lipid metabolism

Introduction

Childhood cancer is a rare condition with about 400,000 new cases worldwide in the age group from 0 to 19 years (1). To date, there are only few established risk factors for the onset of childhood cancer. Besides rare genetic disorders (2–4), exposure to ionizing radiation and specific chemical substances (5) are known to be involved in the development of childhood cancer. Even though treatment options had significantly improved over the past decades, childhood cancer remains a leading cause of morbidity and mortality in this age group (6). As a result of the enhanced therapeutic efficacy, the number of childhood cancer survivors (CCS), and especially long-term CCS, has increased over time (7, 8). However, the incidence in survival is accompanied by adverse late health effects, which are associated with cancer therapy in childhood (9–14). Approximately three out of four CCS suffer from chronic health conditions 30 years after their cancer diagnosis (15), and about 8% of survivors of cancer under the age of 15 in Germany are diagnosed with a second primary malignancy within 30 years of their first diagnosis in Germany (16). In addition, cardiovascular diseases occurring at young ages have become a major cause of morbidity and mortality in CCS (8, 17, 18). In a large American cohort of CCS it has been shown that a reduction in radiation exposure to the heart during therapy reduces long-term effects in adulthood (7). However, cancer therapy may not only directly modulate the risk of cardiovascular diseases itself but also *via* modulation of other risk factors for cardiovascular diseases such as hypertension, dyslipidemia, and diabetes (19–21). Results from the aforementioned survivor cohort showed that former childhood cancer patients were more likely to take medication for the classical risk factors of cardiovascular diseases (hypertension, dyslipidemia, and diabetes) than their healthy siblings (21). It has been proven that, in addition to physical health, the mental health status of adult CCS is also affected by the comprehensive experience in childhood (22–25). The onset of mental health diseases in former childhood cancer patients could be accompanied by alcohol consumption (26, 27). Although former childhood cancer patients are less likely to be heavy drinkers compared to control groups in general (27–29), especially CCS that are living without a partner tend to consume alcohol more often than married ones (28). In addition, alcohol consumption may be associated with the education level, stress, and physical as well as social functionality (28, 29). Along with alcohol consumption, especially with heavy drinking habits, former childhood cancer patients are more likely to smoke (30). However, in the absence of alcohol consumption, the majority of CCS smoked less overall than the control groups

Abbreviations: Adj., Adjusted; BMI, Body mass index; CCS, Childhood cancer survivors; CI, Confidence interval; CO, Cancer-free controls; DAG, Directed Acyclic Graph; FPN, First primary neoplasm; GLMM, Generalized linear mixed model; ISCED, International Standard Classification of Education; KiKme, Krebs im Kindesalter und molekulare Epidemiologie; OR, Odds ratio; SD, Standard deviation; SPN, At least one second primary neoplasm; Unadj., Unadjusted.

(29, 31–33). Both, smoking and drinking are established risk factors for the development of several adverse health effects. Because of the toxins and mutagens present in alcohol, tobacco, and its additives, their use may have additive or even synergistic effects on preexisting risk factors for adverse health effects in CCS (30, 34, 35).

Therefore, the primary endpoint of this study is to provide a comprehensive overview of parameters on clinical information as well as the participants´ lifestyle and to confirm known associations between childhood cancer and late effects within the nested case-control study KiKme (German: "*Krebserkrankungen im Kindesalter und molekulare Epidemiologie*", English: "*Cancer in childhood and molecular epidemiology*") (36). As a secondary endpoint, we aim to generate new hypotheses on novel associations between cancer status, especially regarding CCS of at least one second primary neoplasm (SPN), and adverse late effects of childhood cancer therapies as well as lifestyle parameters in the framework of the KiKme study. To achieve these aims, we will compare CCS with cancer-free controls as well as CCS with FPN with CCS with SPN.

Materials and methods

Study design and participants

All participants of this study were recruited within the population-based nested case-control study KiKme. Detailed recruiting strategies and information on the general data collection were described elsewhere (36). Briefly, the study population consists of 441 CCS, registered at the German Childhood Cancer Registry, and 150 cancer-free controls. In the study, we differentiate between CCS with a first primary neoplasm (FPN, n=340) and CCS with SPN (n=101). FPN CCS were used as cancer controls and were therefore matched to participating SPN CCS by age, sex, cancer site, year of diagnosis, and age at diagnosis to participating SPN CCS. Cancer-free controls were recruited at the Department of Orthopedics and Trauma Surgery at the Johannes Gutenberg-University in Mainz (Germany) and matched by sex and age to the SPN and FPN participants.

Data collection

All information for this study was collected using a questionnaire that was self-completed by the participants. The questionnaire included information on anthropometric and socio-economic factors, medical history, health status, and lifestyle parameters. As anthropometric factors, weight and height were requested. Based on this information, the Body Mass Index (BMI) was calculated by dividing weight in kilograms by height squared in meters (kg/m^2). Normal weight was defined as BMI between 18.5 and <25 kg/m^2, overweight as BMI ≥25 kg/m^2, and obesity as BMI ≥30 kg/m^2 according to the WHO and NIH standards (37). The educational level of the study participants was assessed using the International Standard Classification of Education (ISCED) (38). To assess their medical history and health status, participants were asked whether they take any regular medication and whether they have been diagnosed with one of the following diseases: diabetes, hypercholesterolemia, hypertension, lung diseases such as asthma or bronchitis, hay fever, inflammatory joint or vertebral diseases including arthrosis and rheumatism, neurodermatitis, heart attack, stroke, thyroid diseases, Epstein-Barr virus infections, HIV, Hepatitis, or any other severe disease. Additionally, age at diagnosis for each of the applicable diseases was requested. Smoking and drinking habits were requested, along with consumption of soft drinks, water, coffee, and other drinks, using scaled information per day or week. Using this information, alcoholic beverages per day and pack-years were calculated. In addition, participants were asked about their extent of regular physical activities. Based on all data collected, we then created a score that should depict the general health status of the participants. A maximum of 8 points could be achieved in this health score and the awarding of points were made up as follows: 2 or fewer diseases (1 point), 3 or more diseases (0 points); normal weight defined as BMI between 18.5-30 (1 point), BMI below 18.8 or higher than 30 (0 points); high ISCED defined as upper secondary education or above (1 point), lower secondary and primary education (0 points); never smoker (1 point), current or former smoker (0 points); less than one alcoholic beverage per day (1 point), one or more alcoholic beverages per day (0 points); no consumption of soft drinks (1 point), consumption of soft drinks (0 points); 5 or more hours of physical activity per week (1 point), less than 5 hours physical activity per week (0 points); currently employed or self-employed (1 point), incapacitated or retired (0 points). For the calculation of the health score, at least 4 of the 8 items had to be answered by the participant. If less than 4 items were answered, the health score was set to missing. The total number of points of each participant was then divided by the number of variables that were not missing and the score was divided into 3 categories (<0.75 points, 0.75 points, > 0.75 points).

Statistical analysis

Descriptive analyses were conducted to calculate sample characteristics regarding anthropometric and socio-economic factors, medical history, health status, and lifestyle parameters stratified by cancer status (SPN, FPN, and cancer-free controls). Summary statistics were provided in frequency (N) and proportions (%).

Generalized linear mixed models (GLMM) were applied to estimate the associations between categorical and dichotomous outcome variables, the late effects, with cancer status (SPN vs.

FPN) and with case-control status (CCS vs. cancer-free controls) using odds ratios (OR) and 95% confidence intervals (CIs). We treated the matched groups as random effects and 'age' and 'year of birth' as fixed effects in all models to improve matching efficiency for the variable 'age at recruitment' within the specified 5-year period. Additional adjustment variables for each GLMM were identified *via* Directed Acyclic Graphs (DAGs) that were carefully developed based on prior knowledge using DAGitty 3.0[1] (39) (see Supplementary File 1). All health- and lifestyle-related outcomes that were collected *via* the self-administered questionnaire were taken into account for analyses unless they had less than 5% expression per characteristic across all groups were excluded from the analyses. All statistical analyses for this publication were performed using SAS 9.3 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Study characteristics

The study sample consists of 101 SPN, 340 FPN, and 150 cancer-free controls with 51% females and 49% males (Table 1). However, only the 554 study participants (94%) with sufficient information from self-administered questionnaires could be analyzed depending on the set inclusion criteria for these analyses. The mean age at interview among them was 35.14 years (standard deviation (SD): 7.14; range: 19.90-51.40 years) for CCS of SPN, 34.84 years (SD: 7.68; range: 19.60-54.50 years) for CCS of FPN, and 28.91 years (SD: 7.32; range: 18.70-48.20 years) for cancer-free controls. On average, at the time of the interview, the first cancer diagnosis had occurred 27.26 years (SD: 6.90; range: 5.00-38.00 years) earlier in CCS of SPN and 26.24 years (SD: 6.93; range: 4.00-39.00 years) earlier in CCS of FPN. A total of 90% of study participants indicated their ethnicity as Caucasian. While the CCS included in this study came from all over Germany, the majority of cancer-free controls came from Rhineland-Palatinate due to recruitment at the University Hospital in Mainz. Further characteristics of the study participants including detailed information on health and lifestyle are summarized in Table 1 and Table 2.

Association between cancer status and lifestyle factors

In our study population, we observed that CCS were less likely to be overweight (unadjusted (unadj.): OR=0.59 (95%CI 0.36;0.96), adjusted (adj).: OR=0.56 (95%CI 0.34; 0.92)) or obese (unadj.: OR=0.48 (95%CI 0.27, 0.87), adj.: OR=0.51 (95%CI 0.27, 0.96)) than cancer-free controls (Table 3). In terms of physical activity, former cancer patients were less likely to exercise more than 5 hours per week than cancer-free controls (unadj.: OR=0.47 (95%CI 0.28; 0.82), adj.: OR =0.42 (95% CI 0.24, 0.73)). In addition, SPN and FPN subjects consumed fewer sugar-sweetened beverages than cancer-free controls. This decreased consumption was found to be statistically significant when consumption of less than one drink per day was compared to consumption of no drink (unadj.: OR=0.45 (95%CI 0.24; 0.86), adj.: OR=0.43 (95% CI 0.22; 0.82)). The comparison between CCS with SPN and FPN also showed that CCS with SPN drink more than one sweetened beverage per day less often than CCS with FPN only (unadj.: OR=0.41 (95%CI 0.18; 0.95), adj.: OR=0.42 (95% CI 0.18; 1.00)). We also observed differences in alcohol consumption per day. Here, an association for the comparison between more than one drink and no drink per day could be observed between CCS and cancer-free controls (unadj.: OR=0.34 (95%CI 0.14; 0.80), adj.: OR=0.30 (95% CI 0.12, 0.73)). In addition, a suggested association for the consumption of less than 1 alcoholic drink per day was found in the comparison between the two groups of CCS. However, this association was only significant in the unadjusted model and, when further adjustment variables were included, this result just exceeded the significance limit (SPN versus FPN unadj.: OR=0.46 (95%CI 0.27; 0.79), adj.: OR=0.55 (95% CI 0.29, 1.02)). While a conducted sensitivity analysis, comparing only leukemia CCS to cancer-free controls, also reveals a significant result in the consumption of more than one drink compared to no drink when comparing CCS and cancer-free controls as well as in the consumption of less than one drink compared to no drink when comparing CCS of SPN to CCS of FPN, a sensitivity analysis for CCS of lymphoma did not show any association (Supplementary Table 1). An even stronger association for the comparison of the consumption of more than one drink and no drink per day was found in a stratified analysis including only participants living together with a partner (adj. OR=0.12 (0.03; 0.57). It was also found that CCS were less likely to be current (unadj.: OR=0.45 (95%CI 0.25; 0.82), adj.: OR=0.43 (95%CI 0.24; 0.79)), former (unadj.: OR=0.28 (95%CI 0.17, 0.49), adj.: OR=0.25 (95%CI 0.15; 0.44)), or passive smokers (unadj.: OR=0.47 (95% CI 0.26; 0.85, Table 3 and Table 4) than cancer-free controls. This effect was consistent in all conducted sensitivity analyses and, again, even more pronounced in participants living together with a partner (Supplementary File 1). In the conducted sensitivity analysis including only CCS with lymphoma, moreover, CCS of SPN were found to be more often passive smokers than CCS of FPN (adj. OR=3.83 (1.04; 14.2, Supplementary Table 1). However, such an association was neither found in other stratified analyses nor in the analysis including all study participants. For the models on smoking, no further adjustments were necessary according to the DAGs. Based on the smoking status, it was also found that the number of pack years consumed was lower

1 http://www.dagitty.net/dags.html

TABLE 1 Distribution of cases (SPN and FPN) and controls (CO) of the KiKme study.

	Total (N=591)		SPN (N=101)		FPN (N=340)		CO (N=150)	
	n	%	n	%	n	%	n	%
Questinnaire available								
yes	**554**	**94%**	85	84%	325	96%	144	96%
no	**37**	**6%**	16	16%	15	4%	6	4%
Sex								
female	**301**	**51%**	50	50%	189	56%	62	41%
male	**290**	**49%**	51	50%	151	44%	88	59%
Age at interview	**591**							
<25 years	**100**	**17%**	9	9%	37	11%	54	36%
25-29 years	**106**	**18%**	14	14%	55	16%	37	25%
30-34 years	**113**	**19%**	17	17%	76	22%	20	13%
35-39 years	**111**	**19%**	21	21%	70	21%	20	13%
40 years or more	**124**	**21%**	24	24%	87	26%	13	9%
no questionnaire	**37**	**6%**	16	16%	15	4%	6	4%
Ethnicity								
Caucasian	**533**	**90%**	84	83%	312	92%	137	91%
other ethnicity[1]	**20**	**3%**	0	0%	12	4%	7	5%
no information	**38**	**6%**	17	17%	16	5%	6	4%
State								
Lower Saxony	**36**	**6%**	8	8%	28	8%	0	0%
North Rhine-Westphalia	**101**	**17%**	18	18%	80	24%	3	2%
Hesse	**60**	**10%**	7	7%	29	9%	24	16%
Rhineland-Palatinate	**127**	**21%**	0	0%	18	5%	109	73%
Baden-Wuerttemberg	**60**	**10%**	18	18%	41	12%	1	1%
Bavaria	**83**	**14%**	19	19%	64	19%	0	0%
every other German state[2]	**76**	**13%**	15	15%	59	17%	2	1%
outside Germany	**6**	**1%**	0	0%	6	2%	0	0%
no information	**42**	**7%**	16	16%	15	4%	11	7%
Height								
< 160cm	**51**	**9%**	10	10%	36	11%	5	3%
160 - <170cm	**190**	**32%**	35	35%	124	36%	31	21%
170 - <180cm	**166**	**28%**	21	21%	95	28%	50	33%
180 - <190cm	**113**	**19%**	15	15%	60	18%	38	25%
190cm or more	**33**	**6%**	4	4%	9	3%	20	13%
no information	**38**	**6%**	16	16%	16	5%	6	4%
Weight								
< 60kg	**102**	**17%**	20	20%	70	21%	12	8%
60 - <70kg	**134**	**23%**	19	19%	84	25%	31	21%
70 - <80kg	**98**	**17%**	17	17%	58	17%	23	15%
80 - <90kg	**99**	**17%**	14	14%	54	16%	31	21%
90 - <100kg	**61**	**10%**	10	10%	27	8%	24	16%
100kg or more	**55**	**9%**	4	4%	28	8%	23	15%
no information	**42**	**7%**	17	17%	19	6%	6	4%
Body Mass Index								
normal weight	**297**	**50%**	47	47%	178	52%	72	48%
overweight	**168**	**28%**	28	28%	93	27%	47	31%
obese	**84**	**14%**	9	9%	50	15%	25	17%
no information	**42**	**7%**	17	17%	19	6%	6	4%

(Continued)

TABLE 1 Continued

	Total (N=591)		SPN (N=101)		FPN (N=340)		CO (N=150)	
	n	%	n	%	n	%	n	%
Physical activity (hours per week)								
0 hours	**248**	**42%**	42	42%	149	44%	57	38%
1 - 2 hours	**84**	**14%**	18	18%	53	16%	13	9%
3 - 4 hours	**102**	**17%**	13	13%	67	20%	22	15%
5 hours or more	**110**	**19%**	8	8%	53	16%	49	33%
no information	**47**	**8%**	20	20%	18	5%	9	6%
Consumption of soft drinks per day								
0	**105**	**18%**	26	26%	63	19%	16	11%
<1	**298**	**50%**	43	43%	165	49%	90	60%
1 or more	**92**	**16%**	10	10%	60	18%	22	15%
no information	**96**	**16%**	22	22%	52	15%	22	15%
Alcoholic beverages per day								
0	**124**	**21%**	29	29%	70	21%	25	17%
<1	**359**	**61%**	46	46%	222	65%	91	61%
1 or more	**48**	**8%**	5	5%	24	7%	19	13%
no information	**60**	**10%**	21	21%	24	7%	15	10%
Smoking status								
never smoked	**348**	**59%**	57	56%	224	66%	67	45%
former smoker	**82**	**14%**	14	14%	44	13%	24	16%
current smoker	122	21%	14	14%	56	16%	52	35%
no information	**39**	**7%**	16	16%	16	5%	7	5%
Pack years								
never smoked	**348**	**59%**	57	56%	224	66%	67	45%
<5	**76**	**13%**	10	10%	47	14%	19	13%
5 or more	**103**	**17%**	18	18%	46	14%	39	26%
no information	**64**	**11%**	16	16%	23	7%	25	17%
Passive smoker								
yes	**70**	**12%**	10	10%	31	9%	29	19%
no	**464**	79%	75	74%	281	83%	108	72%
no information	**57**	**10%**	16	16%	28	8%	13	9%
Living situation								
living alone	**144**	**24%**	23	23%	75	22%	46	31%
living without a partner, with children	**23**	**4%**	6	6%	14	4%	3	2%
living with a partner, without children	**142**	**24%**	20	20%	85	25%	37	25%
living with a partner and children	**149**	**25%**	24	24%	102	30%	23	15%
living with parents	**67**	**11%**	11	11%	34	10%	22	15%
living in a shared apartment	**22**	**4%**	1	1%	11	3%	10	7%
other living situation[3]	**4**	**1%**	0	0%	2	1%	2	1%
no information	**40**	**7%**	16	16%	17	5%	7	5%
Main occupation								
still in training	**94**	**16%**	10	10%	38	11%	46	31%
working full time	**300**	**51%**	44	44%	185	54%	71	47%
working part time	**77**	**13%**	12	12%	55	16%	10	7%
housewife/-man	**21**	**4%**	4	4%	14	4%	3	2%
job seeking	**19**	**3%**	5	5%	8	2%	6	4%
pensioner or unemployable	**18**	**3%**	6	6%	10	3%	2	1%
other occupation[4]	**17**	**3%**	2	2%	12	4%	3	2%
no information	**45**	**8%**	18	18%	18	5%	9	6%

(Continued)

TABLE 1 Continued

	Total (N=591)		SPN (N=101)		FPN (N=340)		CO (N=150)	
	n	%	n	%	n	%	n	%
Highest school degree								
Volks-/Hauptschulabschluss	**76**	**13%**	14	14%	44	13%	18	12%
Realschulabschluss/Mittlere Reife	**152**	**26%**	27	27%	91	27%	34	23%
Fachhochschulreife	**75**	**13%**	4	4%	45	13%	26	17%
Abitur/Hochschulreife	**241**	**41%**	38	38%	140	41%	63	42%
no graduation (yet)	**5**	**1%**	2	2%	3	1%	0	0%
no information	**42**	**7%**	16	16%	17	5%	9	6%
Highest vocational education								
completed apprenticeship	**196**	**33%**	28	28%	123	36%	45	30%
graduated from vocational/business school	**72**	**12%**	12	12%	46	14%	14	9%
graduated from a technical college	**47**	**8%**	8	8%	26	8%	13	9%
graduated from college	**151**	**26%**	24	24%	102	30%	25	17%
no graduation (yet)	**71**	**12%**	11	11%	20	6%	40	27%
no information	**54**	**9%**	18	18%	23	7%	13	9%
International Standard Classification of Education								
no graduation (yet)	**5**	**1%**	2	2%	3	1%	0	0%
Sek I	**25**	**4%**	6	6%	6	2%	13	9%
Sek II	**322**	**54%**	45	45%	187	55%	90	60%
academic or equal	**198**	**34%**	32	32%	128	38%	38	25%
no information	**41**	**7%**	16	16%	16	5%	9	6%
Children								
0	**191**	**32%**	36	36%	117	34%	38	25%
1	**71**	**12%**	13	13%	50	15%	8	5%
2	**63**	**11%**	10	10%	45	13%	8	5%
3 or more	**31**	**5%**	6	6%	19	6%	6	4%
no information	**235**	**40%**	36	36%	109	32%	90	60%

cancer-free control (CO), first primary neoplasm (FPN), second primary neoplasm (SPN).
[1]Asian (total = 1%), Latino (1%), Caucasian/Latino (1%), Black (0.3%), North African (0.3%), Caucasian/Asian (0.3%), Caucasian/Black (0.2%).
[2]Schleswig-Holstein (total = 2%), Hamburg (2%), Berlin (2%), Saxony (2%), Bremen (1%), Saarland (1%), Brandenburg (1%), Mecklenburg-Western Pomerania (1%), Thuringia (1%), Saxony-Anhalt (0.3%).
[3]With family and partner (total = 0.3%), with siblings (0.3%).
[4]Parental leave (total = 1%), sheltered workshop (0.3%), internship/volunteering (0.3%), self-employed (0.2%), marginal employment (0.2%), other, not specified (1%).
Significant values were printed in bold.

among CCS than among cancer-free controls (unadj.: OR=0.21 (95%CI 0.12; 0.39), adj.: OR =0.17 (95%CI 0.09; 0.33), Table 3).

Association between cancer status and late adverse health effects

Overall, the CCS in our study had more serious illnesses (cancer excluded) than the cancer-free controls subjects (unadj.: OR =3.55 (95%CI 2.10, 6.01), adj.: OR=3.32 (95%CI 1.95, 5.65), Table 4). In the analysis of individual diseases, it was found that CCS suffer more frequently from thyroid diseases (unadj.: OR=15.01 (95%CI 5.64; 39.95), adj.: OR=14.70 (95%CI 5.49, 39.39)) and hypercholesterolemia (unadj.: OR=6.84 (95%CI 2.03, 23.04), adj.: OR=7.21 (95%CI 2.13; 24.42)) compared to cancer-free controls. In addition, it was found that CCS were more likely to take regular medication than cancer-free controls (unadj.: OR=2.30 (95% CI 1.35; 3.92), no further adjustment according to DAGs necessary). Here, the adjusted comparison between the CCS groups with SPN and with FPN only additionally showed that in our study population CCS of SPN took more medication than those with FPN only (OR=2.53 (95%CI 1.01; 6,30)). All other explorative conducted analyses on cardiovascular, chronic lung, inflammatory bone, allergic, and infectious diseases did not show any associations.

TABLE 2 Distribution of variables on health status of cases (SPN and FPN) and controls (CO).

	Total (N=591)		SPN (N=101)		FPN (N=340)		CO (N=150)	
	n	%	n	%	n	%	n	%
Therapy for FPN								
no cancer therapy	**3**	**1%**	1	1%	2	1%	0	0%
radiotherapy	**4**	**1%**	3	3%	1	0%	0	0%
chemotherapy	**102**	**17%**	15	15%	87	26%	0	0%
radio- and chemotherapy	**209**	**35%**	46	46%	163	48%	0	0%
only other cancer therapy (e.g., operation)	**1**	**0%**	0	0%	1	0%	0	0%
radio- and other cancer therapy	**1**	**0%**	0	0%	1	0%	0	0%
chemo- and other cancer therapy	**18**	**3%**	4	4%	14	4%	0	0%
radio-, chemo- and other cancer therapy	**63**	**11%**	13	13%	50	15%	0	0%
no information	**190**	**32%**	19	19%	21	6%	150	100%
Family with possible Li-Fraumeni syndrome								
yes	**73**	**12%**	21	21%	52	15%	0	0%
no	**483**	**82%**	64	63%	273	80%	146	97%
no information	**35**	**6%**	16	16%	15	4%	4	3%
Regular medication								
yes	**298**	**50%**	65	64%	180	53%	53	35%
no	**247**	**42%**	19	19%	140	41%	88	59%
no information	**46**	**8%**	17	17%	20	6%	9	6%
Any diseases								
yes	**379**	**64%**	70	69%	241	71%	68	45%
no	**174**	**29%**	15	15%	84	25%	75	50%
no information	**38**	**6%**	16	16%	15	4%	7	5%
Number of diseases								
0	**174**	**29%**	15	15%	84	25%	75	50%
1	**182**	**31%**	28	28%	116	34%	38	25%
2	**114**	**19%**	26	26%	68	20%	20	13%
3	**49**	**8%**	10	10%	29	9%	10	7%
4 or more	**34**	**6%**	6	6%	28	8%	0	0%
no information	**38**	**6%**	16	16%	15	4%	7	5%
Diabetes								
yes	**26**	**4%**	4	4%	22	6%	0	0%
no	**519**	**88%**	79	78%	301	89%	139	93%
no information	**46**	**8%**	18	18%	17	5%	11	7%
Thyroid diseases (without cancer)								
yes	**140**	**24%**	18	18%	117	34%	5	3%
no	**384**	**65%**	42	42%	208	61%	134	89%
no information	**67**	**11%**	41	41%	15	4%	11	7%
Hypercholesterolemia								
yes	**66**	**11%**	15	15%	48	14%	3	2%
no	**475**	**80%**	67	66%	274	81%	134	89%
no information	**50**	**8%**	19	19%	18	5%	13	9%
Cardiovascular diseases (hypertension, heart attack, or stroke)								
yes	**69**	**12%**	13	13%	46	14%	10	7%
no	**469**	**79%**	69	68%	271	80%	129	86%
no information	**53**	**9%**	19	19%	23	7%	11	7%

(Continued)

TABLE 2 Continued

	Total (N=591)		SPN (N=101)		FPN (N=340)		CO (N=150)	
	n	%	n	%	n	%	n	%
Hypertension								
yes	**61**	**10%**	11	11%	40	12%	10	7%
no	**482**	**82%**	71	70%	282	83%	129	86%
no information	**48**	**8%**	19	19%	18	5%	11	7%
Heart attack								
yes	**4**	**1%**	0	0%	4	1%	0	0%
no	**540**	**91%**	83	82%	318	94%	139	93%
no information	**47**	**8%**	18	18%	18	5%	11	7%
Stroke								
yes	**6**	**1%**	2	2%	4	1%	0	0%
no	**534**	**90%**	81	80%	314	92%	139	93%
no information	**51**	**9%**	18	18%	22	6%	11	7%
Chronic lung diseases								
yes	**64**	**11%**	**9**	9%	35	10%	20	13%
no	**481**	**81%**	**73**	72%	287	84%	121	81%
no information	**46**	**8%**	19	19%	18	5%	9	6%
Inflammatory bone diseases								
yes	**66**	**11%**	9	9%	42	12%	15	10%
no	**476**	**81%**	72	71%	280	82%	124	83%
no information	**49**	**8%**	20	20%	18	5%	11	7%
Allergic diseases (hay fever or neurodermatitis)								
yes	**156**	**26%**	24	**24%**	91	27%	41	27%
no	**387**	**65%**	59	58%	228	67%	100	67%
no information	**48**	**8%**	18	18%	21	6%	9	6%
Hay fever								
yes	**120**	**20%**	17	17%	72	21%	31	21%
no	**423**	**72%**	66	65%	248	73%	109	73%
no information	**48**	**8%**	18	18%	20	6%	10	7%
Neurodermatitis								
yes	**53**	**9%**	8	8%	32	9%	13	9%
no	**492**	**83%**	75	74%	290	85%	127	85%
no information	**46**	**8%**	18	18%	18	5%	10	7%
Infections (hepatitis, Epstein-Barr virus, or HIV)								
yes	**79**	**13%**	17	17%	51	15%	11	7%
no	**441**	**75%**	66	65%	272	80%	103	69%
no information	**71**	**12%**	18	18%	17	5%	36	24%
Hepatitis								
yes	**11**	**2%**	4	4%	6	2%	1	1%
no	**508**	**86%**	79	78%	316	93%	113	75%
no information	**72**	**12%**	18	18%	18	5%	36	24%
Epstein-Barr virus								
yes	**68**	**12%**	13	13%	45	13%	10	7%
no	**453**	**77%**	70	69%	279	82%	104	69%
no information	**70**	**12%**	18	18%	16	5%	36	24%
HIV								
yes	**1**	**0%**	1	1%	0	0%	0	0%
no	**518**	**88%**	82	81%	322	95%	114	76%
no information	**72**	**12%**	18	18%	18	5%	36	24%

(Continued)

TABLE 2 Continued

	Total (N=591)		SPN (N=101)		FPN (N=340)		CO (N=150)	
	n	%	n	%	n	%	n	%
Health score[1]								
< 0.75 points	**276**	**47%**	44	44%	152	45%	80	53%
0.75 points	**154**	**26%**	23	23%	100	29%	31	21%
> 0.75 points	**123**	**21%**	18	18%	72	21%	33	22%
no information	**38**	**6%**	16	16%	16	5%	6	4%

cancer-free control (CO), first primary neoplasm (FPN), second primary neoplasm (SPN).
[1]Score includes the following variables: number of diseases: 0-2 (1 point (p.)), 3 or more (0 p.); Body Mass Index: <18.5 (0 p.), 18.5-30 (1 p.), >30 (0 p.); International Standard Classification of Education (ISCED): Sek II or academic (1 p.), no graduation or Sek I (0 p.); smoking status: never (1 p.), former or current (0 p.); alcoholic beverages/day: <1 (1 p.), 1 or more (0 p.); consumption of softdrinks: no (1 p.), yes (0 p.); hours of physical activity/week: 5 hours or more (1 p.), 0-4 hours (0 p.); current occupation: occupied (1 p.), unemployable or pensioner (0 p.). At least 4 items of the score have to be answered. To account for missing values, the sum score was divided by the number of answered variables.
Significant values were printed in bold.

Association between cancer status and a calculated overall health score

The majority of the study participants (n=276, 47%) achieved a score below 0.75 points in our health score (Table 2). About a quarter (n=154, 26%) of the participants achieved exactly 0.75 points. Only 123 participants (21%) reached the highest category with a score above 0.75 points. In addition to the subjects without a questionnaire, one additional participant had to be excluded from the health score analysis since the required 4 answers for the calculation of the score were not given. The multinomial logistic regression on cancer status and calculated health score did not show any associations (Table 5).

Discussion

Within the presented study, we attempted to complete the overall picture of the associations between childhood cancer and long-term effects on health and lifestyle factors. We show that CCS and cancer-free controls as well as CCS with and without subsequent SPN differ in terms of their health and lifestyle.

Although the CCS in our study were less likely to exercise extensively, they were less likely to be overweight or obese than cancer-free controls. Even if physical activity is known to reduce the risk of long-term adverse health outcomes after childhood cancer (40, 41) studies have shown that about 50% of CCS in western countries do not meet the recommended time of physical activity per day (42). This reduced time of physical activity among CCS might be due to poorer overall health. In this regard, a Swiss study showed that physical activity was reduced in CCS, particularly when they either had relapse or suffer from musculoskeletal or neurological disorders (42). Similar to our findings on weight status, a cohort of French leukemia survivors identified significant differences regarding the prevalence of metabolic syndrome and BMI between former acute lymphatic leukemia patients and cancer-free controls (43). In addition, they found differences in socio-economic status, education, occupation, and smoking habits. Whereas education was found to be an important adjustment variable in nearly all of our models and was therefore not investigated as an outcome, we were able to identify differences in smoking habits in our sample. Our CCS were less likely to be current, former, or passive smokers. This effect was even more pronounced in participants living together with a partner. Along with this healthier attitude towards smoking habits, there was also reduced consumption of alcohol among the CCS in our study sample. Here again, an even more reduced consumption was found in participants living in a partnership. Similar findings were also reported by Frobisher et al. (28), who reported that CCS living without a partner tend to consume alcohol more often than married ones. Moreover, Brinkman et al. (26) showed that CCS were less likely to be heavy or risky drinkers compared to their siblings. In general, the reduced alcohol consumption might be associated with the identified higher intake of regular medication in the CCS group, since the consumption of alcohol may interact with prescribed medications (44). However, this possible association was taken into account when creating the DAGs and here, it was shown that no further adjustment according to medication intake is necessary for the analysis of the association between cancer status and alcohol consumption. Regarding the increased regular intake of medication in the CCS group, our findings are in line with those from other research groups. Within the large American cohort of the Childhood Cancer Survivor Study it was found that CCS were more likely to take medication for hypertension, dyslipidemia, or diabetes compared to their siblings (21). However, at the same time, they were neither more often obese nor did they show more cardiovascular risk factors than their healthy siblings.

Although some other studies have found evidence of an association between cardiovascular diseases and childhood cancer (17–19), we have not observed such an association in our data. The absence of this known association can have various

TABLE 3 Multinomial logistic regression on cancer status and risk of late effects[1].

Cancer status	n	%	n	%	n	%	n	%	n	%	n	%	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
													adjusted for matchinggroup, birth year, and age at interview			adjusted for matchinggroup, birth year, age at interview, and other variables		
Body Mass Index																		
	total (N=591)		**missings (N=42)**		**normal weight (N=297)**		**overweight (N=168)**		**obesity (N=84)**				**overweight vs. normal weight**	**obesity vs. normal weight**		**overweight vs. normal weight**	**obesity vs. normal weight**	
CO	**150**	**25%**	6	14%	72	24%	47	28%	25	30%			Ref.	Ref.		Ref.[2]	Ref.[2]	
FPN and SPN	**441**	**75%**	36	86%	225	76%	121	72%	59	70%			**0.587 (0.359; 0.96)**	**0.479 (0.265; 0.866)**		**0.561 (0.344; 0.915)**	**0.506 (0.268; 0.958)**	
FPN	**340**	**77%**	19	53%	178	79%	93	77%	50	85%			Ref.	Ref.		Ref.[3]	Ref.[3]	
SPN	**101**	**23%**	17	47%	47	21%	28	23%	9	15%			1.144 (0.659; 1.986)	0.686 (0.305; 1.543)		1.183 (0.659; 2.126)	0.532 (0.198; 1.431)	
Physical activity (hours per week)																		
	total (N=591)		**missings (N=47)**		**0 hours (N=248)**		**1-2 hours (N=84)**		**3-4 hours (N=102)**		**5+ hours (N=110)**		**1-2 hours vs. 0 hours**	**3-4 hours vs. 0 hours**	**5+ hours vs. 0 hours**	**1-2 hours vs. 0 hours**	**3-4 hours vs. 0 hours**	**5+ hours vs. 0 hours**
CO	**150**	**25%**	9	19%	57	23%	13	15%	22	22%	49	45%	Ref.	Ref.	Ref.	Ref.[4]	Ref.[4]	Ref.[4]
FPN and SPN	**441**	**75%**	38	81%	191	77%	71	85%	80	78%	61	55%	1.684 (0.805; 3.524)	1.194 (0.648; 2.199)	**0.474 (0.276; 0.815)**	1.491 (0.691; 3.214)	1.089 (0.577; 2.055)	**0.416 (0.236; 0.734)**
FPN	**340**	**77%**	18	47%	149	78%	53	75%	67	84%	53	87%	Ref.	Ref.	Ref.	Ref.[4]	Ref.[4]	Ref.[4]
SPN	**101**	**23%**	20	53%	42	22%	18	25%	13	16%	8	13%	1.19 (0.614; 2.306)	0.632 (0.304; 1.312)	0.507 (0.211; 1.222)	1.261 (0.63; 2.525)	0.671 (0.315; 1.43)	0.544 (0.22; 1.344)
Consumption of soft drinks per day																		
	total (N=591)		**missing (N=96)**		**0 (N=105)**		**<1 (N=298)**		**1+ (N=92)**				**<1 vs. 0**	**1+ vs. 0**		**<1 vs. 0**	**1+ vs. 0**	
CO	**150**	**25%**	22	23%	16	15%	90	30%	22	24%			Ref.	Ref.		Ref.[4]	Ref.[4]	
FPN and SPN	**441**	**75%**	74	77%	89	85%	208	70%	70	76%			**0.453 (0.24; 0.856)**	0.734 (0.324; 1.664)		**0.426 (0.222; 0.819)**	0.699 (0.303; 1.612)	
FPN	**340**	**77%**	52	70%	63	71%	165	79%	60	86%			Ref.	Ref.		Ref.[4]	Ref.[4]	
SPN	**101**	**23%**	22	30%	26	29%	43	21%	10	14%			0.65 (0.363; 1.166)	**0.41 (0.176; 0.953)**		0.676 (0.378; 1.212)	0.423 (0.179; 1)	
Alcoholic beverages per day																		
	total (N=591)		**missings (N=60)**		**0 (N=124)**		**<1 (N=359)**		**1+ (N=48)**				**<1 vs. 0**	**1+ vs. 0**		**<1 vs. 0**	**1+ vs. 0**	
CO	**150**	**25%**	15	25%	25	20%	91	25%	19	40%			Ref.	Ref.		Ref.[2]	Ref.[2]	
FPN and SPN	**441**	**75%**	45	75%	99	80%	268	75%	29	60%			0.715 (0.421; 1.216)	**0.34 (0.144; 0.8)**		0.663 (0.376; 1.171)	**0.296 (0.121; 0.727)**	
FPN	**340**	**77%**	24	53%	70	71%	222	83%	24	83%			Ref.	Ref.		Ref.[3]	Ref.[3]	
SPN	**101**	**23%**	21	47%	29	29%	46	17%	5	17%			**0.459 (0.267; 0.788)**	0.449 (0.136; 1.482)		0.546 (0.294; 1.015)	0.475 (0.13; 1.74)	

(Continued)

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TABLE 3 Continued

Cancer status	n	%	n	%	n	%	n	%	n	%	n	%	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
													adjusted for matchinggroup, birth year, and age at interview			adjusted for matchinggroup, birth year, age at interview, and other variables		
													Smoking status					
	total (N=591)		missing (N=39)		never (N=348)		former (N=82)		current (N=122)				former vs. never	current vs. never		former vs. never	current vs. never	
CO	**150**	**25%**	7	18%	67	19%	24	29%	52	43%			Ref.	Ref.		Ref.[4]	Ref.[4]	
FPN and SPN	**441**	**75%**	32	82%	281	81%	58	71%	70	57%			**0.283 (0.165; 0.486)**	**0.453 (0.251; 0.816)**		**0.252 (0.146; 0.435)**	**0.43 (0.235; 0.787)**	
FPN	**340**	**77%**	16	50%	224	80%	44	76%	56	80%			Ref.	Ref.		Ref.[5]	Ref.[5]	
SPN	**101**	**23%**	16	50%	57	20%	14	24%	14	20%			0.866 (0.43; 1.742)	1.334 (0.68; 2.617)		0.99 (0.448; 2.186)	1.435 (0.704; 2.925)	
													Pack years					
	total (N=591)		missings (N=64)		never smoked (N=348)		<5 (N=76)		5+ (N=103)				<5 vs. never smoked	5+ vs. never smoked		<5 vs. never smoked	5+ vs. never smoked	
CO	**150**	**25%**	25	39%	67	19%	19	25%	39	38%			Ref.	Ref.		Ref.[4]	Ref.[4]	
FPN and SPN	**441**	**75%**	39	61%	281	81%	57	75%	64	62%			0.814 (0.435; 1.523)	**0.213 (0.115; 0.393)**		0.837 (0.436; 1.608)	**0.173 (0.09; 0.333)**	
FPN	**340**	**77%**	23	59%	224	80%	47	82%	46	72%			Ref.	Ref.		Ref.[5]	Ref.[5]	
SPN	**101**	**23%**	16	41%	57	20%	10	18%	18	28%			0.928 (0.437; 1.97)	1.571 (0.796; 3.1)		1.092 (0.455; 2.622)	1.547 (0.682; 3.509)	

Adjustment variables were selected using directed acyclic graphs.
Confidence interval (CI), cancer-free control (CO), first primary neoplasm (FPN), International Standard Classification for Education (ISCED), odds ratio (OR), second primary neoplasm (SPN).
[1]Missing values are shown but not included in the analysis.
[2]Additionally adjusted for ISCED and ethnicity.
[3]Additionally adjusted for ISCED, ethnicity, and therapy of FPN.
[4]Additionally adjusted for ISCED.
[5]Additionally adjusted for ISCED and therapy of FPN.
Significant values were printed in bold.

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TABLE 4 Comparison of serum IL-4, PGE2 and AGEs in each group.

Cancer status	n	%	n	%	n	%	n	%	OR (95% CI)	OR (95% CI)
									adjusted for matchinggroup, birth year, and age at interview	adjusted for matchinggroup, birth year, age at interview, and other variables
									Passive smoker	
	total (N=591)		**missing (N=57)**		**no (N=464)**		**yes (N=70)**		**yes vs. no**	**yes vs. no**
CO	**150**	**25%**	13	23%	108	23%	29	41%	Ref.	Ref.[2]
FPN and SPN	**441**	**75%**	44	77%	356	77%	41	59%	**0.471 (0.261; 0.849)**	**0.471 (0.261; 0.849)**
FPN	**340**	**77%**	28	64%	281	79%	31	76%	Ref.	Ref.[3]
SPN	**101**	**23%**	16	36%	75	21%	10	24%	1.206 (0.55; 2.646)	1.19 (0.465; 3.048)
									Regular medication	
	total (N=591)		**missing (N=46)**		**no (N=247)**		**yes (N=298)**		**yes vs. no**	**yes vs. no**
CO	**150**	**25%**	9	20%	88	36%	53	18%	Ref.	Ref.[2]
FPN and SPN	**441**	**75%**	37	80%	159	64%	245	82%	**2.301 (1.352; 3.917)**	**2.301 (1.352; 3.917)**
FPN	**340**	**77%**	20	54%	140	88%	180	73%	Ref.	Ref.[3]
SPN	**101**	**23%**	17	46%	19	12%	65	27%	2.938 (0.77; 11.212)	**2.527 (1.013; 6.304)**
									Any disease	
	total (N=591)		**missing (N=70)**		**no (N=175)**		**yes (N=379)**		**yes vs. no**	**yes vs. no**
CO	**150**	**25%**	7	18%	75	43%	68	18%	Ref.	Ref.[4]
FPN and SPN	**441**	**75%**	31	82%	99	57%	311	82%	**3.549 (2.095; 6.014)**	**3.322 (1.952; 5.652)**
FPN	**340**	**77%**	15	48%	84	85%	241	77%	Ref.	Ref.[5]
SPN	**101**	**23%**	16	52%	15	15%	70	23%	1.903 (0.883; 4.101)	1.531 (0.735; 3.189)
									Thyroid diseases (without cancer)	
	total (N=591)		**missing (N=67)**		**no (N=384)**		**yes (N=140)**		**yes vs. no**	**yes vs. no**
CO	**150**	**25%**	11	16%	134	35%	5	4%	Ref.	Ref.[4]
FPN and SPN	**441**	**75%**	56	84%	250	65%	135	96%	**15.007 (5.636; 39.954)**	**14.703 (5.488; 39.387)**
FPN	**340**	**77%**	15	27%	208	83%	117	87%	Ref.	Ref.[5]
SPN	**101**	**23%**	41	73%	42	17%	18	13%	0.702 (0.362; 1.361)	0.658 (0.309; 1.402)
									Hypercholesterolemia	
	total (N=591)		**missing (N=50)**		**no (N=475)**		**yes (N=66)**		**yes vs. no**	**yes vs. no**
CO	**150**	**25%**	13	26%	134	28%	3	5%	Ref.	Ref.[4]
FPN and SPN	**441**	**75%**	37	74%	341	72%	63	95%	**6.836 (2.028; 23.04)**	**7.205 (2.126; 24.424)**
FPN	**340**	**77%**	18	49%	274	80%	48	76%	Ref.	Ref.[5]
SPN	**101**	**23%**	19	51%	67	20%	15	24%	1.215 (0.622; 2.37)	1.29 (<0.001; >999.99)

(Continued)

TABLE 4 Continued

Cancer status	n	%	n	%	n	%	n	%	OR (95% CI) adjusted for matchinggroup, birth year, and age at interview	OR (95% CI) adjusted for matchinggroup, birth year, age at interview, and other variables
Cardiovascular diseases (hypertension, heart attack (SPN=0, FPN=4, CO=0), or stroke (SPN=2, FPN=4, CO=0))										
	total (N=591)		**missing (N=53)**		**no (N=469)**		**yes (N=69)**		**yes vs. no**	**yes vs. no**
CO	**150**	**25%**	11	21%	129	28%	10	14%	Ref.	Ref.[4]
FPN and SPN	**441**	**75%**	42	79%	340	72%	59	86%	1.765 (0.836; 3.725)	1.487 (0.693; 3.192)
FPN	**340**	**77%**	23	55%	271	80%	46	78%	Ref.	Ref.[5]
SPN	**101**	**23%**	19	45%	69	20%	13	22%	1.194 (0.59; 2.417)	1.006 (0.433; 2.336)
Hypertension										
	total (N=591)		**missing (N=48)**		**no (N=482)**		**yes (N=61)**		**yes vs. no**	**yes vs. no**
CO	**150**	**25%**	11	23%	129	27%	10	16%	Ref.	Ref.[4]
FPN and SPN	**441**	**75%**	37	77%	353	73%	51	84%	1.493 (0.69; 3.229)	1.283 (0.582; 2.83)
FPN	**340**	**77%**	18	49%	282	80%	40	78%	Ref.	Ref.[5]
SPN	**101**	**23%**	19	51%	71	20%	11	22%	1.141 (0.529; 2.465)	0.942 (0.379; 2.343)
Chronic lung diseases										
	total (N=591)		**missing (N=46)**		**no (N=481)**		**yes (N=64)**		**yes vs. no**	**yes vs. no**
CO	**150**	**25%**	9	20%	121	25%	20	31%	Ref.	Ref.[4]
FPN and SPN	**441**	**75%**	37	80%	360	75%	44	69%	0.741 (0.394; 1.392)	0.598 (0.305; 1.176)
FPN	**340**	**77%**	18	49%	287	80%	35	80%	Ref.	Ref.[5]
SPN	**101**	**23%**	19	51%	73	20%	9	20%	1.082 (0.485; 2.416)	0.767 (0.275; 2.137)
Inflammatory bone diseases										
	total (N=591)		**missing (N=49)**		**no (N=476)**		**yes (N=66)**		**yes vs. no**	**yes vs. no**
CO	**150**	**25%**	11	22%	124	26%	15	23%	Ref.	Ref.[4]
FPN and SPN	**441**	**75%**	38	78%	352	74%	51	77%	0.737 (0.383; 1.421)	0.776 (0.397; 1.516)
FPN	**340**	**77%**	18	47%	280	80%	42	82%	Ref.	Ref.[5]
SPN	**101**	**23%**	20	53%	72	20%	9	18%	0.877 (0.401; 1.919)	0.79 (0.313; 1.996)
Allergic diseases (hay fever or neurodermatitis)										
	total (N=591)		**missing (N=48)**		**no (N=387)**		**yes (N=156)**		**yes vs. no**	**yes vs. no**
CO	**150**	**25%**	9	19%	100	26%	41	26%	Ref.	Ref.[4]
FPN and SPN	**441**	**75%**	39	81%	287	74%	115	74%	0.916 (0.572; 1.465)	0.83 (0.509; 1.352)
FPN	**340**	**77%**	21	54%	228	79%	91	79%	Ref.	Ref.[5]
SPN	**101**	**23%**	18	46%	59	21%	24	21%	1.1 (0.63; 1.921)	0.993 (0.495; 1.994)

(Continued)

TABLE 4 Continued

Cancer status	n	%	n	%	n	%	n	%	OR (95% CI) adjusted for matchinggroup, birth year, and age at interview	OR (95% CI) adjusted for matchinggroup, birth year, age at interview, and other variables
									Hay fever	
	total (N=591)		**missing (N=48)**		**no (N=423)**		**yes (N=120)**		**yes vs. no**	**yes vs. no**
CO	**150**	**25%**	10	21%	109	26%	31	26%	Ref.	Ref.[4]
FPN and SPN	**441**	**75%**	38	79%	314	74%	89	74%	0.898 (0.539; 1.494)	0.817 (0.481; 1.388)
FPN	**340**	**77%**	20	53%	248	79%	72	81%	Ref.	Ref.[5]
SPN	**101**	**23%**	18	47%	66	21%	17	19%	0.944 (0.51; 1.747)	0.88 (0.093; 8.297)
									Neurodermatitis	
	total (N=591)		**missing (N=46)**		**no (N=492)**		**yes (N=53)**		**yes vs. no**	**yes vs. no**
CO	**150**	**25%**	10	22%	127	26%	13	25%	Ref.	Ref.[4]
FPN and SPN	**441**	**75%**	36	78%	365	74%	40	75%	1.264 (0.609; 2.622)	1.225 (0.577; 2.601)
FPN	**340**	**77%**	18	50%	290	79%	32	80%	Ref.	Ref.[5]
SPN	**101**	**23%**	18	50%	75	21%	8	20%	1.046 (0.445; 2.463)	0.816 (0.267; 2.493)
									Infections (hepatitis (SPN=4, FPN=6, CO=1), Epstein-Barr virus, or HIV (SPN=1, FPN=0, CO=0))	
	total (N=591)		**missing (N=71)**		**no (N=441)**		**yes (N=79)**		**yes vs. no**	**yes vs. no**
CO	**150**	**25%**	36	51%	103	23%	11	14%	Ref.	Ref.[4]
FPN and SPN	**441**	**75%**	35	49%	338	77%	68	86%	1.757 (0.874; 3.533)	1.831 (0.9; 3.725)
FPN	**340**	**77%**	17	49%	272	80%	51	75%	Ref.	Ref.[5]
SPN	**101**	**23%**	18	51%	66	20%	17	25%	1.306 (0.701; 2.43)	1.43 (0.689; 2.97)
									Epstein-Barr virus infection	
	total (N=591)		**missing (N=70)**		**no (N=453)**		**yes (N=68)**		**yes vs. no**	**yes vs. no**
CO	**150**	**25%**	36	51%	104	23%	10	15%	Ref.	Ref.[4]
FPN and SPN	**441**	**75%**	34	49%	349	77%	58	85%	1.637 (0.791; 3.392)	1.689 (0.805; 3.544)
FPN	**340**	**77%**	16	47%	279	80%	45	78%	Ref.	Ref.[5]
SPN	**101**	**23%**	18	53%	70	20%	13	22%	1.039 (0.529; 2.039)	1.16 (0.529; 2.543)

Adjustment variables were selected using directed acyclic graphs.
Confidence interval (CI), cancer-free control (CO), first primary neoplasm (FPN), human immunodeficiency virus (HIV), International Standard Classification for Education (ISCED), odds ratio (OR), second primary neoplasm (SPN).
[1]Missing values are shown but not included in the analysis.
[2]DAGs identified no additional adjustment variables for this model.
[3]Additionally adjusted for therapy of FPN.
[4]Additionally adjusted for families with possible Li-Fraumeni syndrome.
[5]Additionally adjusted for therapy of FPN and families with possible Li-Fraumeni syndrome.
Significant values were printed in bold.

reasons: On the one hand, with a mean age of 32 years, we have a relatively young cohort (36). Moreover, we overall observed only very few cardiovascular events in our cohort, of which most were related to the presence of hypertension. However, due to the young cohort, in the course of the advancing observation period and the ongoing survival time, further cardiovascular diseases could occur. It can already be seen in our cohort that CCS more often suffer from disorders of the lipid metabolism, which is one of the main risk factors for the development of cardiovascular diseases (19, 45). Besides a higher prevalence of lipid metabolism

TABLE 5 Multinomial logistic regression on cancer status and calculated health score[1].

Cancer status	n	%	n	%	n	%	n	%	n	%	OR (95% CI) adjusted for matchinggroup, birth year, and age at interview	OR (95% CI) adjusted for matchinggroup, birth year, and age at interview[2]	OR (95% CI) adjusted for matchinggroup, birth year, and age at interview	OR (95% CI) adjusted for matchinggroup, birth year, and age at interview[2]
Health score (max. 1 point (p.); all participants)														
	total (N=591)		**missings (N=37)**		**< 0.75 p. (N=276)**		**0.75 p. (N=154)**		**> 0.75 p. (N=124)**		**< 0.75 p. vs. > 0.75 p.**	**0.75 p. vs. > 0.75 p.**	**< 0.75 p. vs. > 0.75 p.**	**0.75 p. vs. > 0.75 p.**
CO	**150**	**25%**	6	16%	80	29%	31	20%	33	27%	Ref.	Ref.	Ref.	Ref.
FPN and SPN	**441**	**75%**	31	84%	196	71%	123	80%	91	73%	0.745 (0.445; 1.247)	1.616 (0.871; 2.998)	0.687 (0.4; 1.181)	1.425 (0.744; 2.731)
FPN	**340**	**77%**	15	48%	152	78%	100	81%	73	80%	Ref.	Ref.	Ref.	Ref.
SPN	**101**	**23%**	16	52%	44	22%	23	19%	18	20%	1.112 (0.595; 2.078)	0.913 (0.456; 1.827)	1.06 (0.563; 1.995)	0.78 (0.382; 1.592)
Health score (max. 1 p.; females)														
	total (N=301)		**missings (N=14)**		**< 0.75 p. (N=134)**		**0.75 p. (N=93)**		**> 0.75 p. (N=60)**		**< 0.75 p. vs. > 0.75 p.**	**0.75 p. vs. > 0.75 p.**	**< 0.75 p. vs. > 0.75 p.**	**0.75 p. vs. > 0.75 p.**
CO	**62**	**21%**	3	21%	31	23%	17	18%	11	18%	Ref.	Ref.	Ref.	Ref.
FPN and SPN	**239**	**79%**	11	79%	103	77%	76	82%	49	82%	0.701 (0.308; 1.598)	1.088 (0.442; 2.681)	0.597 (0.247; 1.443)	1.103 (0.412; 2.95)
FPN	**189**	**79%**	5	45%	80	78%	62	82%	42	86%	Ref.	Ref.	Ref.	Ref.
SPN	**50**	**21%**	6	55%	23	22%	14	18%	7	14%	1.681 (0.655; 4.312)	1.397 (0.514; 3.794)	1.623 (0.624; 4.22)	1.318 (0.479; 3.631)
Health score (max. 1 p.; males)														
	total (N=290)		**missings (N=23)**		**< 0.75 p. (N=142)**		**0.75 p. (N=61)**		**> 0.75 p. (N=64)**		**< 0.75 p. vs. > 0.75 p.**	**0.75 p. vs. > 0.75 p.**	**< 0.75 p. vs. > 0.75 p.**	**0.75 p. vs. > 0.75 p.**
CO	**88**	**30%**	3	13%	49	35%	14	23%	22	34%	Ref.	Ref.	Ref.	Ref.
FPN and SPN	**202**	**70%**	20	87%	93	65%	47	77%	42	66%	0.812 (0.404; 1.631)	1.839 (0.759; 4.46)	0.679 (0.263; 1.752)	1.274 (0.491; 3.308)
FPN	**151**	**75%**	10	50%	72	77%	38	81%	31	74%	Ref.	Ref.	Ref.	Ref.
SPN	**51**	**25%**	10	50%	21	23%	9	19%	11	26%	0.765 (0.304; 1.926)	0.626 (0.204; 1.922)	0.739 (0.283; 1.929)	0.491 (0.152; 1.585)

Confidence interval (CI), cancer-free control (CO), first primary neoplasm (FPN), odds ratio (OR), second primary neoplasm (SPN).

[1]Score includes the following variables: number of diseases: 0-2 (1 point (p.)), 3 or more (0 p.); Body Mass Index: <18.5 (0 p.), 18.5-30 (1 p.), >30 (0 p.); International Standard Classification for Education (ISCED): Sek II or academic (1 p.), no graduation or Sek I (0 p.); smoking status: never (1 p.), former or current (0 p.); alcoholic beverages/day: <1 (1 p.), 1 or more (0 p.); consumption of soft drinks: no (1 p.), yes (0 p.); hours of physical activity/week: 5 hours or more (1 p.), 0-4 hours (0 p.); current occupation: occupied (1 p.), unemployable or pensioner (0 p.). At least 4 items of the score have to be answered. To account for missing values, the sum score was divided by the number of answered variables. Missing values are shown but not included in the analysis.

[2]All models were additionally adjusted for ethnicity and families with possible Li-Fraumeni syndrome. Adjustment variables were selected using directed acyclic graphs.

Significant values were printed in bold.

disorders, the CCS of the KiKme study suffered from thyroid disorders significantly more frequently than cancer-free controls. Thyroid disorders are well-known adverse late health effects of cancer therapies and especially of cancer therapies in childhood (46). This known association between thyroid diseases and cancer therapy is well illustrated in our data as well since the strong effect observed when comparing CCS and cancer-free controls disappears when comparing SPN and FPN, both of whom received some type of cancer therapy.

Besides the confirmation of known associations in our data, we attempted to generate new hypotheses on novel associations between cancer status and adverse late health effects of childhood cancer as well as lifestyle parameters. Within the comparison between cases and controls, no new hypotheses could be generated. However, to the best of our knowledge, this study is the first one to investigate differences between CCS with a single diagnosis in childhood and CCS with multiple primary malignancies.

This comparison between CCS groups showed that CCS with SPN took more medication than those with FPN. This result complements the previously described hypothesis of reduced alcohol consumption with regular medication intake. It was also shown here, even if the result just exceeds the significance limit in the adjusted model (Table 3), that CCS with SPN, who take significantly more medication, drink alcoholic beverages (less than 1 drink/day compared to no drink) less frequently than CCS with FPN only. No difference was found for higher amounts (more than one drink/day) of alcohol per day. In addition, there

was also a difference in the consumption of more than 1 soft drink per day, even if the significance limit in the adjusted model was also slightly exceeded here. Again, CCS with SPN were found to drink sugar-sweetened beverages less frequently than CCS with FPN (Table 3). This could be an indication of a more conscious lifestyle in general. These findings, however, need to be confirmed in larger studies.

Regarding the associations between cancer status and a calculated overall health score, other than expected from us, no difference was observed by cancer status in our study. This null result may be explained by the fact that, as our results showed, the cases might have a higher disease burden but live a healthier lifestyle overall. The cancer-free control group, on the other hand, appears to be healthier but have an unhealthier lifestyle. Thus, for the health score, which includes both health and lifestyle factors, the total scores obtained by cases and controls may annihilate.

Strengths and limitations

Regarding strengths and limitations, this unique cohort of CCS with and without subsequent SPN and cancer-free controls is the first to carry out differentiated analyzes on cancer and late health effects as well as on differences in lifestyle, also at the level of different numbers of cancer diagnoses.

All information for the conducted analyses was self-reported by the participants and therefore might underlie a certain recall bias. However, by collecting self-reported data, we were able to get information on a large number of variables that enables us to extensively adjust our models. Moreover, we succeeded to collect not only information from the subjects themselves but also collected information on the family history of severe diseases, which allows us to adjust for familial predispositions to some extent.

As with all self-reported epidemiological studies, our study underlies an inherent survivor bias as only living patients could be recruited. Severe cases with high mortality (e.g. acute myeloid leukemia after acute lymphocytic leukemia or 2 diagnoses in quick succession) cannot be covered to a full extent by this study. Moreover, a selection bias cannot be ruled out in this study, as individuals with serious health problems may be less motivated to participate and the recruitment of cancer-free controls was regionally limited due to logistic reasons. Moreover, cancer-free controls were found to be slightly younger then participating CCS. In addition, the statistical power of the study is limited by the sample size. The number of available former childhood cancer patients was restricted by the number of CCS meeting the inclusion criteria that were registered at the German Childhood Cancer Registry. The sample size and the rather short follow-up time of CCS result in a small number of adverse health outcomes, especially for rare diseases such as heart attack, stroke, or serious infectious diseases. However, the number of late adverse health outcomes may increase during the further follow-up of our cohort. The cohort thus offers the possibility for extensive analyzes of late effects of childhood cancer in the future. With an increasing number of outcomes, more differentiated investigations, e.g., concerning the type, number, and localization of received therapies, can also be considered.

Conclusion

Overall, a different general state of health and different health behaviors could be identified between CCS and cancer-free controls. Although CCS seem to have healthier lifestyles than cancer-free controls, including less soft drink and alcohol consumption as well as less tobacco smoking and lower body mass index, they are more likely to have serious illnesses. In detail, the results of this study conducted on German CCS and cancer-free controls, confirm that thyroid diseases without thyroid cancer and disorders of the lipid metabolism may be more common in CCS than in cancer-free controls. As a consequence of the higher disease burden, CCS, particularly those with SPN, may take more regular medication. In addition, CCS seem to be less physically active than cancer-free controls, which might be explained by their higher disease burden. The higher overall disease burden is likely related to previous cancer therapies. Based on these findings, research into improving cancer therapies and starting points for reducing long-term consequences should continue in the future. Moreover, we recommend that former childhood cancer patients be closely monitored by their treating physicians, not only with regard to cancer follow-up, but especially with regard to possible potential risk factors for the development of late adverse health effects. Here in particular, lipid metabolism disorders should be treated to prevent the development of cardiovascular disease. In addition, survivors should be encouraged to achieve the recommended time of physical activity, as this has been identified in the past as protective for the development of various adverse health outcomes in cancer survivors.

Data availability statement

The datasets presented in this article are not readily available because of ethic and data protection reasons, but are available from the corresponding author on reasonable request. Requests to access the datasets should be directed to the Leibniz Institute for Prevention Research and Epidemiology – BIPS, Bremen, Germany.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethik-Kommission of the Landesärztekammer Rheinland-Pfalz, Mainz, Germany. The patients/participants provided their written informed consent to participate in this study.

Authors contributions

MM is the principal investigator of the KiKme study and developed its design, which was implemented and monitored by MM and LB. DG supported the development of strategies for the recruitment of former childhood cancer patients. MM, LB, DGa, and SZ conducted the recruitment of the participants, which was organized and planned by MM and LB. MM, LB, and HS monitored the recruitment of cancer-free controls. LB, MM, RF, and AP developed the analysis pipelines for the project. HSz, DGa, SZ, TH, ML, AP, DGr, MB, and HS contributed to the writing process, which was initially prepared by LB, RF, and MM. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.1037276/full#supplementary-material

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Publication 5

Self-administered questionnaire assessing childhood cancer treatments and associated risks for adverse health outcomes – The KiKme Study (First Author)

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Self-administered questionnaire assessing childhood cancer treatments and associated risks for adverse health outcomes - The KiKme study

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Background: Childhood cancer survivors (CCS) are at particularly high risk for therapy-related late sequelae, with secondary primary neoplasms (SPN) being the most detrimental. Since there is no standardized questionnaire for retrospective assessment of associations between prior cancer treatments and late health effects, we developed a self-administered questionnaire and validated it in a cohort of CCS.

Methods: CCS of a first primary neoplasm (FPN, N=340) only or with a subsequent SPN (N=101) were asked whether they had received cancer therapies. Self-reports were compared to participants' medical records on cancer therapies from hospitals and clinical studies (N=242). Cohen's Kappa (κ) was used to measure their agreement and logistic regression was used to identify factors influencing the concordance. Associations between exposure to cancer therapies and late health effects (overweight/obesity, diseases of the lipid metabolism and the thyroid gland, cardiovascular diseases, occurrence of SPN) were analyzed in all participants by applying generalized linear mixed models to calculate odds ratios (OR) and 95% confidence intervals (95%CI).

Results: For CCS of SPN, a perfect agreement was found between self-reports and medical records for chemotherapy (CT, κ=1.0) while the accordance for radiotherapy (RT) was lower but still substantial (κ=0.8). For the CCS of FPN the accordance was less precise (CT: κ=0.7, RT: κ=0.3). Cancer status, tumors of the central nervous system, sex, age at recruitment, vocational training, follow-up time, and comorbidities had no impact on agreement. CCS with exposure to CT

were found to be less often overweight or obese compared to those without CT (OR=0.6 (95%CI 0.39; 0.91)). However, they were found to suffer more likely from thyroid diseases excluding thyroid cancers (OR=9.91 (95%CI 4.0; 24.57)) and hypercholesterolemia (OR=4.45 (95%CI 1.5; 13.23)). All other analyses did not show an association.

Conclusion: Our new questionnaire proved reliable for retrospective assessment of exposure to CT and RT in CCS of SPN. For the CCS of FPN, self-reported RT was very imprecise and should not be used for further analyses. We revealed an association between late health outcomes occurring as hypercholesterolemia and thyroid diseases, excluding thyroid cancer, and the use of CT for the treatment of childhood cancer.

KEYWORDS

childhood cancer survivors (CCS), second malignancies, radiotherapy, chemotherapy, body mass index - BMI, thyroid diseases, lipid metabolism, validation

1 Background

Patients with childhood cancer are often treated with radiotherapy (RT) and/or chemotherapy (CT) (1). Over the last decades, these therapies for childhood cancer have improved significantly, which have been accompanied by improvements in long-term survival (2, 3). However, since these therapies not only affect the tumor but also healthy tissues, they are known factors associated with the development of second primary neoplasms after childhood cancer (4) or can result in several late adverse health effects (5). Despite similar therapies, not all childhood cancer survivors (CCS) suffer from long-term health effects. Data from 2018 showed that around 8% of the CCS listed in the German Childhood Cancer Registry develop a second primary neoplasm (SPN) within the next 35 years (6). In addition to this particularly serious late adverse health outcome after primary cancer treatment in childhood, CCS are at increased risk for chronic cardiovascular or lung diseases, as well as infertility (7–12). The risk of such late-occurring health issues seems to be associated with the dose of RT and CT (11, 13, 14).

In a large cohort of CCS it has been shown that a reduction in radiation exposure during therapy leads to fewer cardiac events in adulthood (2). In particular, irradiation of the mediastinum or spinal cord, for example in the context of treatment for Hodgkin's lymphoma or tumors of the central nervous system (CNS), is considered as a risk factor for the development of cardiac disease later in life (15). Similarly, CCS are at increased risk of developing restrictive lung diseases after thoracic RT. Due to the formation of the lung alveoli in the first few years of life, exposure to ionizing radiation at this age is moreover associated with reduced lung capacity (15).

In the case of both RT and CT, the dose of therapy received is of importance for the development of heart and lung diseases later in life (16). While treatment with high doses of CT agents is associated with an increased risk of cardiac events (17), lower doses are associated primarily with conditions considered to be risk factors for the development of late cardiac events (18).

RT and CT are used not only as definitive treatments/monotherapy, but also as part of multimodal therapy strategies. A combination of RT and CT has become established as the standard treatment for many cancer sites (19), as the combination of systemically acting CT and RT often achieves better therapeutic results (20). In this combination, the systematically acting CT acts by a radiosensitization mechanism that involves making tumor cells more sensitive to RT (21, 22). Due to an additive or synergistic effect of this multimodal therapy, CCS are at increased risk for the development of late adverse health effects (e.g., cardiovascular, hematological, neurological, pulmonary, and renal conditions) compared to CCS treated with monotherapy (22).

In addition to RT and CT or the combination of these two therapeutic strategies, childhood cancers are now also treated with targeted and cancer-specific approaches. Immunologic and targeted therapies are increasingly finding their way into the treatment of pediatric cancers because, unlike CT and RT, they are cancer-specific and not genotoxic, and thus may reduce the risk of late effects (23).

Malignant diseases of the hematopoietic or lymphatic system, which occur particularly frequently in childhood, are often successfully treated with hematopoietic stem cell transplantation (23, 24). In order to prevent rejection reactions such as graft-versus-host diseases after transplantation, post-transplant treatments with

Abbreviations: CCS, Childhood cancer survivors; CI, Confidence interval; CO, Cancer-free controls; CT, Chemotherapy; DAG, Directed acyclic graph; FPN, Childhood cancer survivors with a first primary neoplasm only; GLMM, Generalized linear mixed model; NPV, Negative predictive value; OR, Odds ratio; RT, Radiotherapy; PPV, Positive predictive value; SPN, Childhood cancer survivors with at least one second primary neoplasm.

immunosuppressants are required. Due to this combination of therapies, recipients of stem cell transplants have a further increased risk of late adverse health effects such as diseases of the kidney and liver, development of SPN, as well as overall reduced quality of life (24).

Despite the important role of cancer therapies in the development of late adverse health effects after surviving childhood cancer, research on accurate exposure measures of cancer therapies in childhood often remains challenging due to a lack of valid information on cancer therapies in many epidemiologic studies. To our knowledge, the only attempt of asking young adults about their exposure to cancer therapies in childhood was done *via* telephone interviews within the Childhood Cancer Survivor Study in the early 2000s (25). However, until now, there is no established self-administered questionnaire to retrospectively assess exposures to RT, CT, or other cancer therapies as well as to diagnostic procedures in childhood among a population of young adults. Particularly in countries where the linkage of different medical data (e.g., from hospitals, outpatient care, registries, and health insurance companies) has so far only been possible to a limited extent and often only at great expense for reasons of data protection (26) or infrastructural issues, such a questionnaire would be of great benefit to be able to ask study participants in an uncomplicated way for information on cancer therapies received. Therefore, a new self-administered questionnaire, which consists of a total of 62 items in total, was developed and applied within the population of the nested case-control study KiKme (27). Nine of the questionnaire items collect detailed information about lifetime medical exposures to radiation and cancer therapies.

To validate this new questionnaire, this study aims, first, to compare self-reported exposure to cancer therapies with information on cancer treatment from medical records. Therefore, a subsample of study participants with complete information from both questionnaires and medical records from hospitals and therapy-optimizing studies will be used. Secondly, influencing factors on concordance between the questionnaire and medical records will be analyzed. Finally, reliable self-reported information from our questionnaire will be used to estimate possible associations of exposure to cancer therapies with the risk of late adverse health effects within the KiKme study population.

2 Materials and methods

2.1 Study design and participants

The study participants were recruited within the population-based nested case-control study KiKme. Detailed information on recruiting strategies and data collection can be found in the study protocol (27). In brief, the KiKme study population included 441 CCS, registered at the German Childhood Cancer Registry. CCS were grouped into survivors with a first primary neoplasm (FPN, n=340) only and survivors with a subsequent SPN (n=101). CCS with FPN only were used as cancer controls and were matched to participating CCS with an SPN by age, sex, cancer site, year of diagnosis, and age at diagnosis. In addition, the study population includes 150 cancer-free controls that were recruited at the Department of Orthopedics and Trauma Surgery at the Johannes Gutenberg-University in Mainz (Germany). Cancer-free controls were matched by sex and age to the SPN and FPN survivors.

2.2 Data collection

The data collection in the study was done using our newly developed questionnaire which was self-administered by all participants. In 62 questions the study participants were asked to provide information about their demographics, health and health-related behaviors, regular medication, as well as severe diseases in their families. The study participants were also allowed to obtain information from others, e.g., their parents, in order to answer the questionnaire.

Based on anthropometric information on weight and height, normal weight was defined as Body Mass Index (BMI) of 18.5 and < 25 kg/m^2, overweight as BMI ≥25 kg/m^2, and obesity as BMI ≥30 kg/m^2 according to the WHO standards. To assess their medical history and health status, participants were asked whether they had been diagnosed with any severe disease, including hypercholesterolemia, hypertension, heart attack, stroke, and thyroid diseases. Additionally, age at diagnosis was requested.

Besides the questions on anthropometric factors and health, the questionnaire included nine questions on medical therapies and lifetime exposure to ionizing radiation. Within these nine questions participants were asked whether they had ever received cancer therapy (RT, CT, or other cancer therapy). If so, they were asked in what year, at what age, how often, and with what doses they were treated. Also, information on affected body regions and substances was collected. They were asked whether they had ever had diagnostic or interventional exposures, including radiographic examination, such as for fractures, pneumonia, surgery, or dental examinations, computed tomography, positron and single photon emission computed tomography, magnetic resonance imaging, minimally invasive radiological intervention, and thyroid radioiodine therapy.

To validate the information from the questionnaire, we used available data from medical records on cancer therapies recorded by treating hospitals or therapy-optimizing studies from a subsample of our participants and compared them with participants' self-reported information.

2.3 Statistical *analysis*

Descriptive analyses were performed on age, sex, cancer diagnoses, subsequent therapies, and exposure to medical diagnostics. Results were stratified by cancer status (SPN, FPN, and cancer-free controls) and frequency (N) and proportions (%) were provided for summary statistics.

A quality assessment was performed to determine the validity and agreement of self-reported information on therapy (received RT/CT: yes/no) with the information from the medical records.

This was measured by Cohen's kappa coefficient (κ) (28). Influencing factors (sex, age, number of neoplasms, tumors of the CNS, vocational training, comorbidities, time since cancer treatment) on the concordance between the questionnaire and medical records were analyzed using logistic regression.

We applied generalized linear mixed models (GLMM) to the self-reported information from questionnaires to analyze the statistical association between exposure to cancer therapies and risk of later occurring adverse health effects as well as to calculate odds ratios (OR) and 95% confidence intervals (95%CI). For the analysis of adverse health effects after exposure to cancer therapies, CCS of FPN and CCS of SPN were aggregated to ensure a sufficiently large cell population for each adverse health effect occurring after the FPN and prior to a possible SPN. For CCS of SPN, only therapies for the FPN were included in analyses. For the analysis of the occurrence of an SPN later in life, cancer-free control patients were excluded. In our models, each matching group was treated as a random effect. Additionally, 'age' and 'year of birth' were included as fixed effects in all models to improve matching efficiency for the variable 'age at recruitment' within the specified 5-year period (27). Possible additional adjustment variables were considered after drawing a Directed Acyclic Graph (DAG) that was carefully developed based on prior knowledge using DAGitty (version 3.0)[1] (see Supplementary File 1).

Survival analysis using Kaplan-Meier curves was applied to describe and compare the cumulative incidence curves of the onset of late adverse health effects by cancer site (leukemia, lymphoma, and tumors of the central nervous system) and stratified by self-reported cancer therapy. For this purpose, the year of diagnosis of the late adverse disease was subtracted from the year of reported therapy. All statistical analyses for this publication were performed using SAS 9.3 (SAS Institute Inc., Cary, North Carolina, USA).

3 Results

3.1 Study characteristics

This study includes 591 participants of which 51% were females (Table 1). The mean age at the interview was 35.14 years (standard deviation (SD): 7.14; range: 19.90-51.40 years) for CCS of SPN, 34.84 years (SD: 7.68; range: 19.60-54.50 years) for CCS of FPN, and 28.91 years (SD: 7.32; range: 18.70-48.20 years) for cancer-free controls. The interviews were conducted on average 27.26 years (SD: 6.90; range 5.0-38.0 years) after the first cancer diagnosis in CCS of SPN and 26.24 years (SD: 6.93; range: 4-39 years) after the first diagnosis in CCS of FPN. Leukemia and lymphoma were most commonly treated with both RT and CT in our study (leukemia: N=105, 50%, lymphoma: N=85, 47%, Supplementary Figures 1A, B), regardless of the chronological order of the two therapies. For tumors of the CNS either RT and CT or a combination with an additional therapy (e.g., stem cell transplantation) was most likely (N=17, 29% for both, Supplementary Figure 1C). Further characteristics of the study participants including detailed information on cancer diagnoses and treatment as well as on exposure to medical diagnostics are summarized in Table 1. Participants who did not provide information in self-administered questionnaires (N=37, 6%) were excluded from the analyses. For 272 (46%, 93 CCS of SPN and 179 CCS of FPN) of the KiKme study participants information was available from medical records (Table 2). Of these participants, 235 (86%) had received RT or CT, five (2%) participants had only undergone a stem cell transplant, and for another two (1%) participants no therapy was indicated. For the remaining 30 (11%) study participants, information on cancer therapies from medical records was not available.

3.2 Association between self-reported cancer therapies and medical records

A perfect agreement (κ=1.0) was found between self-reports on CT from CCS of SPN and their corresponding information from medical records (Table 3). Overall, 71 (97%) CCS of SPN reported receiving CT and only two (3%) reported not receiving CT. The agreement for RT was lower but substantial (κ=0.77). Three (5%) CCS of SPN misreported on RT, while there was an agreement between both data sources for the remaining 59 (95%) CCS of SPN. Overall, the group of CCS of FPN reported less accurately. For CT, a moderate agreement was observed (κ=0.66). However, only one (1%) CCS of FPN misreported by indicating no CT in the self-reported questionnaire whereas there was information on CT available in the medical records. The other 140 (99%) CCS of SPN with available information on CT reported correctly. The lowest and only fair agreement was found for RT in CCS of FPN (κ=0.31). Whereas 105 (93%) CCS of FPN reported correctly on RT, a total of eight (7%) CCS of FPN reported that they did not receive RT while RT was documented in their medical records. Using logistic regression, none of the variables (cancer status, sex, age at recruitment, tumors of the CNS, vocational training, follow-up time, and comorbidities) had significant impact on the agreement between self-reported or clinically documented RT (Table 4).

3.3 Exposure to cancer therapies and risk of later occurring adverse health effects

Since CCS provided valid self-reports of CT, we analyzed potential associations of this treatment with adverse health effects. The results showed that CCS treated with CT were found to be less often overweight or obese compared to CCS without CT (OR=0.6 (95%CI 0.39; 0.91), Table 5). A total of 140 (24%) of the study participants reported on diseases of the thyroid gland (Table 1). Here, only non-malignant diseases were considered as thyroid diseases and malignant diseases of the thyroid gland were considered as SPN. Thyroid diseases occurred more often in participants with CT (OR=9.91 (95% CI 4.0; 24.57), Table 5). Similar results were obtained for hypercholesterolemia: Participants treated with CT were found to suffer more likely from such disorders of lipid

1 http://www.dagitty.net/dags.html.

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TABLE 1 Description of the study population.

	CCS of SPN (N=101)		CCS of FPN (N=340)		CO (N=150)		Total (N=591)	
	n	%	n	%	n	%	n	%
Questionnaire available								
Yes	85	84%	325	96%	144	96%	554	94%
No	16	16%	15	4%	6	4%	37	6%
Sex								
Female	50	50%	189	56%	62	41%	301	51%
Male	51	50%	151	44%	88	59%	290	49%
Age at interview								
< 25 years	9	9%	37	11%	54	36%	100	17%
25-29 years	14	14%	55	16%	37	25%	106	18%
30-34 years	17	17%	76	22%	20	13%	113	19%
35-39 years	21	21%	70	21%	20	13%	111	19%
≥ 40 years	24	24%	87	26%	13	9%	124	21%
Cancer site of FPN								
Leukemia	41	41%	166	49%	–	–	207	35%
Lymphoma	41	41%	135	40%	–	–	176	30%
Brain & CNS	15	15%	35	10%	–	–	50	8%
Other tumors	4	4%	4	1%	–	–	8	1%
Cancer site of SPN								
Thyroid cancer	30	30%	–	–	–	–	30	5%
Skin carcinoma	32	32%	–	–	–	–	32	5%
Malignant melanoma	4	4%	–	–	–	–	4	1%
Leukemia	9	9%	–	–	–	–	9	2%
Lymphoma	6	6%	–	–	–	–	6	1%
Brain & CNS	9	9%	–	–	–	–	9	2%
Breast cancer	3	3%	–	–	–	–	3	1%
Other unspecific carcinoma	7	7%	–	–	–	–	7	1%
Sarcoma	2	2%	–	–	–	–	2	0%
Radiotherapy								
Ever	68	67%	222	65%	3	2%	293	50%
For FPN diagnosis	62	61%	215	63%	–	–	277	47%
For SPN diagnosis	7	7%	2	1%	–	–	9	2%
For other diseases	0	0%	0	0%	3	2%	3	1%
Never	15	15%	95	28%	140	93%	250	42%
No information	18	18%	23	7%	7	5%	48	8%
Chemotherapy								
Ever	82	81%	314	92%	0	0%	396	67%
For FPN diagnosis	78	77%	314	92%	–	–	392	66%

(Continued)

TABLE 1 Continued

	CCS of SPN (N=101)		CCS of FPN (N=340)		CO (N=150)		Total (N=591)	
	n	%	n	%	n	%	n	%
For SPN diagnosis	14	14%	0	0%	–	–	14	2%
Never	2	2%	7	2%	114	76%	123	21%
No information	17	17%	19	6%	36	24%	72	12%
Other cancer therapies								
Ever	59	58%	79	23%	0	0%	138	23%
For FPN diagnosis	17	17%	66	19%	–	–	83	14%
Surgery	*17*	*17%*	*56*	*16%*	-	-	*73*	*12%*
Other cancer therapies[1]	*2*	*2%*	*9*	*3%*	-	-	*11*	*2%*
For SPN diagnosis	52	51%	4	1%	–	–	56	9%
Surgery	*47*	*47%*	*4*	*1%*	-	-	*51*	*9%*
Other cancer therapies[1]	*2*	*2%*	*0*	*0%*	-	-	*2*	*0%*
For further cancer diagnosis	5	5%	0	0%	–	–	5	1%
Surgery	*3*	*3%*	*0*	*0%*	-	-	*3*	*1%*
Never	23	23%	226	66%	113	75%	362	61%
No information	19	19%	35	10%	37	25%	91	15%
X-ray examinations								
Ever	84	83%	310	91%	141	94%	535	91%
Never	0	0%	5	1%	3	2%	8	1%
No information	17	17%	25	7%	6	4%	48	8%
Computed tomography examinations								
Ever	73	72%	237	70%	68	45%	378	64%
Never	6	6%	56	16%	67	45%	129	22%
No information	22	22%	47	14%	15	10%	84	14%
Positron emission tomography								
Ever	29	29%	69	20%	0	0%	98	17%
Never	32	32%	186	55%	138	92%	356	60%
No information	40	40%	85	25%	12	8%	137	23%
Magnetic resonance imaging								
Ever	75	74%	229	67%	109	73%	413	70%
Never	5	5%	56	16%	32	21%	93	16%
No information	21	21%	55	16%	9	6%	85	14%
Minimally invasive radiological intervention								
Ever	13	13%	30	9%	7	5%	50	8%
Never	58	57%	261	77%	142	95%	461	78%
No information	30	30%	49	14%	1	1%	80	14%
Thyroid radioiodine therapy								
Ever	22	22%	26	8%	1	1%	49	8%

(Continued)

TABLE 1 Continued

	CCS of SPN (N=101)		CCS of FPN (N=340)		CO (N=150)		Total (N=591)	
	n	%	n	%	n	%	n	%
Never	58	57%	261	77%	142	95%	461	78%
No information	21	21%	53	16%	7	5%	81	14%
Weight status								
Underweight (BMI < 18.5 kg/m²)	2	2%	11	3%	3	2%	16	3%
Normal weight (BMI ≥ 18.5 - < 25 kg/m²)	45	45%	167	49%	69	46%	281	48%
Overweight (BMI ≥ 25 - < 30 kg/m²)	28	28%	93	27%	47	31%	168	28%
Obesity (BMI ≥ 30 kg/m²)	9	9%	50	15%	25	17%	84	14%
No information	17	17%	19	6%	6	4%	42	7%
Thyroid diseases (without cancer)[2]								
Yes	18	18%	117	34%	5	3%	140	24%
No	42	42%	208	61%	134	89%	384	65%
No information	41	41%	15	4%	11	7%	67	11%
Hypercholesterolemia								
Yes	15	15%	48	14%	3	2%	66	11%
No	67	66%	274	81%	134	89%	475	80%
No information	19	19%	18	5%	13	9%	50	8%
Cardiovascular diseases[3]								
Yes	13	13%	46	14%	10	7%	69	12%
No	69	68%	271	80%	129	86%	469	79%
No information	19	19%	23	7%	11	7%	53	9%

BMI, body mass index; CCS, childhood cancer survivors; CO, cancer-free control; FPN, first primary neoplasm; SPN, second primary neoplasm.
[1]Other cancer therapies include stem cell transplantation and other medication.
[2]Malignant thyroid diseases were considered as SPN.
[3]Including hypertension, heart attack, or stroke.

metabolism (OR=4.45 (95%CI 1.5; 13.23)). No difference was found for the occurrence of cardiovascular diseases (OR=1.46 (95%CI 0.71; 3.01)) and second primary neoplasms (OR=0.28 (95%CI 0.05; 1.47)). The association between exposure to RT and late adverse health effects in the group of CCS of SPN could not be calculated due to small sample sizes (Table 6).

397 CCS with RT and/or CT were included in the Kaplan-Meier analysis. Figures 1, 2 illustrate the cumulative incidence curves for late adverse diseases of the thyroid gland (excluding thyroid cancer) after exposure to cancer therapy for leukemia, lymphoma, and CNS tumors. The median follow-up time was 26.48 years (SD: 6.84 years, range: 4.0-36.0 years). After RT or CT, 26 (25%) or 38 (23%) CCS of leukemia, 54 (52%) or 56 (39%) CCS of lymphoma and 16 (52%) or 16 (47%) CCS of CNS tumors developed a non-malignant thyroid disease, respectively. The 20-year disease-free survival after primary cancer diagnosis was 54%, 64% in the group of leukemia CCS, 52% in CCS of lymphoma and 37% in CCS of CNS tumors. There were no remarkable differences between cancer sites in long-time survival for the other late adverse health outcomes.

4 Discussion

This study was successful in the validation of the newly developed self-administered questionnaire on the retrospective assessment of exposure to cancer therapies in childhood, especially regarding CT. Based on the data collected in this way, we demonstrated an impact of CT on health-related late effects in the cohort of CCS of the KiKme study. CCS with CT in childhood were found to suffer more likely from diseases of the thyroid gland and lipid metabolism. They were also less likely to be overweight or obese compared to CCS without CT. Self-reporting of RT in childhood was too imprecise to investigate associations with potential late effects.

4.1 Agreement between self-reported exposure and medical records

Similar analyses on the agreement between self-reported cancer therapy and medical records were previously conducted on

TABLE 2 Available therapy information from medical records of KiKme study participants.

	CCS of SPN (N=93)		CCS of FPN (N=179)		Total (N=272)	
	n	%	n	%	n	%
Radio- and/or chemotherapy	86	92%	149	83%	235	86%
Only radiotherapy	1	1%	3	2%	4	1%
Only chemotherapy	20	22%	31	17%	51	19%
Radiochemotherapy	65	70%	115	64%	180	66%
Stem cell transplantation[1]	10	11%	9	5%	19	7%
Only stem cell transplantation	0	0%	5	3%	5	2%
Stem cell transplantation with radio-/chemotherapy	10	11%	4	2%	14	5%
No therapy	2	2%	0	0%	2	1%
Missing data	5	5%	25	14%	30	11%

CCS, childhood cancer survivors; FPN, first primary neoplasm; SPN, second primary neoplasm.
[1]Information on stem cell transplantation was not actively collected, available data are incidental findings. The actual number of transplantations is probably higher.

survivors of adult breast cancer (29–35) or several other tumor entities (25, 32, 36).

The studies from other research groups showed good to very good agreements between self-reported exposure to CT and data from medical records. However, the follow-up period of the other studies was rather short. A study on breast cancer survivors by Kool et al. (35) found a high agreement for exposure to CT (κ=0.95) in a sample of 350 study participants after a short follow-up of 9 to 18 months after tumor surgery. An even shorter follow-up period was found in the study by Gupta and colleagues (34), where breast cancer survivors were asked about their disease and therapy approximately 6.5 months after their diagnosis. Considering that CT starts about 1 month after diagnosis and lasts for about one month, the time between last CT and interview was only about 4.5 months. The authors found moderate to excellent agreement for CT (81.7-98.0%). Besides the short time span between therapy and interview, patients in this study were provided with detailed information about their disease and therapy when they are discharged from the hospital, which might have contributed to the good agreement. In our study, study participants were asked about their exposure to CT about 27 years after the first cancer diagnosis in childhood. Nonetheless, we found similar rates of agreement for CT using our new developed questionnaire. The generally high compliance with CT might be attributed to recollection of the severe acute side effects of this treatment. This was also assumed in a recent review of self-reported medication in cancer survivors from Brüne et al. (32).

Contrary to the good agreement for CT, the self-reported exposure to RT was less precise in our study. Similarly, Gupta et al. (34) also report poor agreement with respect to RT. While 32.1% of participants reported RT, it was applied in only 4.9% of cases based on their medical records. Gupta et al. justify this phenomenon with the fact that RT is not used as first-line therapy in the curative treatment of breast cancer. Because RT is only used as a palliative or second/third-line therapy when surgery and CT were not able to control tumor growth or metastatic spread at a later time after diagnosis, it may not have been as well remembered by participants as CT. In contrast to our results and the results from Gupta et al., the study on breast cancer survivors by

TABLE 3 Concordance between self-reported exposure to cancer therapies and data from medical records.

	Information from medical records											
	CCS of SPN				CCS of FPN				Total			
	Received chemo-therapy		*Received radia-tion therapy*		*Received chemo-therapy*		*Received radia-tion therapy*		*Received chemo-therapy*		*Received radia-tion therapy*	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Questionnaire data												
Received therapy												
Yes	71	0	53	2	139	0	103	0	210	0	156	2
No	0	2	1	6	1	1	8	2	1	3	9	8
K	1.00		0.77		0.66		0.31		0.85		0.56	

CCS, childhood cancer survivors; FPN, first primary neoplasm; SPN, second primary neoplasm.

TABLE 4 Influencing factors on the correlation between self-reported exposure to radiotherapy and data from medical records of participants from the KiKme study.

	Total (n=175)		Not concordant (n=11)		Concordant (n=164)		OR (95% CI)
	n	%	n	%	n	%	
Second primary neoplasm							
No	113	65%	8	73%	105	64%	Ref.
Yes	62	35%	3	27%	59	36%	1.08 (0.25; 4.75)
Sex							
Female	102	58%	9	82%	93	57%	Ref.
Male	73	42%	2	18%	71	43%	2.43 (0.46; 12.88)
Age							
< 35 years	74	42%	5	45%	69	42%	Ref.
≥ 35 years	101	58%	6	55%	95	58%	1.14 (0.16; 7.97)
Tumors of the CNS							
No	152	87%	10	91%	142	87%	Ref.
Yes	23	13%	1	9%	22	13%	1.35 (0.13; 13.99)
Vocational training							
Non-academic	98	56%	6	55%	92	56%	Ref.
Academic	64	37%	3	27%	61	37%	1.26 (0.29; 5.43)
Missing	13	7%	2	18%	11	7%	
Follow-up time							
< 25 years	67	38%	5	45%	62	38%	Ref.
≥ 25 years	108	62%	6	55%	102	62%	2.24 (0.34; 14.54)
Comorbidities[1]							
No	39	22%	2	18%	37	25%	Ref.
Yes	136	78%	9	82%	127	75%	1.39 (0.27; 7.34)

CI, confidence interval; CNS, central nervous system; OR, odds ratio.
[1]Comorbidities included diabetes, hypercholesterolemia, hypertension, lung diseases such as asthma or bronchitis, hay fever, inflammatory joint or vertebral diseases including arthrosis and rheumatism, neurodermatitis, heart attack, stroke, thyroid diseases, Epstein-Barr virus infections, HIV, Hepatitis, or any other severe disease.

Kool et al. (35) found a high agreement for exposure to RT (κ=0.94) 9 to 18 months after tumor surgery. In addition, Roberts et al. (36) also reported a high agreement for exposure to RT (92%). However, they only investigated pelvic RT in the course of impact of cancer treatments on fertility in 101 young adult female cancer survivors. One possible cause for the differences in agreement regarding RT between the study of Roberts et al. and our study could be their underlying research question and the associated study population. They examined the impact of cancer and cancer treatments on reproductive health. In this context, pelvic RT as a potential cause of infertility might be particularly remembered by the respondents.

However, with the exception of the study by Roberts et al. (36), which includes survivors that were diagnosed during childhood or early adulthood, all other beforementioned validation studies have been conducted in adults. To the best of our knowledge, the only other study that ever requested information from young adults about their exposure to cancer therapies in childhood was the Childhood Cancer Survivor Study (25). In the early 2000s they completed telephone interviews and compared information from their study participants to therapy information assessed at the baseline survey. In total, they found a high agreement for exposure to CT (94%) and RT (89%) in their survey. In their validation study, participants who received input from others during interviews were excluded. In contrast, we gave our self-administered questionnaires to our study participants and gave them as much time as they needed to fill them out. They were also allowed to gather as much information from others as they wanted. Likely, many of the survivors who suffered from cancer in their early childhood cannot remember the therapy exactly when they are adults (25). In contrast, memory is likely to be very present in relatives, especially parents, for many years after therapy. To obtain the most accurate information possible, we encouraged our

TABLE 5 Self-reported exposure to chemotherapy and risk of later adverse health effects in participants of the KiKme case-control study.

	n	%	n	%	n	%	OR (95% CI)
Weight status							
	Total (N=538)		Underweight or normal weight (N=292)		Overweight or obese (N=246)		Overweight/obesity vs. underweight/normal weight
Chemotherapy	388	72%	215	74%	173	70%	0.60 (0.39; 0.91)
No chemotherapy	150	28%	77	26%	73	30%	Ref.
Thyroid diseases (without cancer)							
	Total (N=497)		Yes (N=117)		No (N=380)		Yes vs. no
Chemotherapy	354	71%	111	95%	243	64%	9.91 (4.00; 24.57)
No chemotherap	143	29%	6	5%	137	36%	Ref.
Hypercholesterolemia							
	Total (N=520)		Yes (N=56)		No (N=464)		Yes vs. no
Chemotherapy	377	73%	52	93%	325	70%	4.45 (1.50; 13.23)
No chemotherapy	143	28%	4	7%	139	30%	Ref.
Cardiovascular diseases							
	Total (N=519)		Yes (N=60)		No (N=459)		Yes vs. no
Chemotherapy	374	72%	49	82%	325	71%	1.46 (0.71; 3.01)
No chemotherapy	145	28%	11	18%	134	29%	Ref.
Second primary neoplasm[1]							
	Total (N=398)		Yes (N=81)		No (N=317)		Yes vs. no
Chemotherapy	392	98%	78	96%	314	99%	0.28 (0.05; 1.47)
No chemotherapy	6	2%	3	4%	3	1%	Ref.

All analyses were adjusted for the matching group, birth year, and age at the interview. For CCS of SPN only chemotherapy for the first primary neoplasm was included in analyses. Participants with missing information were excluded from analysis.
CCS, childhood cancer survivors; CI, confidence interval; OR, odds ratio; SPN, second primary neoplasm.
[1]Cancer-free control patients were excluded from this analysis.

participants to also obtain information from others (e.g., from parents). In this way, we were able to retrospectively collect particularly accurate information about cancer therapies in early childhood.

Regarding factors that may have an influence on the agreement between self-reports and medical records, none of the chosen variables in our study (cancer status, sex, age at recruitment, tumors of the CNS, vocational training, follow-up time, and comorbidities) were found to be associated with the agreement. Also, Kool and colleagues investigated factors influencing concordance. In line with our findings, age had no significant impact on agreement for RT and CT in their study. Moreover, they could not demonstrate any influence of CT and endocrine therapy (35). In addition, in three studies (29, 30, 33) included in the review by Brüne et al. (32) neither age nor education had a significant effect on agreement regarding CT. Only the group by Roberts et al. (36) found significant associations between agreement and age as well as cancer recurrence. Here, younger age at diagnoses and cancer recurrence was associated with a higher risk of misreporting. These identified influencing factors seem reasonable to us since memory may not be as good for diagnoses at a younger age and therapies may have been mixed up by participants with multiple diagnoses. By encouraging our study participants to obtain information from others, we seemed to be able to successfully circumvent this effect in our study.

4.2 Late adverse health effects after cancer therapy

Previously, we investigated associations between cancer status and the occurrence of tumor therapy-related late adverse health effects in CCS of the KiKme study (37). In these analyses, however, cancer therapies were only considered as potential confounders. We found associations between cancer status and individual diseases including body mass index, hypercholesterolemia, and thyroid diseases excluding thyroid cancer. In detail, we observed that CCS of FPN and SPN were less likely to be overweight or obese than

TABLE 6 Self-reported exposure to radiotherapy and late adverse health effects in CCS of SPN from the KiKme case-control study.

	n	%	n	%	n	%
Weight status						
	Total (N=80)		Underweight or normal weight (N=46)		Overweight or obese (N=34)	
Radiotherapy	62	78%	36	78%	26	76%
No radiotherapy	18	23%	10	22%	8	24%
Thyroid diseases (without cancer)						
	Total (N=54)		Yes (N=15)		No (N=39)	
Radiotherapy	38	70%	15	100%	23	59%
No radiotherapy	16	30%	0	0%	16	41%
Hypercholesterolemia						
	Total (N=73)		Yes (N=9)		No (N=64)	
Radiotherapy	56	77%	9	100%	47	73%
No radiotherapy	17	23%	0	0%	17	27%
Cadiovascular diseases						
	Total (N=76)		Yes (N=9)		No (N=67)	
Radiotherapy	58	76%	6	67%	52	78%
No radiotherapy	18	24%	3	33%	15	22%

For CCS of SPN only radiotherapy for the first primary neoplasm was included in analyses. Participants with missing information were excluded.
CCS, childhood cancer survivors; SPN, second primary neoplasm.

cancer-free controls. In an analysis of individual diseases, it was found that CCS suffer more frequently from thyroid diseases other than thyroid cancer and hypercholesterolemia compared to controls. Since these strong effects were only observed when comparing CCS to cancer-free controls and disappeared when comparing CCS of SPN to CCS of FPN, we hypothesized that the effect may be driven by cancer therapies and conducted the present study.

Our current analyses show that thyroid disease was significantly more common in CCS with CT than in CCS without CT. A recent literature review on thyroid disease after childhood cancer therapy concludes that it is unclear whether CT itself is a risk for the

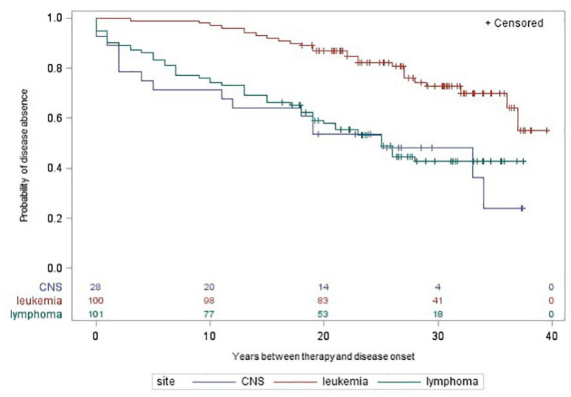

FIGURE 1
Time between self-reported radiotherapy and onset of late adverse diseases of the thyroid gland by cancer site in participants of the nested case-control study KiKme. Participants were included, if they received radiotherapy for a first primary cancer diagnosis (n=277). Thyroid cancers occurring as second primary neoplasms were excluded for this analysis. CNS, central nervous system.

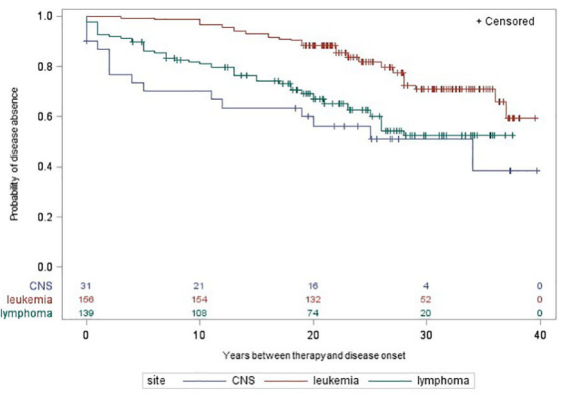

FIGURE 2
Time between self-reported chemotherapy and onset of late adverse diseases of the thyroid gland by cancer site in participants of the nested case-control study KiKme. Participants were included, if they received radiotherapy for a first primary cancer diagnosis (n=392). Thyroid cancers occurring as second primary neoplasms were excluded for this analysis. CNS, central nervous system.

development of thyroid disease or whether it adds to the well-known risk of RT (38). Thyroid disorders are most frequently observed after irradiation of the neck or spinal cord (15) with the highest risk after childhood exposure (38). Due to a large number of CCS in our study population who received both RT and CT, we are unfortunately not able to differentiate our results in this regard either. Moreover, we were unable to unravel an effect of RT on the risk of thyroid diseases due to the lack of precise information from the CCS of FPN and a limited case number.

We were also able to assign the previously observed association between cancer status and dyslipidemia as therapy-related. Prolonged CT or overall reduced physical fitness due to disease and therapy were previously discussed as possible causes of such metabolic changes (18, 39). In the long term, the presence of disorders of lipid metabolism is one of the main risk factors for the development of cardiovascular diseases later in life (40, 41). Therefore, the analysis of cardiovascular outcomes after cancer therapy in childhood was particularly important to us, even if we could not observe a significant association between childhood cancer and the occurrence of cardiovascular diseases in our previously published analysis (37). In addition, in the present study, we observed no association between cardiovascular diseases and CT in childhood although such an association was reported by several other studies (17, 41, 42). As different cytostatic drugs could have different cardiotoxic effects (12, 17), the cause of this unobserved effect in our study could be the imprecision of our data. In the present study, moreover, the risk of cardiovascular diseases after RT in childhood could not be estimated due to the low number of cases. However, with regard to the latency of cardiovascular diseases and second primary malignancies, we expect an increase in these therapy-related sequalae with extended follow-up and older age.

4.3 Strengths and limitations

This study has several strengths. Hitherto, to the best of our knowledge, only one other validation study on retrospective assessment of cancer therapies in childhood was conducted (25). Contrary to their methods, we allowed our participants to obtain as much information as possible about previous cancer therapies before answering our self-administered questionnaire. In addition, we had access to valid information on cancer therapies from the treating hospitals of the participants as well as from therapy-optimizing studies. Therefore, this unique study sample provides the basis for the first validation of therapy information from self-administered questionnaires. The newly developed questionnaire enables in particular researchers who cannot link their study data to clinical or registry data due to infrastructural or data protection reasons to collect valid information for important research questions in the field of tumor therapy-related late sequelae in a cost-effective and efficient way. In the long term, information obtained with this questionnaire can be used to forward research on therapy-associated late effects.

However, because we used self-administered data from long-term survivors of CCS, our analysis is subject to inherent survivor bias. Severe cancer cases with high mortality, e.g., acute myeloid leukemia following acute lymphoblastic leukemia or with two consecutive cancer diagnoses in a very short time, could not be considered. Moreover, a surveillance bias cannot be excluded in our study, as former cancer patients may be diagnosed more frequently with late sequelae due to regular follow-up examinations. Since we used information from the self-reports of the participants our results might be subject to a certain recall bias, especially regarding the information on occurred adverse health effects. In addition, a selection bias cannot be ruled out and the sample size was not sufficient enough to provide enough statistical power for specific research questions, in particular regarding late adverse health effects after RT in CCS of FPN. Due to the short follow-up period and the corresponding young age of our CCS cohort, only a small number of health-related late effects have occurred so far. However, prolonged follow-up of this unique cohort of CCS and cancer-free control subjects will ensure an important and highly relevant increase in knowledge about treatment-related late effects in long-term CCS.

5 Conclusion

Our new self-reported questionnaire for CCS is reliable for a retrospective assessment of a general exposure to tumor therapies in childhood, particularly for CT and RT in CCS with at least one SPN. However, the self-reported information on RT provided by study participants in the FPN group was too imprecise and could not be used. Nevertheless, our questionnaire offers a simple and cost-effective way to collect valid therapy information from long-term cancer survivors. This allowed us to demonstrate an association between CT in childhood and the occurrence of some late health effects, including thyroid and lipid metabolism disorders.

Data availability statement

The datasets presented in this article are not readily available because of ethic and data protection reasons. We are happy to support other research groups in their research projects by providing the newly developed questionnaire. Requests to access the questionnaires should be directed to the Leibniz Institute for Prevention Research and Epidemiology – BIPS, Bremen, Germany.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethik-Kommission of the Landesärztekammer Rheinland-Pfalz, Mainz, Germany. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MM is the principal investigator of the KiKme study and developed the study design and the questionnaires. The study was implemented and monitored by MM and LB. CS supported the development of strategies for the recruitment of childhood cancer survivors. MM, LB, DG, and SZ conducted the recruitment of the participants, which was organized and planned by MM and LB. MM, LB, and HSchm monitored the recruitment of controls. LB, MM, RF, and AP developed the analyses pipelines for the project. HSchw, DG, SZ, TH, AP, CS, MB, and HSchm contributed to the writing process, which was initially prepared by LB, MM, and RF. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1150629/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Type of self-reported cancer therapy by cancer site and year of diagnosis in participants of the nested case-control study KiKme.

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Questionnaires used in the KiKme study

General questionnaire

FRAGEBOGEN

Bei Teilnahme an der Studie möchten wir Sie bitten, den ausgefüllten Fragebogen an uns zurückzugeben.

AUSFÜLLHINWEISE FÜR STUDIENTEILNEHMER

Bitte beachten Sie beim Ausfüllen des Fragebogens die folgenden Punkte:

- Versuchen Sie die Antworten so genau wie möglich zu geben und den Fragebogen bis zum Ende zu bearbeiten.
- Die Fragen und Erläuterungstexte wurden grau hinterlegt, die Antwortmöglichkeiten finden Sie in den weiß hinterlegten Feldern.
- Es gibt keine richtigen oder falschen Antworten. Beantworten Sie die Fragen so, wie diese auf Sie persönlich zutreffen. Wenn Sie eine Frage nicht beantworten können, dann kreuzen Sie bitte „unbekannt“ an.
- Verwenden Sie zum Ausfüllen bitte nur Kugel- oder Filzschreiber und füllen Sie die Textfelder möglichst leserlich aus. In den meisten Fällen ist ein Kästchen anzukreuzen. Falls Sie versehentlich das falsche Kästchen angekreuzt haben, färben Sie bitte das falsche Kästchen vollständig ein und markieren Sie das richtige Kästchen mit einem Kreuz. *Beispiel:* ☒ *ja* ☐ *nein* → ■ *ja* ☒ *nein*
- In der Regel ist für jede Frage nur eine Antwort zulässig, ansonsten finden Sie im Fragetext die Anmerkung „Mehrfachnennungen möglich“.
- Gelegentlich können Sie einen Teil der Fragen überspringen. Sie sollten dies aber nur tun, wenn ein entsprechender Hinweis gegeben wird, zum Beispiel:

Nehmen Sie regelmäßig Medikamente (mindestens einmal in der Woche oder häufiger)?	☐ ja (➔ Frage 3.2.) ☐ nein (➔ Frage 3.3.)

➔ Nehmen Sie regelmäßig Medikamente, dann beantworten Sie bitte anschließend die Frage 3.2.!

➔ Nehmen Sie nicht regelmäßig Medikamente, dann gehen Sie bitte weiter zur Frage 3.3.!

Ausfülldatum: |__|__|/|__|__|/|__|__|__|__|

1. ANGABEN ZUR PERSON

1.1. Wann sind Sie geboren? **Wie groß waren Sie bei der Geburt?** **Wie viel wogen Sie bei der Geburt?**	|__|__| / |__|__| / |1|9|__|__| (Tag/Monat/Jahr) ca. |__|__| cm (Größe bei Geburt) ca. |__|__|__|__| g (Gewicht bei der Geburt)
1.2. Welches Geschlecht haben Sie?	☐ weiblich ☐ männlich ☐ anderes
1.3. Wie würden Sie sich ethnisch hauptsächlich einordnen?	☐ Weißer (Kaukasier) ☐ Schwarzer ☐ Asiate ☐ Latino ☐ andere, welche: _____ ☐ unbekannt / keine Angabe
1.4. Wie groß sind Sie derzeit?	ca. |__|__|__| cm (Aktuelle Größe)
1.5. Wie viel wiegen Sie derzeit?	ca. |__|__|__|,|__| kg (Aktuelles Gewicht)
1.6. Wo ist ihr derzeitiger Wohnort?	|__|__|__|__|__| _____ (PLZ / Ort)

1.7. Wie ist Ihre derzeitige Lebenssituation?	☐ allein lebend ☐ ohne Partner, mit Kind(ern) lebend ☐ mit Partner, ohne Kinder lebend ☐ mit Partner, mit Kind(ern) lebend ☐ mit Eltern(teil) lebend ☐ mit sonstigen Personen lebend, welche: _____ ☐ keine Angabe
1.8. Wie ist Ihre derzeitige Beschäftigungs- / Ausbildungssituation? Mehrfachnennungen möglich	☐ noch in schulischer / beruflicher Ausbildung, welche: _____ ☐ Vollzeit berufstätig ☐ Teilzeit berufstätig: _____% ☐ Hausfrau /-mann ☐ arbeitssuchend ☐ berufsunfähig ☐ Rentner/in ☐ Sonstiges (bitte angeben): _____ ☐ unbekannt / keine Angabe
1.9. Welchen höchsten allgemein bildenden Schulabschluss haben Sie derzeit?	☐ Haupt- oder Volksschulabschluss ☐ Realschulabschluss / Mittlere Reife / Fachschulreife / Polytechnische Oberschule ☐ Fachhochschulreife / Abschluss einer Fachoberschule ☐ Abitur / allgemeine Hochschulreife ☐ anderer Schulabschluss (bitte angeben): _____ ☐ (noch) kein Schulabschluss ☐ unbekannt / keine Angabe
1.10. Welchen höchsten beruflichen Ausbildungsabschluss bzw. Hochschulabschluss haben Sie derzeit?	☐ Abschluss einer Lehre (beruflich-betriebliche Ausbildung) ☐ Abschluss an einer Berufsfachschule, Handelsschule (beruflich-schulische Ausbildung) ☐ Abschluss an einer Fachschule (Meister-, Technikerschule, Berufs- oder Fachakademie) ☐ Abschluss an einer Hochschule (Universität, Fachhochschule) ☐ Sonstiger Abschluss (bitte angeben): _____ ☐ (noch) kein Berufsabschluss ☐ unbekannt / keine Angabe

2. ALLGEMEINE GESUNDHEITSASPEKTE

2.1. Haben Sie in den letzten 12 Monaten regelmäßig Sport getrieben?	☐ nein ☐ ja etwa |__|__| Stunden pro Woche
2.2. Haben Sie früher geraucht oder rauchen Sie zurzeit?	☐ ich habe noch nie geraucht bzw. weniger als 1 mal pro Woche für weniger als ein Jahr (➔ **Frage 2.7.**) ☐ ich habe früher geraucht, aber jetzt nicht mehr (➔ **Frage 2.3.**) ☐ ich rauche zur Zeit (➔ **Frage 2.3.**)
2.3. RAUCHER und EXRAUCHER: **Wie alt waren Sie, als Sie begonnen haben regelmäßig zu rauchen (wenn auch in kleinen Mengen)?**	|__|__| Jahre alt
2.4. NUR RAUCHER: **Wie viel haben Sie in den letzten 12 Monaten gewöhnlich pro Tag <u>oder</u> pro Woche geraucht?** Mehrfachnennungen möglich	pro Tag / pro Woche Zigaretten mit Filter: |__|__|__| oder |__|__|__| Zigaretten ohne Filter: |__|__|__| oder |__|__|__| Zigarren, Zigarillos, Pfeifen: |__|__|__| oder |__|__|__|
2.5. RAUCHER und EXRAUCHER: **Wie viel haben Sie früher gewöhnlich pro Tag oder pro Woche geraucht?** Mehrfachnennungen möglich	pro Tag / pro Woche Zigaretten mit Filter: |__|__|__| oder |__|__|__| Zigaretten ohne Filter: |__|__|__| oder |__|__|__| Zigarren, Zigarillos, Pfeifen: |__|__|__| oder |__|__|__|
2.6. NUR EXRAUCHER: **Wie alt waren Sie, als Sie aufgehört haben zu rauchen?**	|__|__| Jahre alt
2.7. Sind Sie täglich mehr als 2 Stunden zusammen mit einem oder mehreren aktiven Rauchern in einem Raum?	☐ nein ☐ ja

2.8. Welche dieser Getränke trinken Sie üblicherweise in welcher Menge?

Durchschnittliche Anzahl an jeweils genannten Getränken bitte ankreuzen (bitte nur ein Feld pro Zeile):

	nie	pro Woche				pro Tag				
		< 1	1-2	3-4	5-6	1	2	3	4	5 +
Wasser (0,5 l)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fruchtsäfte (0,2 l)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Milch (0,2 l)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Kaffee (Tassen)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tee (Tassen)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Limonaden/Cola/Softgetränke (0,2 l)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Bier (0,3 l)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Wein (0,2 l)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Verdünnt Hochprozentiges (0,3 l) (Cocktails, Alkopops …)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Unverdünnt Hochprozentiges (2cl) (Whiskey, Wodka …)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

3. MEDIZINISCHE THERAPIEN UND STRAHLENEXPOSITIONEN

3.1 Wurde bei Ihnen jemals eine Strahlentherapie durchgeführt?
Bei einer Strahlentherapie werden radioaktive Strahlen zur Behandlung von z.B. Krebserkrankungen eingesetzt. Zum Teil werden auch gutartige Erkrankungen wie Arthrose, Fersensporn oder Morbus Bechterew mit Strahlentherapie behandelt.

nein	ja	weiß nicht	wie oft insgesamt	in welchem Jahr (circa)	oder in welchem Alter (circa)	wie oft	Körperregion
☐	☐	☐	|__|__|	|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________

3.2 Wurde bei Ihnen jemals eine Chemotherapie durchgeführt?

nein	ja	weiß nicht	wie oft insgesamt	in welchem Jahr (circa)	oder in welchem Alter (circa)	wie oft	Medikament bzw. Substanz
☐	☐	☐	|__|__|	|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________

3.3 Wurde bei Ihnen jemals eine andere Krebstherapie durchgeführt?

nein	ja	weiß nicht	wie oft insgesamt	in welchem Jahr (circa)	oder in welchem Alter (circa)	wie oft	Art der anderen Therapie z.B. Chirurgische Tumorentfernung, Hormontherapie
☐	☐	☐	|__|__|	|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________

3.4 Wurde bei Ihnen jemals eine Röntgenuntersuchung durchgeführt?
z.B. wegen Knochenbrüchen, Lungenentzündungen, Krankenhausaufenthalten oder Operationen. Bitte auch zahnärztliche Röntgenuntersuchungen aufführen. Bitte geben Sie auch an, ob für die Untersuchung ein Kontrastmittel verabreicht wurde (z.B. zur Untersuchung von Hohlorganen wie der Magen-Darm-Trakt, die Gallenwege oder Blutgefässe).

nein	ja	weiß nicht	wie oft ins-gesamt	in welchem Jahr (circa)	oder in welchem Alter (circa)	wie oft	Körperregion	Kontrast-mittel ja	Kontrast-mittel nein
☐	☐	☐	|__|__|	|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________	☐	☐
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________	☐	☐
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________	☐	☐
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________	☐	☐
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________	☐	☐
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________	☐	☐
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________	☐	☐
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________	☐	☐
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________	☐	☐
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	____________	☐	☐

3.5 Wurde bei Ihnen jemals eine Computertomographie (CT) durchgeführt?

z.B. wegen schweren Verkehrsunfällen, Schädel-Hirn-Trauma, Halswirbelsäulenverletzungen, Knochenbrüche, Bandscheibenvorfälle, Gelenkentzündungen bzw. Arthritis oder Wirbelsäulenverkrümmung. Kann auch durchgeführt werden bei entzündlichen Erkrankungen wie Lungenentzündungen/Blinddarmentzündungen, sowie bei schweren Erkrankungen wie Krebs. Ebenso werden CTs im Zusammenhang mit Operationen durchgeführt. Im CT sind leise und klickende Geräusche während der Bewegung der Röhre zu hören.

nein	ja	weiß nicht	wie oft insgesamt	in welchem Jahr (circa)	oder in welchem Alter (circa)	wie oft	Körperregion
☐	☐	☐	|__|__|	|__|__|__|__|	|__|__| Jahre alt	|__|__|	_____
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	_____
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	_____
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	_____
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	_____

3.6 Wurde bei Ihnen jemals eine PET (Positronen-Emissionstomographie) oder eine SPECT (Single photon emission computed tomographie) durchgeführt?

Es handelt sich hierbei um eine Computertomographie, bei der gleichzeitig eine radioaktive Substanz gespritzt wird, um z.B. einen Tumor sichtbar zu machen oder um Funktionsstörungen von Organen, z.B. der Schilddrüse, der Lunge, der Knochen oder des Herzens bildlich darzustellen. Ein Computer erstellt ein meist mehrfarbiges Bild, ein sogenanntes Szintigramm (Szintigraphie).

nein	ja	weiß nicht	wie oft insgesamt	in welchem Jahr (circa)	oder in welchem Alter (circa)	wie oft	Körperregion
☐	☐	☐	|__|__|	|__|__|__|__|	|__|__| Jahre alt	|__|__|	_____
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	_____
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	_____
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	_____
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	_____

3.7 Wurde bei Ihnen jemals eine Kernspin/Magnetresonanz-Tomographie (MRT) durchgeführt?

Prinzipiell wird ein MRT bei den gleichen Erkrankungen durchgeführt, wie unter 4.2. Computertomographie beschrieben. Während der Untersuchung im MRT erzeugen die Magnetfelder sehr laute, klopfende Geräusche.

nein	ja	weiß nicht	wie oft insgesamt	in welchem Jahr (circa)	oder in welchem Alter (circa)	wie oft	Körperregion
☐	☐	☐	|__|__|	|__|__|__|__|	|__|__| Jahre alt	|__|__|	_____
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	_____
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	_____
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	_____
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	_____

3.8 Wurde bei Ihnen jemals ein minimal-invasiver radiologischer Eingriff durchgeführt?

Darunter versteht man Behandlungen, die unter radiologischer Kontrolle durchgeführt werden. Zum Beispiel Herzkatheteruntersuchungen oder das Einsetzen von Gefäßstützen, sog. Stents in ein Blutgefäß, in die Atemwege oder die Gallenwege um diese „offen“ zu halten. Oder wurde bei Ihnen jemals eine Embolisation durchgeführt?

nein	ja	weiß nicht	wie oft insgesamt	in welchem Jahr (circa)	oder in welchem Alter (circa)	wie oft	Körperregion
☐	☐	☐	|__|__|	|__|__|__|__|	|__|__| Jahre alt	|__|__|	_____
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	_____
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	_____
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	_____
				|__|__|__|__|	|__|__| Jahre alt	|__|__|	_____

3.9 Wurde bei Ihnen jemals eine Schilddrüsen-Radiojodtherapie durchgeführt?						
nein	ja	weiß nicht	wie oft insgesamt	in welchem Jahr (circa)	oder in welchem Alter (circa)	wie oft
☐	☐	☐	|__|__|	|__|__|__|__|	|__|__| Jahre alt	|__|__|
				|__|__|__|__|	|__|__| Jahre alt	|__|__|
				|__|__|__|__|	|__|__| Jahre alt	|__|__|
				|__|__|__|__|	|__|__| Jahre alt	|__|__|
				|__|__|__|__|	|__|__| Jahre alt	|__|__|

4. FRAGEN ZU EIGENEN MEDIKAMENTEN UND EIGENEN SPEZIELLEN ERKRANKUNGEN

4.1 Nehmen Sie regelmäßig Medikamente (mindestens einmal in der Woche oder häufiger)?	☐ nein (➔ **Frage 4.3.**) ☐ ja (➔ **Frage 4.2.**)

4.2 Welche Medikamente haben Sie in der letzten Woche an mindestens einem Tag eingenommen? Bitte nennen Sie jeweils Name oder Inhaltsstoff, Dosis und Tagesmenge und kreuzen Sie an, an wie vielen Tagen Sie es eingenommen haben.

Name des Medikaments: z.B. Aspirin/Acetylsalicylsäure/ASS, Hydrochlorothiazid, Paracetamol, Bisoprolol	Dosis: (z.B. 100 mg)	Anzahl / Tag (z.B. 1 Tablette oder 20 Tropfen)	an 1-2 Tagen	an 3-4 Tagen	an 5-6 Tagen	täglich
a)		|__|__| _____	☐	☐	☐	☐
b)		|__|__| _____	☐	☐	☐	☐
c)		|__|__| _____	☐	☐	☐	☐
d)		|__|__| _____	☐	☐	☐	☐
e)		|__|__| _____	☐	☐	☐	☐
f)		|__|__| _____	☐	☐	☐	☐
g)		|__|__| _____	☐	☐	☐	☐
h)		|__|__| _____	☐	☐	☐	☐

4.3 Wurden bei Ihnen jemals eine oder mehrere der folgenden Krankheiten von einem Arzt festgestellt?	nein	ja	Falls ja, in welchem Alter (circa):	Falls ja, auch noch zurzeit aktuell? nein	ja
a) Diabetes (erhöhte Urin- oder Blutzuckerwerte)	☐	☐	|__|__| Jahre alt	☐	☐
b) Erhöhtes Cholesterin im Blut	☐	☐	|__|__| Jahre alt	☐	☐
c) Erhöhter Blutdruck	☐	☐	|__|__| Jahre alt	☐	☐
d) chronische Lungenerkrankung wie Asthma, Bronchitis	☐	☐	|__|__| Jahre alt	☐	☐
e) Heuschnupfen	☐	☐	|__|__| Jahre alt	☐	☐
f) entzündliche Gelenk- oder Wirbelsäulenerkrankungen, Arthrosen, Rheuma	☐	☐	|__|__| Jahre alt	☐	☐
g) Neurodermitis (atopische Dermatitis)	☐	☐	|__|__| Jahre alt	☐	☐
h) Herzinfarkt	☐	☐	|__|__| Jahre alt	☐	☐
i) Schlaganfall	☐	☐	|__|__| Jahre alt	☐	☐
j) Schilddrüsenerkrankungen	☐	☐	|__|__| Jahre alt	☐	☐
k) Pfeiffersches Drüsenfieber / Epstein-Barr-Virus	☐	☐	|__|__| Jahre alt	☐	☐
l) AIDS Erkrankung / Humanes Immundefizienz-Virus (HIV)	☐	☐	|__|__| Jahre alt	☐	☐
m) Hepatitis, wenn ja welcher Typ (a, b, c, d, e): _____	☐	☐	|__|__| Jahre alt	☐	☐

n) Sonstige gravierende Erkrankungen (bitte angeben):	Alter (circa):	nein	ja
- _____	|__|__| Jahre alt	☐	☐
- _____	|__|__| Jahre alt	☐	☐
- _____	|__|__| Jahre alt	☐	☐
- _____	|__|__| Jahre alt	☐	☐
- _____	|__|__| Jahre alt	☐	☐
- _____	|__|__| Jahre alt	☐	☐

5. FRAGEN ZU ERKRANKUNGEN IN IHRER FAMILIE

5.1 Traten in Ihrer Familie schwerwiegende Erkrankungen oder Syndrome auf, außer Tumorerkrankungen? (z.B. Diabetes, Asthma, Schlaganfall, Neurodermitis…)

Nicht leibliche Familienangehörige (z.B. adoptiert oder Stiefgeschwister) führen Sie bitte NICHT auf!

Familiengrad	nein	ja	unbe-kannt	Wenn ja, tragen Sie hier bitte alle Erkrankungen dieser Person in dieser Spalte ein
Mutter	☐	☐	☐	
Vater	☐	☐	☐	
Großmutter mütterlicherseits (Mutter der Mutter)	☐	☐	☐	
Großvater mütterlicherseits (Vater der Mutter)	☐	☐	☐	
Großmutter väterlicherseits (Mutter des Vaters)	☐	☐	☐	
Großvater väterlicherseits (Vater des Vaters)	☐	☐	☐	
Eigene Kinder	Anzahl eigener Kinder: |__|__|			
☐ Tochter ☐ Sohn	☐	☐	☐	
☐ Tochter ☐ Sohn	☐	☐	☐	
☐ Tochter ☐ Sohn	☐	☐	☐	
☐ Tochter ☐ Sohn	☐	☐	☐	
☐ Tochter ☐ Sohn	☐	☐	☐	
Eigene Geschwister	Anzahl eigener Geschwister: |__|__|			
☐ Schwester ☐ Bruder	☐	☐	☐	
☐ Schwester ☐ Bruder	☐	☐	☐	
☐ Schwester ☐ Bruder	☐	☐	☐	
☐ Schwester ☐ Bruder	☐	☐	☐	
☐ Schwester ☐ Bruder	☐	☐	☐	

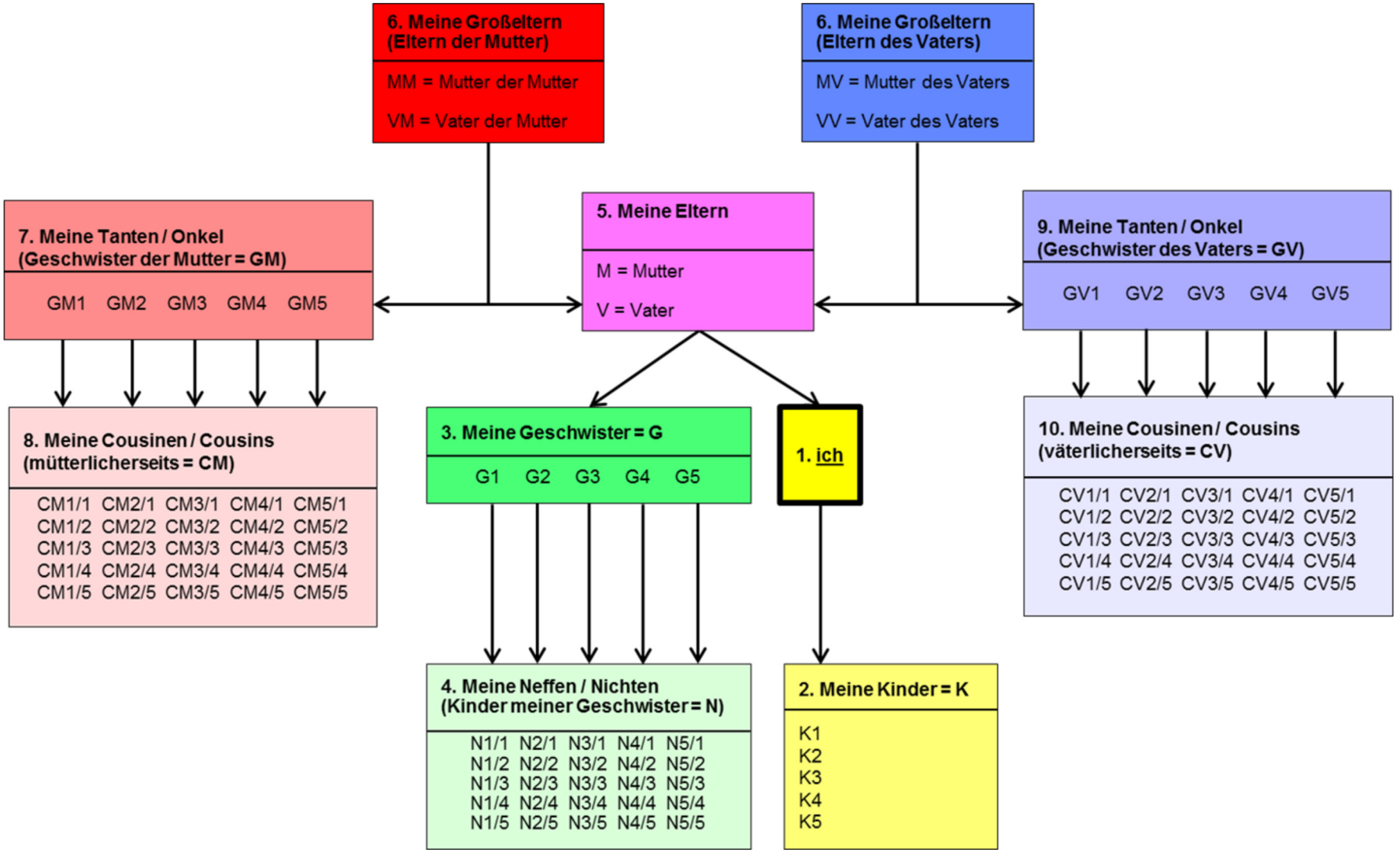
VIELEN HERZLICHEN DANK!

Family history of cancer

FRAGEBOGEN ZU KREBSERKRANKUNGEN IN DER FAMILIE

Im Folgenden werden Ihnen Fragen zu Krebserkrankungen in Ihrer Familie gestellt. Bitte teilen Sie uns mit, falls Sie oder eines Ihrer Familienmitglieder jemals an einer Krebserkrankung erkrankt ist. Für die Grundlagenforschung über Krebserkrankungen ist dies eine sehr wichtige Information.

Zur übersichtlichen Auswertung wird jedem Familienmitglied eine Kennnummer zugeordnet (z.B. K1 für Ihr erstes Kind oder M für Ihre Mutter). Zum besseren Verständnis ist hier ein Stammbaum dargestellt. Gerne können Sie in diesem Stammbaum Ihre Verwandten einkreisen, um eine bessere Übersicht zu gewinnen.



REK-ID: «rekid»

Ausfüllhinweise für Interviewer/in:

- Sollte die Anzahl der Spalten/Personen in den Fragen 1–10 nicht ausreichen (wenn Teilnehmer/in z. B. 6 Geschwister hat), dann werden diese Familienangehörige separat auf der letzten Seite eingetragen (Frage 11 – z.B. 6. Geschwisterteil).
- Treten im Familien-Stammbaum Halbgeschwister auf, dann werden diese ebenfalls separat auf der letzten Seite eingetragen (Frage 11 – z.B. Halbbruder väterlicherseits).
- Adoptierte Familienangehörige im Stammbaum werden auch deutlich gekennzeichnet auf der letzten Seite eingetragen (Frage 11 – z. B. adoptierter Bruder).

Ausfüllbeispiel

Kennnummer	Zum Beispiel: M (Mutter)
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja → |__|__| Jahre *(Lebensalter)* ☒ nein → |5|2| Jahre *(Alter bei Tod)* ☐ unb. (unbekannt)
Geburtsjahr *(+/- 5 Jahre)*	|1|9|5|8| **(+/- 5 Jahre)** ☐ unb. (unbekannt)
Geschlecht	☒ w (weiblich) ☐ m (männlich) ☐ unb. (unbekannt)
Krebserkrankung	☒ ja ☐ nein ☐ unb. (unbekannt)
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung	Brustkrebs *(1. Krebserkrankung)*
Erkrankungsalter *(in Jahre)*	|4|2| Jahre ☐ unb. *(mit)*
2. Krebserkrankung	Ovarialkarzinom *(2. Krebserkrankung)*

Ausfülldatum: |__|__|/|__|__|/|__|__|__|__|

1. Angaben zu Ihrer Person

Ist bei Ihnen jemals eine Krebserkrankung festgestellt worden?

Kennnummer	Ich
Krebserkrankung	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung	
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.
2. Krebserkrankung	
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.

Initialen Interviewer	|__|__|
Anmerkungen/besondere Bemerkungen Interviewer/Arzt	

2. Angaben zu ihren leiblichen Kindern

Haben Sie leibliche Kinder? ☐ nein (➔ Frage 3) ☐ ja, wie viele |__| (➔ Folgefelder ausfüllen)

Kennnummer	**K1** = Kind 1	**K2** = Kind 2	**K3** = Kind 3	**K4** = Kind 4	**K5** = Kind 5
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.
Geschlecht	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.
Ist bei Ihren Kindern jemals eine Krebserkrankung festgestellt worden?	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.
2. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.

3. Angaben zu Ihren leiblichen Geschwistern = G

Haben Sie leibliche Geschwister? ☐ nein (➔ Frage 5) ☐ ja, wie viele |__| (➔ Folgefelder ausfüllen)

Kennnummer	**G1** = Geschwister 1	**G2** = Geschwister 2	**G3** = Geschwister 3	**G4** = Geschwister 4	**G5** = Geschwister 5
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.
Geschlecht	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.
Ist bei Ihren Geschwistern jemals eine Krebserkrankung festgestellt worden?	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung					
Erkrankungsalter	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.
2. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.

4.1 Angaben zu leiblichen Neffen / Nichten = N ≙ leibliche Kinder von Geschwisterteil 1 = G1

Hat Ihr Geschwisterteil 1 = G1 leibliche Kinder? ☐ nein (➔ Frage 4.2) ☐ ja, wie viele |__| (➔ Folgefelder ausfüllen)

Kennnummer	N1/1 = Kind 1 v. Geschwister 1	N1/2 = Kind 2 v. Geschwister 1	N1/3 = Kind 3 v. Geschwister 1	N1/4 = Kind 4 v. Geschwister 1	N1/5 = Kind 5 v. Geschwister 1
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.
Geschlecht	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.
Ist bei Ihren Neffen/Nichten jemals eine Krebserkrankung festgestellt worden?	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.
2. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.

4.2 Angaben zu leiblichen Neffen / Nichten = N ≙ leibliche Kinder von Geschwisterteil 2 = G2

Hat Ihr Geschwisterteil 2 = G2 leibliche Kinder? ☐ nein (➔ Frage 4.3) ☐ ja, wie viele |__| (➔ Folgefelder ausfüllen)

Kennnummer	N2/1 = Kind 1 v. Geschwister 2	N2/2 = Kind 2 v. Geschwister 2	N2/3 = Kind 3 v. Geschwister 2	N2/4 = Kind 4 v. Geschwister 2	N2/5 = Kind 5 v. Geschwister 2
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.
Geschlecht	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.
Ist bei Ihren Neffen/Nichten jemals eine Krebserkrankung festgestellt worden?	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.
2. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.

4.3 Angaben zu leiblichen Neffen / Nichten = N ≙ leibliche Kinder von Geschwisterteil 3 = G3

Hat Ihr Geschwisterteil 3 = G3 leibliche Kinder? ☐ nein (➔ Frage 4.4) ☐ ja, wie viele |__| (➔ Folgefelder ausfüllen)

Kennnummer	**N3/1 =** Kind 1 v. Geschwister 3	**N3/2 =** Kind 2 v. Geschwister 3	**N3/3 =** Kind 3 v. Geschwister 3	**N3/4 =** Kind 4 v. Geschwister 3	**N3/5 =** Kind 5 v. Geschwister 3
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.
Geschlecht	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.
Ist bei Ihren Neffen/Nichten jemals eine Krebserkrankung festgestellt worden?	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.
2. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.

4.4 Angaben zu leiblichen Neffen / Nichten = N ≙ leibliche Kinder von Geschwisterteil 4 = G4

Hat Ihr Geschwisterteil 4 = G4 leibliche Kinder? ☐ nein (➔ Frage 4.5) ☐ ja, wie viele |__| (➔ Folgefelder ausfüllen)

Kennnummer	**N4/1 =** Kind 1 v. Geschwister 4	**N4/2 =** Kind 2 v. Geschwister 4	**N4/3 =** Kind 3 v. Geschwister 4	**N4/4 =** Kind 4 v. Geschwister 4	**N4/5 =** Kind 5 v. Geschwister 4
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.
Geschlecht	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.
Ist bei Ihren Neffen/Nichten jemals eine Krebserkrankung festgestellt worden?	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.
2. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.

4.5 Angaben zu leiblichen Neffen / Nichten = N ≙ leibliche Kinder von Geschwisterteil 5 = G5

Hat Ihr Geschwisterteil 5 = G5 leibliche Kinder? ☐ nein (➔ Frage 5) ☐ ja, wie viele ___ (➔ Folgefelder ausfüllen)

Kennnummer	N5/1 = Kind 1 v. Geschwister 5	N5/2 = Kind 2 v. Geschwister 5	N5/3 = Kind 3 v. Geschwister 5	N5/4 = Kind 4 v. Geschwister 5	N5/5 = Kind 5 v. Geschwister 5
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja → ___ Jahre ☐ nein → ___ Jahre ☐ unb.	☐ ja → ___ Jahre ☐ nein → ___ Jahre ☐ unb.	☐ ja → ___ Jahre ☐ nein → ___ Jahre ☐ unb.	☐ ja → ___ Jahre ☐ nein → ___ Jahre ☐ unb.	☐ ja → ___ Jahre ☐ nein → ___ Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	____ ☐ unb.	____ ☐ unb.	____ ☐ unb.	____ ☐ unb.	____ ☐ unb.
Geschlecht	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.
Ist bei Ihren Neffen/Nichten jemals eine Krebserkrankung festgestellt worden?	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	___ Jahre ☐unb.	___ Jahre ☐unb.	___ Jahre ☐unb.	___ Jahre ☐unb.	___ Jahre ☐unb.
2. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	___ Jahre ☐ unb.	___ Jahre ☐ unb.	___ Jahre ☐ unb.	___ Jahre ☐ unb.	___ Jahre ☐ unb.

5./6. Angaben zu Ihren leiblichen Eltern und leiblichen Großeltern

	Eltern		Großeltern mütterlicherseits		Großeltern väterlicherseits	
Kennnummer	Mutter	Vater	MM = Mutter der Mutter	VM = Vater der Mutter	MV = Mutter des Vaters	VV = Vater des Vaters
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja → ___ Jahre ☐ nein → ___ Jahre ☐ unb.	☐ ja → ___ Jahre ☐ nein → ___ Jahre ☐ unb.	☐ ja → ___ Jahre ☐ nein → ___ Jahre ☐ unb.	☐ ja → ___ Jahre ☐ nein → ___ Jahre ☐ unb.	☐ ja → ___ Jahre ☐ nein → ___ Jahre ☐ unb.	☐ ja → ___ Jahre ☐ nein → ___ Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	____ ☐ unb.	____ ☐ unb.	____ ☐ unb.	____ ☐ unb.	____ ☐ unb.	____ ☐ unb.
Ist bei Ihren Eltern/Großeltern jemals eine Krebserkrankung festgestellt worden?	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung						
Erkrankungsalter	___ Jahre ☐unb.	___ Jahre ☐unb.	___ Jahre ☐unb.	___ Jahre ☐unb.	___ Jahre ☐unb.	___ Jahre ☐unb.
2. Krebserkrankung						
Erkrankungsalter *(in Jahre)*	___ Jahre ☐ unb.	___ Jahre ☐ unb.	___ Jahre ☐ unb.	___ Jahre ☐ unb.	___ Jahre ☐ unb.	___ Jahre ☐ unb.

7. Angaben zu Ihren leiblichen Onkel und Tanten = GM (≙ leiblichen Geschwistern Ihrer Mutter)

Hat Ihre Mutter leibliche Geschwister? ☐ nein (➔ Frage 9) ☐ ja, wie viele |__| (➔ Folgefelder ausfüllen)

Kennnummer	**GM1 =** Geschwister 1 der Mutter	**GM2 =** Geschwister 2 der Mutter	**GM3 =** Geschwister 3 der Mutter	**GM4 =** Geschwister 4 der Mutter	**GM5 =** Geschwister 5 der Mutter
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.
Geschlecht	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.
Ist bei den Geschwistern Ihrer Mutter jemals eine Krebserkrankung festgestellt worden?	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.
2. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.

8.1 Angaben zu leiblichen Cousinen / Cousins mütterlicherseits = CM (≙ Kinder von Geschwister 1 der Mutter = GM1)

Hat Geschwister 1 Ihrer Mutter leibliche Kinder? ☐ nein (➔ Frage 8.2) ☐ ja, wie viele |__| (➔ Folgefelder ausfüllen)

Kennnummer	**CM1/1 =** Kind 1 von Geschwister 1 der Mutter = GM1	**CM1/2 =** Kind 2 von Geschwister 1 der Mutter = GM1	**CM1/3 =** Kind 3 von Geschwister 1 der Mutter = GM1	**CM1/4 =** Kind 4 von Geschwister 1 der Mutter = GM1	**CM1/5 =** Kind 5 von Geschwister 1 der Mutter = GM1
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.
Geschlecht	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.
Ist bei Ihren Cousinen/Cousins mütterlicherseits jemals eine Krebserkrankung festgestellt worden?	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.
2. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.

8.2 Angaben zu leiblichen Cousinen / Cousins mütterlicherseits = CM (≙ Kinder von Geschwister 2 der Mutter = GM2)

Hat Geschwister 2 Ihrer Mutter leibliche Kinder? ☐ nein (➔ Frage 8.3) ☐ ja, wie viele |__| (➔ Folgefelder ausfüllen)

Kennnummer	**CM2/1** = Kind 1 von Geschwister 2 der Mutter = GM2	**CM2/2** = Kind 2 von Geschwister 2 der Mutter = GM2	**CM2/3** = Kind 3 von Geschwister 2 der Mutter = GM2	**CM2/4** = Kind 4 von Geschwister 2 der Mutter = GM2	**CM2/5** = Kind 5 von Geschwister 2 der Mutter = GM2
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.
Geschlecht	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.
Ist bei Ihren Cousinen/Cousins mütterlicherseits jemals eine Krebserkrankung festgestellt worden?	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.
2. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.

8.3 Angaben zu leiblichen Cousinen / Cousins mütterlicherseits = CM (≙ Kinder von Geschwister 3 der Mutter = GM3)

Hat Geschwister 3 Ihrer Mutter leibliche Kinder? ☐ nein (➔ Frage 8.4) ☐ ja, wie viele |__| (➔ Folgefelder ausfüllen)

Kennnummer	**CM3/1** = Kind 1 von Geschwister 3 der Mutter = GM3	**CM3/2** = Kind 2 von Geschwister 3 der Mutter = GM3	**CM3/3** = Kind 3 von Geschwister 3 der Mutter = GM3	**CM3/4** = Kind 4 von Geschwister 3 der Mutter = GM3	**CM3/5** = Kind 5 von Geschwister 3 der Mutter = GM3
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.
Geschlecht	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.
Ist bei Ihren Cousinen/Cousins mütterlicherseits jemals eine Krebserkrankung festgestellt worden?	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.
2. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.

8.4 Angaben zu leiblichen Cousinen / Cousins mütterlicherseits = CM (≙ Kinder von Geschwister 4 der Mutter = GM4)

Hat Geschwister 4 Ihrer Mutter leibliche Kinder? ☐ nein (➔ Frage 8.5) ☐ ja, wie viele |__| (➔ Folgefelder ausfüllen)

Kennnummer	**CM4/1** = Kind 1 von Geschwister 4 der Mutter = GM4	**CM4/2** = Kind 2 von Geschwister 4 der Mutter = GM4	**CM4/3** = Kind 3 von Geschwister 4 der Mutter = GM4	**CM4/4** = Kind 4 von Geschwister 4 der Mutter = GM4	**CM4/5** = Kind 5 von Geschwister 4 der Mutter = GM4
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.
Geschlecht	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.
Ist bei Ihren Cousinen/Cousins mütterlicherseits jemals eine Krebserkrankung festgestellt worden?	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.
2. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.

8.5 Angaben zu leiblichen Cousinen / Cousins mütterlicherseits = CM (≙ Kinder von Geschwister 5 der Mutter = GM5)

Hat Geschwister 5 Ihrer Mutter leibliche Kinder? ☐ nein (➔ Frage 9) ☐ ja, wie viele |__| (➔ Folgefelder ausfüllen)

Kennnummer	**CM5/1** = Kind 1 von Geschwister 5 der Mutter = GM5	**CM5/2** = Kind 2 von Geschwister 5 der Mutter = GM5	**CM5/3** = Kind 3 von Geschwister 5 der Mutter = GM5	**CM5/4** = Kind 4 von Geschwister 5 der Mutter = GM5	**CM5/5** = Kind 5 von Geschwister 5 der Mutter = GM5
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.
Geschlecht	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.
Ist bei Ihren Cousinen/Cousins mütterlicherseits jemals eine Krebserkrankung festgestellt worden?	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.
2. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.

9. Angaben zu Ihren leiblichen Onkel und Tanten väterlicherseits = GV (≙ Liebliche Geschwister Ihres Vaters)

Hat Ihr Vater leibliche Geschwister? ☐ nein (➔ Frage 11) ☐ ja, wie viele |__| (➔ Folgefelder ausfüllen)

Kennnummer	**GV1 =** Geschwister 1 des Vaters	**GV2 =** Geschwister 2 des Vaters	**GV3 =** Geschwister 3 des Vaters	**GV4 =** Geschwister 4 des Vaters	**GV5 =** Geschwister 5 des Vaters
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	|_|_|_|_| ☐ unb.	|_|_|_|_| ☐ unb.	|_|_|_|_| ☐ unb.	|_|_|_|_| ☐ unb.	|_|_|_|_| ☐ unb.
Geschlecht	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.
Ist bei den Geschwistern Ihres Vaters jemals eine Krebserkrankung festgestellt worden?	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.
2. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.

10.1 Angaben zu leiblichen Cousinen / Cousins väterlicherseits = CV (≙ Kinder von Geschwister 1 des Vaters = GV1)

Hat Geschwister 1 Ihres Vaters leibliche Kinder? ☐ nein (➔ Frage 10.2) ☐ ja, wie viele |__| (➔ Folgefelder ausfüllen)

Kennnummer	**CV1/1 =** Kind 1 von Geschwister 1 des Vaters = GM1	**CV1/2 =** Kind 2 von Geschwister 1 des Vater = GM1	**CV1/3 =** Kind 3 von Geschwister 1 des Vater = GM1	**CV1/4 =** Kind 4 von Geschwister 1 des Vater = GM1	**CV1/5 =** Kind 5 von Geschwister 1 des Vater = GM1
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	|_|_|_|_| ☐ unb.	|_|_|_|_| ☐ unb.	|_|_|_|_| ☐ unb.	|_|_|_|_| ☐ unb.	|_|_|_|_| ☐ unb.
Geschlecht	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.
Ist bei Ihren Cousinen/Cousins väterlicherseits jemals eine Krebserkrankung festgestellt worden?	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.
2. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.

10.2 Angaben zu leiblichen Cousinen / Cousins väterlicherseits = CV (≙ Kinder von Geschwister 2 des Vaters = GV2)

Hat Geschwister 2 Ihres Vaters leibliche Kinder? ☐ nein (➔ Frage 10.3) ☐ ja, wie viele |__| (➔ Folgefelder ausfüllen)

Kennnummer	**CV2/1** = Kind 2 von Geschwister 2 des Vaters = GM2	**CV2/2** = Kind 2 von Geschwister 2 des Vater = GM2	**CV2/3** = Kind 3 von Geschwister 2 des Vater = GM2	**CV2/4** = Kind 4 von Geschwister 2 des Vater = GM2	**CV2/5** = Kind 5 von Geschwister 2 des Vater = GM2
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.
Geschlecht	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.
Ist bei Ihren Cousinen/Cousins väterlicherseits jemals eine Krebserkrankung festgestellt worden?	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.
2. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.

10.3 Angaben zu leiblichen Cousinen / Cousins väterlicherseits = CV (≙ Kinder von Geschwister 3 des Vaters = GV3)

Hat Geschwister 3 Ihres Vaters leibliche Kinder? ☐ nein (➔ Frage 10.4) ☐ ja, wie viele |__| (➔ Folgefelder ausfüllen)

Kennnummer	**CV3/1** = Kind 3 von Geschwister 3 des Vaters = GM3	**CV3/2** = Kind 2 von Geschwister 3 des Vater = GM3	**CV3/3** = Kind 3 von Geschwister 3 des Vater = GM3	**CV3/4** = Kind 4 von Geschwister 3 des Vater = GM3	**CV3/5** = Kind 5 von Geschwister 3 des Vater = GM3
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.
Geschlecht	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.
Ist bei Ihren Cousinen/Cousins väterlicherseits jemals eine Krebserkrankung festgestellt worden?	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.
2. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.

10.4 Angaben zu leiblichen Cousinen / Cousins väterlicherseits = CV (≙ Kinder von Geschwister 4 des Vaters = GV4)

Hat Geschwister 4 Ihres Vaters leibliche Kinder? ☐ nein (➔ Frage 10.5) ☐ ja, wie viele |__| (➔ Folgefelder ausfüllen)

Kennnummer	**CV4/1** = Kind 4 von Geschwister 4 des Vaters = GM4	**CV4/2** = Kind 2 von Geschwister 4 des Vater = GM4	**CV4/3** = Kind 3 von Geschwister 4 des Vater = GM4	**CV4/4** = Kind 4 von Geschwister 4 des Vater = GM4	**CV4/5** = Kind 5 von Geschwister 4 des Vater = GM4
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.
Geschlecht	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.
Ist bei Ihren Cousinen/Cousins väterlicherseits jemals eine Krebserkrankung festgestellt worden?	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.
2. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.

10.5 Angaben zu leiblichen Cousinen / Cousins väterlicherseits = CV (≙ Kinder von Geschwister 5 des Vaters = GV5)

Hat Geschwister 5 Ihres Vaters leibliche Kinder? ☐ nein (➔ Frage 11) ☐ ja, wie viele |__| (➔ Folgefelder ausfüllen)

Kennnummer	**CV5/1** = Kind 5 von Geschwister 5 des Vaters = GM5	**CV5/2** = Kind 2 von Geschwister 5 des Vater = GM5	**CV5/3** = Kind 3 von Geschwister 5 des Vater = GM5	**CV5/4** = Kind 4 von Geschwister 5 des Vater = GM5	**CV5/5** = Kind 5 von Geschwister 5 des Vater = GM5
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.	☐ ja ➔ |__|__| Jahre ☐ nein ➔ |__|__| Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.
Geschlecht	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.
Ist bei Ihren Cousinen/Cousins väterlicherseits jemals eine Krebserkrankung festgestellt worden?	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.
2. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.

11. Wenn die Anzahl der Felder in den Fragen 1 – 10 nicht ausgereicht haben sollte, da Teilnehmer/in z.B. mehr als 5 Geschwister hat, dann diese bitte mit Bezeichnung separat in folgende Tabellen eintragen. Falls es im Familienstammbaum Halbgeschwister geben sollte diese bitte ebenfalls mit Bezeichnung in folgende Tabellen eintragen. (siehe auch Ausfüllbeispiel)

Kennnummer	z.B.: Halbgeschwister väterlicherseits				
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☒ ja |1|5| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	|1|9|9|7| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.
Geschlecht	☒ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.
Ist jemals eine Krebserkrankung festgestellt worden?	☐ ja ☒ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.
2. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.

Kennnummer					
Lebt wenn ja, dann Lebensalter in Jahre wenn nein, dann Alter bei Tod	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.	☐ ja → |__|__| Jahre ☐ nein → |__|__| Jahre ☐ unb.
Geburtsjahr *(+/- 5 Jahre)*	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.	|__|__|__|__| ☐ unb.
Geschlecht	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.	☐ w. ☐ m. ☐ unb.
Ist jemals eine Krebserkrankung festgestellt worden?	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.	☐ ja ☐ nein ☐ unb.
Wenn Krebs ☒ ja, dann	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**	**Wenn Krebs ☒ ja, dann**
1. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.	|__|__| Jahre ☐unb.
2. Krebserkrankung					
Erkrankungsalter *(in Jahre)*	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.	|__|__| Jahre ☐ unb.

VIELEN DANK FÜR IHRE TEILNAMEBEREITSCHAFT UND IHRE GEDULD!

Participation registration

TEILNAHMEERFASSUNG - KLINIK

Liebe Kollegen, wir möchten Sie bitten folgenden Fragebogen für alle potentiellen Studienteilnehmer an der KiKme Studie auszufüllen und an die Studienleitung weiterzuleiten.

Ausfülldatum: |__|__|/|__|__|/|__|__|__|__|

1. Hauptdiagnose für Klinikaufenthalt (nur CO)

ICD-Code der Hauptdiagnose mit Beschreibung	_____ _____

2. Angaben aus Antwortblatt (für SN, PN und CO)

Proband ist bereit an dem genannten Vorhaben teilzunehmen. Er hat die Studieninformation gelesen und hat verstanden, dass die Teilnahme an der Studie mit dem Ausfüllen eines Fragebogens und mit einer kleinen Hautbiopsie sowie einer Speichelprobe verbunden ist. Er ist damit einverstanden, dass seine Kontaktdaten (Telefonnummer und/oder E-Mail-Adresse) an die Klinik für Strahlentherapie und Radioonkologie weitergegeben werden.

- ☐ **Proband möchte inkl. Hautbiopsie, Speichelprobe und Fragebogen an Studie teilnehmen.**
- ☐ **Proband möchte mit Hautbiopsie aber ohne Speichelprobe und mit Fragebogen an Studie teilnehmen.**
- ☐ **Proband möchte ohne Hautbiopsie aber mit Speichelprobe und Fragebogen an Studie teilnehmen.**
- ☐ **Proband möchte ohne Hautbiopsie und ohne Speichelprobe aber mit Fragebogen an Studie teilnehmen.**

☐ **Studienteilnahme in Mainz**

☐ **Studienteilnahme vor Ort (PLZ, Ort)** |__|__|__|__|__| _____

☐ **Proband möchte zunächst weitere Informationen** und ist damit einverstanden, dass seine Kontaktdaten (Telefonnummer und/oder E-Mail-Adresse) an die Klinik für Strahlentherapie und Radioonkologie weitergegeben werden.

☐ **Proband möchte nicht teilnehmen** und ist aber bereit die folgenden Punkte zu beantworten.

Der Grund der Nichtteilnahme ist:

- ☐ kein Interesse
- ☐ zeitlicher Aufwand
- ☐ weiter Anfahrtsweg
- ☐ gesundheitliche Gründe
- ☐ kein persönlicher Nutzen bei Teilnahme an Studie
- ☐ keine ausreichenden Deutschkenntnisse
- ☐ unverständliche Aufklärung über Studieninhalte
- ☐ Sinn und Zweck der Studie fraglich
- ☐ wegen kleiner Hautprobe
- ☐ sonstige Gründe, welche: _____
- ☐ nicht erreicht
- ☐ bereits entlassen
- ☐ verstorben, wann: |__|__|/|__|__|/|__|__|__|__|
- ☐ keine Angabe

Das Geburtsdatum (Tag/Monat/Jahr) ist: |__|__| / |__|__| / |__|__||__|__|

Das Geschlecht ist: ☐ weiblich ☐ männlich ☐ anderes

Der Wohnort (PLZ/Ort) ist: |__|__|__|__|__|__| _____

☐ **Proband möchte nicht teilnehmen** und keine Angaben zu seiner Person machen.

☐ **Proband möchte ärztliche Beratung in Anspruch nehmen.**

Datum der Antwort: |__|__|/|__|__|/|__|__|__|__|

VIELEN HERZLICHEN DANK!

Sampling protocol – Part 1

PROBENENTNAHMEPROTOKOLL – TEIL 1

Nach der Probenentnahme das Probenentnahmeprotokoll Teil1 ausfüllen und die gesamte Mappe an die Studienleitung weiterleiten.
In der Klinik verbleiben die Einverständniserklärung des Probanden und die Kontaktdaten des Probanden (klare Folie) und das Probenentnahmeprotokoll – Teil 2 bis zur Gefrierlagerung der Zelllinien.

1. Initialen Arzt	|__|__|
2. Probenentnahmedatum / Ausfülldatum:	|__|__|/|__|__|/|__|__|__|__| (Tag/Monat/Jahr)
3. Ethnische Zuordnung (nach Einschätzung des Arztes)	☐ Weißer (Kaukasier) ☐ Schwarzer ☐ Asiate ☐ Latino ☐ andere, welche: _____
4. Gesundheitszustand erlaubt aus Sicht des behandelnden Arztes eine Teilnahme. (ohne schwerwiegende Erkrankung oder akute Entzündungen)	☐ nein ☐ ja
5. Informationen wurden vom Probanden gelesen und die Risiken sind dem Probanden bekannt.	☐ nein ☐ ja
6. Einwilligungserklärung des Probanden liegt vor. **Datum der Einwilligung**	☐ nein ☐ ja |__|__|/|__|__|/|__|__|__|__| (Tag/Monat/Jahr)
7. Blutentnahme durchgeführt?	☐ nein ☐ ja
8. Hautbiopsie durchgeführt?	☐ nein ☐ ja
9. Speichelprobe durchgeführt?	☐ nein ☐ ja
10. Komplikationen erkennbar?	☐ nein ☐ ja Wenn ja, welche:_____ _____ _____
11. Uhrzeit bei Probenentnahme:	|__|__| : |__|__| (hh:mm)
12. Temperatur bei Probenentnahme:	|__|__|,|__| °C
13. Luftfeuchtigkeit bei Probenentnahme:	|__|__| %
14. Art der Probe und Menge	☐ Hautbiopsie: _____ Stück Biopsie aus Körperteil: _____ ☐ Speichel: _____ ml ☐ Blut: _____ ml

VIELEN HERZLICHEN DANK!

REK-ID: |_|_|_|_|_|

Sampling protocol – Part 2

PROBENENTNAHMEPROTOKOLL – TEIL 2

Nach dem Kultivieren der Zelllinien bitte Probenentnahmeprotokoll – Teil 2 an die Studienleitung weiterleiten, damit die Proben-ID auf die Proben geklebt werden können.

15. Anzahl gefriergelagerter Proben von Zelllinien	|__|__|
16. Datum der Gefrierlagerung der Zelllinien	|__|__|/|__|__|/|__|__|__|__| (Tag/Monat/Jahr)
17. Anzahl gefriergelagerter Blutproben	|__|__|
18. Datum der Gefrierlagerung der Blutproben	|__|__|/|__|__|/|__|__|__|__| (Tag/Monat/Jahr)
19. Anzahl gefriergelagerter Proben des Speichels	|__|__|
20. Datum der Gefrierlagerung des Speichels	|__|__|/|__|__|/|__|__|__|__| (Tag/Monat/Jahr)

VIELEN HERZLICHEN DANK!