Published in final edited form as:

Eur J Cancer. 2017 February ; 72: 177–185. doi:10.1016/j.ejca.2016.11.022.

Neuroblastoma Survivors are at Increased Risk for Second Malignancies: A Report from the International Neuroblastoma Risk Group Project

Mark A. Applebaum, MD¹, Zalman Vaksman, PhD², Sang Mee Lee, PhD³, Eric A. Hungate, PhD¹, Tara O. Henderson, MD, MPH¹, Wendy B. London, PhD⁴, Navin Pinto, MD⁵, Samuel L. Volchenboum, MD, PhD¹, Julie R. Park, MD⁵, Arlene Naranjo, PhD⁶, Barbara Hero, MD⁷, Andrew D. Pearson, MD⁸, Barbara E. Stranger, PhD^{9,10}, Susan L. Cohn, MD¹, and Sharon J. Diskin, PhD^{2,11}

¹Department of Pediatrics, University of Chicago, Chicago, Illinois

²Division of Oncology and Center for Childhood Cancer Research, Children's Hospital of Philadelphia, Philadelphia, PA; Department of Biomedical and Health Informatics, Children's Hospital of Philadelphia, Philadelphia, PA

³Department of Public Health Sciences, University of Chicago, Chicago, Illinois

⁴Boston Children's Hospital and Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

⁵Department of Pediatrics, Seattle Children's Hospital, University of Washington, Seattle, WA

⁶Children's Oncology Group Statistics and Data Center, Department of Biostatistics, University of Florida, Gainesville, FL

⁷Children's Hospital, Department of Pediatric Oncology and Hematology, University of Cologne, Cologne, Germany

⁸Institute of Cancer Research and Royal Marsden Hospital, Surrey, United Kingdom - Retired

⁹Section of Genetic Medicine, Department of Medicine, University of Chicago, Chicago, Illinois

¹⁰Institute for Genomics and Systems Biology, Center for Data Intensive Science, University of Chicago, Chicago, Illinois

¹¹Department of Pediatrics and Abramson Family Cancer Research Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

Abstract

Corresponding Authors: Sharon J. Diskin, PhD, Department of Pediatrics, University of Pennsylvania, 3501 Civic Center Blvd., Room 3026 CTRB, Philadelphia, PA 19104, Telephone: 215-590-9160, Facsimile: 267-426-0685, diskin@email.chop.edu.

Conflict of interest statement

The authors have no conflicts to disclose

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Background—The incidence of SMN within the first ten years of diagnosis in high-risk neuroblastoma patients treated with modern, intensive therapy is unknown. Further, the underlying germline genetics that contribute to SMN in these survivors are not known.

Methods—The International Neuroblastoma Risk Group (INRG) database of patients diagnosed from 1990–2010 was analyzed. SMN risk was assessed by cumulative incidence, standardized incidence ratios (SIR), and absolute excess risk (AER). A candidate gene-based association study evaluated genetic susceptibility to SMN in neuroblastoma survivors.

Results—Of the 5,987 patients in the INRG database with SMN data enrolled in a clinical trial, 43 (0.72%) developed a SMN. The 10-year cumulative incidence of SMN for high-risk patients was 1.8% (95% CI 1.0–2.6%) compared to 0.38% (95% CI: 0.22–0.94%) for low-risk patients ($P=0.01$). High-risk patients had an almost 18-fold higher incidence of SMN compared to age and sex matched controls (SIR=17.5 (95% CI: 11.4–25.3), AER=27.6). For patients treated on high- and intermediate-risk clinical trials, the SIR of acute myelogenous leukemia (AML) was 106.8 (95% CI: 28.7–273.4) and 127.7 (95%CI: 25.7–373.3), respectively. Variants implicating DNA repair genes *XRCC3* (rs861539: $P=0.006$; Odds Ratio: 2.04, 95%CI: 1.19–3.46) and *MSH2* (rs17036651: $P=0.009$; Odds Ratio: 0.26, 95% CI: 0.08–0.81) were associated with SMN.

Conclusion—The intensive multi-modality treatment strategy currently used to treat high-risk neuroblastoma is associated with a significantly increased risk of secondary AML. Defining the interactions of treatment exposures and genetic factors that promote the development of SMN is critical for optimizing survivorship care

Keywords

Neuroblastoma; second malignant neoplasms; INRG; genetic association study

Introduction

Modern, risk-adapted treatment approaches for neuroblastoma have led to an increasing numbers of survivors (Supplementary Table 1) [1–3]. Survivors, treated in the 1970's and 1980's when neuroblastoma treatment modalities differed considerably from modern approaches, have a higher risk of developing second malignant neoplasms (SMN) compared to the general population, with cumulative incidences between 3.6% and 10% [1, 4–7]. Although low- and intermediate-risk patients currently have minimal exposure to chemotherapy, modern chemotherapeutic regimens used to treat high-risk patients includes alkylating agents, topoisomerase II inhibitors, and platinum-based drugs which are known to induce treatment-related leukemia [8, 9]. In addition, radiation therapy and myeloablative therapy with stem cell rescue are included as part of standard-of-care treatment, further increasing the risk of treatment-related hematologic malignancies.

Because treatment-related leukemia often presents within five years from diagnosis [7], these SMN would not be captured in studies that define survivors as patients who are at least five years from their initial diagnosis. To investigate the prevalence of SMN in patients treated with modern, risk-based treatment and identify the second cancers that occur within the first five years from diagnosis, we analyzed data from Children's Oncology Group (COG) patients included in the International Neuroblastoma Risk Group (INRG) database.

We also performed a candidate gene/variant-based association study using germline single nucleotide polymorphism (SNP) genotype data to investigate the genetic etiology of SMN in neuroblastoma survivors.

Patients and Methods

Patients and Variables

The International Neuroblastoma Risk Group (INRG) database was analyzed [10]. SMN data were available only on COG patients diagnosed after 1990, and to ensure follow-up time of at least five years, we restricted the study cohort to the 9,173 patients diagnosed from 1990–2010. To investigate the incidence of SMN, the study cohort was limited to the 5,987 patients who were enrolled on risk-adapted therapeutic trials. Low-risk patients with INSS stage 1 or 2 disease diagnosed after 2004 who were enrolled on the ANBL00B1 biology study were included in this cohort, as there was no available therapeutic trial for these patients during this time period [11]. For the genomic association studies, the analytic cohort was comprised of all patients within the 9,173 patient cohort with available genotype data, which included 3,186 patients who were only enrolled on COG biology studies but not a clinical trial. (Figure 1, Supplementary Table 2).

Classification of SMN was accrued through required institutional reporting for patients on COG biology or therapeutic neuroblastoma trials. Patients were categorized as not having a SMN if the database coded the histology as neuroblastoma (n=5), or non-melanomatous skin cancer (n=5). Histology of the SMN is indicated by ICD-O-10 coding in the database. No patients had multiple SMN documented.

Statistical Methods

The impact of clinical features on the development of a SMN was assessed by comparing patients with SMN to those without. For each risk factor competing risks regression (CRR) based on Fine and Gray's proportional subhazards models were performed [12].

The time to SMN was calculated from neuroblastoma diagnosis until the time of SMN diagnosis, death, or last contact. The cumulative incidences of SMN were estimated, with death as a competing risk, and Gray's test compared SMN curves by clinical trial group [13]. Standardized incidence ratio (SIR) compared the risk of SMN in our cohort to the risk of developing any cancer in age and sex matched controls according to the SEER database. Confidence intervals on the SIRs were calculated using the Wilson-Hilferty method [14]. Absolute excess risk (AER), of extra cancers per 10,000 person-years of follow up was calculated using SEER population controls. Statistical analyses were performed using R v3.2.2.

Genomic Association Studies

To test for genetic susceptibility variations associated with SMN, germline DNA of neuroblastoma patients was assayed as previously described [15]. To improve the power to detect these variations, we included all patients within the 9,173 patient cohort with available genotype data (Figure 1). All cases (survivors with SMN) and controls (survivors

without SMN) were matched by risk group and genetic ancestry as described previously [15]. Patients were eligible for the control group if they had not developed an SMN and had at least five years of follow up. An admixed population of African and European descent was analyzed as previously described [16], estimating the proportion of African admixture using ADMIXTURE [17]. We attempted to match cases to controls in a 1:10 ratio. When intermediate-risk cases could not be matched to 10 intermediate-risk controls, high-risk controls were utilized.

Genome-wide genotype imputation was performed using IMPUTE2 v2.3.1 [18] for all SNPs and indel variants annotated in 1000 Genomes Phase I Release 3. Association testing was performed under an additive genetic effect model using the frequentist likelihood score method in SNPTEST [19] using risk group and proportion African admixture as covariates. We defined a set of candidate variants to evaluate based on 1) variants in genes previously reported to be associated with the development of SMN [20] and 2) quantitative trait loci (eQTLs) for these genes. We used the GTEx consortium browser [21] to identify eQTLs in any tissue. Candidate SNPs and eQTLs were extracted from SNPTEST results and evaluated for association with SMN in neuroblastoma survivors.

Results

Clinical trial enrollment and SMN

Of the 5,987 patients enrolled on neuroblastoma risk-based therapeutic trials, 43 (0.72%) developed a SMN. The median follow-up time for the 5,987 patient cohort was 63 months consisting of 34,382 person-years and 87 months for neuroblastoma survivors. The median age at diagnosis of a SMN was 6.7 years (range 9 months–23.6 years) with a median latency from primary neuroblastoma diagnosis of 41 months (range 1–223 months). Increased risk of SMN was associated with stage 4 disease, unfavorable histology, and diagnosis prior to 2006 (Table 1). Twenty-four SMN were diagnosed within five years of the primary neuroblastoma diagnosis, and 17 were hematologic malignancies, including eight cases of AML. Nine of the 17 hematologic SMN developed in patients treated on high-risk clinical trials (Table 2, Supplementary Table 3).

The cumulative incidence of SMN at ten years for high-risk patients was 1.8% (95% CI 1.0–2.6%) compared to 0.38% (95% CI: 0.22–0.94%) for low-risk patients ($P=0.01$, Figure 2). The cumulative incidence of SMN for high-risk patients appeared to be greater than for intermediate-risk patients at this time point (0.56% (95% CI: 0.34–1.3%)), though this difference did not reach statistical significance ($P=0.053$). Several patients with high-risk disease were diagnosed with a SMN ten years after their initial neuroblastoma diagnosis while late SMN occurrences were not observed in low- or intermediate-risk patients.

The SIR of patients treated on high-risk clinical trials was 17.5 (95% CI: 11.4–25.3) with an AER of 27.6 of second malignancies compared to the risk of developing a primary cancer in the general population per 10,000 person years of follow up (Table 3). Patients on low-risk protocols had an SIR of 3.1 (95% CI: 1.4–6.2) and AER of 3.7. Within the overall cohort, the SIR was 7.5 (95% CI: 5.4–10.0) with an AER of 10.8. We also evaluated the SIR of AML within the first five years from diagnosis of neuroblastoma. For high- and

intermediate-risk patients, the SIR of AML in this time frame was 106.8 (95% CI: 28.7–273.4) and 127.7 (95% CI: 25.7–373.3), respectively. Patients enrolled on low-risk trials did not appear to have an increased risk of this outcome as the confidence interval of the SIR included 1.0.

Genetic association study of second malignant neoplasms

Within the 9,173 COG patient cohort, genotype data was available on 5,149 patients, including 2,585 patients who were also enrolled on clinical trials. Thirty-one patients with genotype data developed a SMN, including 22 high-risk patients (Supplementary Table 4). Twenty-one of the 31 patients were enrolled on clinical trials and included in the SMN incidence analyses. The types of SMNs included leukemia and lymphoma (15, including 11 cases of AML), sarcoma (7), carcinoma (5), brain tumors (2), neuroblastoma (1), and hepatoblastoma (1). The genomic inflation was 1.02 (Supplementary Figure 2).

For the genetic association study, the 31 patients with SMN were matched to 309 control patients without SMN (Supplementary Table 5, Supplementary Figure 1). Based on an analysis of 66 candidate SNPs from 47 unique genes, we identified rs861539, a non-synonymous missense variant (Thr241Met) in the X-Ray Repair Complementing Defective Repair in Chinese Hamster Cells 3 gene, *XRCC3*, most associated with SMN ($P=0.006$, Odds Ratio: 2.04, 95% CI: 1.20–3.46 Table 4, Supplementary Table 6). Specifically, the T allele (reverse strand) was over-represented in SMN cases (45%) compared to controls (29%). This association remained the most significant variant when we restricted the analysis to individuals of European ancestry ($P=0.006$, Odds Ratio: 1.86, 95% CI: 1.12–3.09; Supplementary Table 7).

Next, we identified 1,038 eQTLs [21] associated with 19 of the candidate SMN-associated genes (Supplementary Table 8), 927 of which had minor allele frequencies greater than 10%. Association testing of the 927 variants with SMN identified rs17036651 at 2p21, which maps to intron 10 of the DNA mismatch repair gene MutS Homolog 2, *MSH2*, as the most significant SNP ($P=0.009$, Odds Ratio: 0.26, 95% CI: 0.09–0.81; Table 4, Supplementary Table 9), though this did not meet the Bonferroni-corrected threshold of 5×10^{-5} . The G allele of rs17036651 is associated with increased expression of *MSH2* in 14 tissue types (Supplementary Table 10), and the G allele was present in a lower frequency in cases with SMN (5.4%) compared to controls (17.7%), similar to the overall population frequency (15.7%) in the 1000 Genomes Project. The association of rs17036651 with SMN remained as the most significantly associated variant when we restricted our analysis to individuals of European ancestry ($P=0.006$, Odds Ratio: 0.30, 95% CI: 0.11–0.82; Supplementary Table 11).

Conclusion

In this analysis of 5,987 neuroblastoma patients, we show that high-risk survivors have a nearly eighteen-fold increase in risk of SMN compared to the development of primary malignancies in the general population. Additionally, we demonstrate for the first time a markedly high prevalence of secondary AML in high- and intermediate-risk survivors within five years from their initial diagnosis of neuroblastoma, with SIRs of 106.8 and 127.7. The

higher incidence of SMN in high-risk patients compared to intermediate- or low-risk patients was an expected finding and supports previous studies [6]. However, the high rate of AML observed in the intermediate-risk cohort of patients, who receive relatively low doses of alkylating agents and topoisomerase inhibitors, is surprising. Interestingly, low-risk neuroblastoma patients, largely treated with surgery alone, also had higher rates of SMN than expected, with a SIR of 3.8. Thus, even with limited exposures to cytotoxic treatments, neuroblastoma survivors are at increased risk for developing SMN, suggesting underlying genetic susceptibilities.

This is the largest neuroblastoma cohort ever evaluated for SMN risk, and comprehensively describes the cumulative incidence of SMN within five years of the primary neuroblastoma diagnosis. By definition, these early SMN are excluded from survivor studies that only include patients surviving five-years or more. Although the 10 year cumulative incidence of SMN of 1.8% in our high-risk cohort is lower than noted in other neuroblastoma studies, the incidence will likely increase with additional follow-up (Table 5). Further, because there was no effort to re-contact patients in our study who were lost to follow-up, the risk of SMN may be higher than what we observed. A significantly higher incidence of SMN is seen in survivors of other cancers like Hodgkin lymphoma [32, 33], indicating that the risk of developing a SMN is influenced by the primary diagnosis in addition to treatment exposures.

We also evaluated the potential link between germline genetic variation and the development of SMN in neuroblastoma survivors and identified multiple variants implicating genes involved in DNA repair. The rs861539 SNP causes a Thr241Met change in the DNA repair gene *XRCC3*. The T allele of rs861539 has been associated with increased risk of SMN in patients with head and neck cancer [34] as well as the development of therapy related AML (t-AML) in survivors of other types of cancer [23]. Consistent with these studies, the T allele is over-represented in neuroblastoma survivors who developed SMN. Further, eQTLs analysis for the candidate SMN-related genes, revealed a lower proportion of patients who developed SMN harbored the rs17036651 G allele, which is associated with increased expression of the mismatch repair gene *MSH2*. Decreased function of *MSH2* has been associated with increased risk of t-AML [35]. Thus, the reduction in G allele frequency of rs17036651 in the cohort who developed SMN suggests that this increased risk may be influenced by a decrease in *MSH2* expression. While these findings imply a gene-exposure interaction, the observed associations did not achieve statistical significance when corrected for multiple testing. To increase statistical power and confidence in our results, larger datasets of patients with known therapy exposure will be required to fully elucidate this connection. Sequencing of the host genome in patients who developed SMN may also reveal new germline mutations influencing risk of SMN in neuroblastoma survivors.

Although our analysis of SMN risk was conducted using the cohort of patients enrolled on COG risk-based clinical trials, a significant limitation of this study is that we were not able to capture treatment received. This is because COG only maintains data regarding assigned treatment and does not collect cumulative treatment dose received. Thus, the specific effects of radiation therapy, whether it be local or total body, or other treatment modalities cannot be evaluated. The COG is developing a neuroblastoma survivorship study (ALTE15N2) that will systematically collect treatment exposure data from high-risk COG patients diagnosed

after January 2000 (personal communication Dr. Tara Henderson, University of Chicago). We anticipate this study will elucidate the association of specific treatment exposures on SMN development as well as other adverse events.

Our findings show that the risk for SMN in neuroblastoma survivors is significant in the first ten years from diagnosis and that this cohort has high rates of secondary AML. Additionally, the recently released results of the COG high-risk study (ANBL0532) have demonstrated the efficacy of tandem courses of myeloablative chemotherapy with stem cell rescue leading to a new standard-of-care [36]. This increase in treatment intensity is likely to further enhance the risk of SMNs in the high-risk cohort, emphasizing the need for close risk-based follow-up of these patients as recommended by the Institute of Medicine and the COG Long Term Follow Up Guidelines [37, 38]. We also identified germline genetic variants in DNA repair genes as potential genomic risk factors for developing SMN. Further research focused on confirming these findings and identifying additional gene-environment interactions that account for the second cancers is warranted. Appropriate screening and surveillance studies could then be performed on patients determined to be at high risk for SMN, which may ultimately lead to improved outcome for neuroblastoma survivors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The INRG database is supported in part by the William Guy Forbeck Research Foundation, the St. Baldrick's Foundation, the Little Heroes Cancer Research Fund, Children's Neuroblastoma Cancer Foundation, Neuroblastoma Children's Cancer Foundation, the Super Jake Foundation, and the Alex's Lemonade Stand Foundation. Data included in the INRG database were provided by Children's Oncology Group [COG], Pediatric Oncology Group [POG], Children's Cancer Study Group [CCSG], German Gesellschaft für Pädiatrische Onkologie und Hämatologie [GPOH], European Neuroblastoma Study Group [ENSG], International Society of Paediatric Oncology Europe Neuroblastoma Group [SIOPEN], Japanese Neuroblastoma Study Group [JNBSG], Japanese Infantile Neuroblastoma Co-operative Study Group [JINCS], Spanish Neuroblastoma Group and the Italian Neuroblastoma Group. This work was supported by the National Institutes of Health (R01CA124709, R00 CA151869 to S.J.D, and K12CA139160 and T32GM007019 to M.A.A.). The contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

References

1. Moroz V, Machin D, Faldum A, Hero B, Iehara T, Mosseri V, et al. Changes over three decades in outcome and the prognostic influence of age-at-diagnosis in young patients with neuroblastoma: a report from the International Neuroblastoma Risk Group Project. *Eur J Cancer*. 2011; 47(4):561–71. [PubMed: 21112770]
2. Baker DL, Schmidt ML, Cohn SL, Maris JM, London WB, Buxton A, et al. Outcome after reduced chemotherapy for intermediate-risk neuroblastoma. *N Engl J Med*. 2010; 363(14):1313–23. [PubMed: 20879880]
3. Pinto NR, Applebaum MA, Volchenboum SL, Matthay KK, London WB, Ambros PF, et al. Advances in Risk Classification and Treatment Strategies for Neuroblastoma. *J Clin Oncol*. 2015; 33(27):3008–17. [PubMed: 26304901]
4. Laverdiere C, Liu Q, Yasui Y, Nathan PC, Gurney JG, Stovall M, et al. Long-term outcomes in survivors of neuroblastoma: a report from the Childhood Cancer Survivor Study. *J Natl Cancer Inst*. 2009; 101(16):1131–40. [PubMed: 19648511]

5. Rubino C, Adjadj E, Guerin S, Guibout C, Shamsaldin A, Dondon MG, et al. Long-term risk of second malignant neoplasms after neuroblastoma in childhood: role of treatment. *Int J Cancer*. 2003; 107(5):791–6. [PubMed: 14566829]
6. Applebaum MA, Henderson TO, Lee SM, Pinto N, Volchenboum SL, Cohn SL. Second malignancies in patients with neuroblastoma: The effects of risk-based therapy. *Pediatr Blood Cancer*. 2015; 62(1):128–133. [PubMed: 25251613]
7. Godley LA, Larson RA. Therapy-related myeloid leukemia. *Semin Oncol*. 2008; 35(4):418–29. [PubMed: 18692692]
8. Kreissman SG, Seeger RC, Matthay KK, London WB, Spoto R, Grupp SA, et al. Purged versus non-purged peripheral blood stem-cell transplantation for high-risk neuroblastoma (COG A3973): a randomised phase 3 trial. *Lancet Oncol*. 2013; 14(10):999–1008. [PubMed: 23890779]
9. Sandoval C, Pui CH, Bowman LC, Heaton D, Hurwitz CA, Raimondi SC, et al. Secondary acute myeloid leukemia in children previously treated with alkylating agents, intercalating topoisomerase II inhibitors, and irradiation. *J Clin Oncol*. 1993; 11(6):1039–45. [PubMed: 8388919]
10. Cohn SL, Pearson AD, London WB, Monclair T, Ambros PF, Brodeur GM, et al. The International Neuroblastoma Risk Group (INRG) classification system: an INRG Task Force report. *J Clin Oncol*. 2009; 27(2):289–97. [PubMed: 19047291]
11. Strother DR, London WB, Schmidt ML, Brodeur GM, Shimada H, Thorner P, et al. Outcome after surgery alone or with restricted use of chemotherapy for patients with low-risk neuroblastoma: results of Children's Oncology Group study P9641. *J Clin Oncol*. 2012; 30(15):1842–8. [PubMed: 22529259]
12. Fine JP, Gray RJ. A Proportional Hazards Model for the Subdistribution of a Competing Risk. *Journal of the American Statistical Association*. 1999; 94(446):496–509.
13. Gray RJ. A Class of K-Sample Tests for Comparing the Cumulative Incidence of a Competing Risk. *The Annals of Statistics*. 1988; 16(3):1141–1154.
14. Wilson EB, Hilferty MM. The Distribution of Chi-Square. *Proc Natl Acad Sci U S A*. 1931; 17(12):684–8. [PubMed: 16577411]
15. Diskin SJ, Capasso M, Schnepf RW, Cole KA, Attiyeh EF, Hou C, et al. Common variation at 6q16 within HACE1 and LIN28B influences susceptibility to neuroblastoma. *Nat Genet*. 2012; 44(10):1126–30. [PubMed: 22941191]
16. Gamazon ER, Pinto N, Konkashbaev A, Im HK, Diskin SJ, London WB, et al. Trans-population analysis of genetic mechanisms of ethnic disparities in neuroblastoma survival. *J Natl Cancer Inst*. 2013; 105(4):302–9. [PubMed: 23243203]
17. Alexander DH, Novembre J, Lange K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res*. 2009; 19(9):1655–64. [PubMed: 19648217]
18. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet*. 2009; 5(6):e1000529. [PubMed: 19543373]
19. Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat Genet*. 2007; 39(7):906–13. [PubMed: 17572673]
20. Bhatia S. Genetic variation as a modifier of association between therapeutic exposure and subsequent malignant neoplasms in cancer survivors. *Cancer*. 2015; 121(5):648–63. [PubMed: 25355167]
21. Consortium GT. The Genotype-Tissue Expression (GTEx) project. *Nat Genet*. 2013; 45(6):580–5. [PubMed: 23715323]
22. Maris JM, Hogarty MD, Bagatell R, Cohn SL. Neuroblastoma. *Lancet*. 2007; 369(9579):2106. [PubMed: 17586306]
23. Seedhouse C, Faulkner R, Ashraf N, Das-Gupta E, Russell N. Polymorphisms in genes involved in homologous recombination repair interact to increase the risk of developing acute myeloid leukemia. *Clin Cancer Res*. 2004; 10(8):2675–80. [PubMed: 15102670]
24. Bolufer P, Collado M, Barragan E, Calasanz MJ, Colomer D, Tormo M, et al. Profile of polymorphisms of drug-metabolising enzymes and the risk of therapy-related leukaemia. *Br J Haematol*. 2007; 136(4):590–6. [PubMed: 17367411]

25. Hosking FJ, Feldman D, Bruchim R, Olver B, Lloyd A, Vijayakrishnan J, et al. Search for inherited susceptibility to radiation-associated meningioma by genomewide SNP linkage disequilibrium mapping. *Br J Cancer*. 2011; 104(6):1049–54. [PubMed: 21364586]
26. Jin L, Sturgis EM, Zhang Y, Huang Z, Wei P, Guo W, et al. Genetic variants in p53-related genes confer susceptibility to second primary malignancy in patients with index squamous cell carcinoma of head and neck. *Carcinogenesis*. 2013; 34(7):1551–7. [PubMed: 23508638]
27. Garaventa A, Gambini C, Villavecchia G, Di Cataldo A, Bertolazzi L, Pizzitola MR, et al. Second malignancies in children with neuroblastoma after combined treatment with 131I-metaiodobenzylguanidine. *Cancer*. 2003; 97(5):1332–8. [PubMed: 12599242]
28. Kushner BH, Kramer K, Modak S, Qin LX, Yataghena K, Jhanwar SC, et al. Reduced risk of secondary leukemia with fewer cycles of dose-intensive induction chemotherapy in patients with neuroblastoma. *Pediatr Blood Cancer*. 2009; 53(1):17–22. [PubMed: 19148951]
29. Martin A, Schneiderman J, Helenowski IB, Morgan E, Dilley K, Danner-Koptik K, et al. Secondary malignant neoplasms after high-dose chemotherapy and autologous stem cell rescue for high-risk neuroblastoma. *Pediatr Blood Cancer*. 2014; 61(8):1350–6. [PubMed: 24634399]
30. Federico SM, Allewelt HB, Spunt SL, Hudson MM, Wu J, Billups CA, et al. Subsequent malignant neoplasms in pediatric patients initially diagnosed with neuroblastoma. *J Pediatr Hematol Oncol*. 2015; 37(1):e6–e12. [PubMed: 24633303]
31. Huibregtse KE, Vo KT, DuBois SG, Fetzko S, Neuhaus J, Batra V, et al. Incidence and risk factors for secondary malignancy in patients with neuroblastoma after treatment with (131)I-metaiodobenzylguanidine. *Eur J Cancer*. 2016; 66:144–52. [PubMed: 27573428]
32. van Leeuwen FE, Klokman WJ, Veer MB, Hagenbeek A, Krol AD, Vetter UA, et al. Long-term risk of second malignancy in survivors of Hodgkin's disease treated during adolescence or young adulthood. *J Clin Oncol*. 2000; 18(3):487–97. [PubMed: 10653864]
33. Schaapveld M, Aleman BM, van Eggermond AM, Janus CP, Krol AD, van der Maazen RW, et al. Second Cancer Risk Up to 40 Years after Treatment for Hodgkin's Lymphoma. *N Engl J Med*. 2015; 373(26):2499–511. [PubMed: 26699166]
34. Gal TJ, Huang WY, Chen C, Hayes RB, Schwartz SM. DNA repair gene polymorphisms and risk of second primary neoplasms and mortality in oral cancer patients. *Laryngoscope*. 2005; 115(12):2221–31. [PubMed: 16369171]
35. Rund D, Krichevsky S, Bar-Cohen S, Goldschmidt N, Kedmi M, Malik E, et al. Therapy-related leukemia: clinical characteristics and analysis of new molecular risk factors in 96 adult patients. *Leukemia*. 2005; 19(11):1919–28. [PubMed: 16167058]
36. Park, JR., Kreissman, SG., London, W., Naranjo, A., Cohn, SL., Hogarty, M., et al. A phase III randomized clinical trial (RCT) of tandem myeloablative autologous stem cell transplant (ASCT) using peripheral blood stem cell (PBSC) as consolidation therapy for high-risk neuroblastoma (HR-NB): A Children's Oncology Group (COG) study. *J Clin Oncol*; ASCO Annual Meeting; 2016; Chicago, IL; 2016.
37. Simone, JV., Weiner, SL., Hewitt, M. *Childhood Cancer Survivorship:: Improving Care and Quality of Life*. National Academies Press; 2003.
38. American Academy of Pediatrics Section on Hematology/Oncology Children's Oncology G. Long-term follow-up care for pediatric cancer survivors. *Pediatrics*. 2009; 123(3):906–15. [PubMed: 19255020]

Highlights

- High-risk neuroblastoma survivors are at 17.5-fold greater risk of second cancers.
- There is a high prevalence of secondary leukemia in neuroblastoma survivors.
- Variants in DNA repair genes are most closely associated with second malignancies.

Figure 1.
Flow diagram of patients in the INRG database highlighting those with second malignancy and genotype data available.

Figure 2.
Cumulative incidence of second malignant neoplasms from time of diagnosis evaluated by clinical trial enrollment in 5,987 patients with neuroblastoma.

Table 1

Characteristics of 43 patients with neuroblastoma who developed a second malignant neoplasm compared by competing risk regression with 5,944 patients with neuroblastoma who did not develop second malignancies.

Feature	Sample Size (SMN) No. ^a	SMN Formed, %	No SMN Formed, %	P-value
Clinical Characteristic				
Median age at initial diagnosis	5,987 (43)	27.5 months	18.0 months	0.22
Age at diagnosis	5,987 (43)			
18 mo		58.1	50.0	0.27
< 18 mo		41.9	50.0	
Sex	5,987 (43)			
Male		37.2	53.4	0.07
Female		62.8	46.6	
Race	5,210(37)			
White		81.1	84.9	0.08
Black		8.1	11.8	
Asian/Native		10.8	3.3	
Ethnicity	4,984 (35)			
Non-Hispanic		91.4	88.5	0.88
Hispanic		8.6	11.5	
INSS Stage	5,885 (40)			
4		57.5	40.1	0.05
Not 4		42.5	59.9	
INSS Stage	5,885 (40)			
4		57.5	40.1	0.08
4S		2.5	4.9	
3		15.0	14.7	
2		15.0	18.2	
1		10.0	22.1	
Risk^b	5,697 (40)			
High		62.5	37.3	0.01
Intermediate		17.5	23.7	
Low		20.0	39.0	
Primary tumor site	3,374 (26)			
Nonadrenal		57.7	56.9	0.81
Adrenal		42.3	43.1	
Primary tumor site	3,374 (26)			
Nonthoracic		84.6	82.1	0.66
Thoracic		15.4	17.9	
LDH	765 (9)			

Feature	Sample Size (SMN) No. ^a	SMN Formed, %	No SMN Formed, %	P-value
< 587 u/L		55.6	42.1	0.43
≥ 587 u/L		44.4	57.9	
Serum Ferritin	1,144 (11)			
< 92 ng/mL		72.7	56.7	0.21
≥ 92 ng/mL		27.3	43.3	
Era of Diagnosis	5,993 (43)			
1997–2006		72.1	68.0	0.01
1990–1996		28.9	32.0	
Pathology/biology features				
MYCN	5,353 (40)			
Non-Amplified		85.0	85.4	0.82
Amplified		15.0	14.6	
INPC	4,770 (33)			
Unfavorable		69.7	38.8	0.001
Favorable		30.3	61.2	
Ploidy	4,180 (36)			
Hyperdiploid		66.7	68.1	0.79
Hypodiploid		33.3	31.9	
Tumor diagnosis	3,832 (23)			
Neuroblastoma		91.3	81.2	0.29
Ganglioneuroblastoma or ganglioneuroma		8.7	18.8	
Grade of differentiation	3,570 (22)			
Undifferentiated/poorly differentiated		95.5	86.1	0.22
Differentiating		4.5	13.9	
MKI	3,465 (21)			
Low/Intermediate		61.9	83.9	0.11
High		38.1	16.1	
LOH at 1p	999 (3)			
Absent		66.7	78.7	0.54
Present		33.3	21.3	
Aberration at 11q	1,691 (8)			
Absent		75.0	86.3	0.36
Present		25.0	13.7	
Gain of 17q	201 (0)			
Absent		0	69.7	N/A
Present		0	30.3	

Abbreviations: INPC, International Neuroblastoma Pathology Classification; INSS, International Neuroblastoma Staging System; LDH, Lactate dehydrogenase; LOH, loss of heterozygosity; MKI, mitosis-karyorrhexis index; N/A, not applicable.

^aThe sample size is the total number of patients and those with a SMN in parenthesis with data for the given row feature

^b Risk group was assigned according to the COG classification system [38] based on INSS stage, age, tumor histology, ploidy, and *MYCN* status.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 2

Number of second malignancies by histology according to time from original neuroblastoma diagnosis.

Histology	SMN <5 years from diagnosis (%)	SMN 5–10 years from diagnosis (%)	Total SMN in the cohort (%)
ALL or lymphoma	9 (37.5)	0 (0)	9 (20.9)
AML	8 (33.3)	2 (16.7)	10 (23.2)
Sarcoma	2 (8.3)	6 (50)	12 (27.9)
Carcinoma	3 (12.5)	1 (8.3)	6 (14)
Brain	2 (8.3)	3 (25)	6 (14)
Total	24	12	43

Table 3

Standardized incidence ratios and absolute excess risk of SMN in 5987 patients with neuroblastoma calculated by Era and risk group.

Characteristic	Number of patients	Person Years at Risk	Number of SMNs		SIR	95% CI on SIR	AER
			Observed	Expected ^a			
All SMN histology for total cohort	5987	34 390	43	5.8	7.5	5.4–10.0	10.8
Low-risk trial	2334	14 696	8	2.5	3.1	1.4–6.2	3.7
Intermediate-risk trial	1492	10 465	8	1.7	4.8	2.1–9.4	6.0
High-risk trial	2161	9229	27	1.6	17.5	11.4–25.3	27.6
AML within five years of neuroblastoma diagnosis	5987	12 247	8	0.1	96.1	46.0–176.7	8.1
Low-risk	2334	4939	1	0.04	23.2	0.3–128.9	1.9
Intermediate-risk	1492	2388	3	0.02	127.7	25.7–373.3	12.5
High-risk	2161	4920	4	0.04	106.8	28.7–273.4	8.1

^aNumber of expected SMNs according to the SEER database

Table 4

Variants with most significant *P*-values in the candidate gene association and eQTL association studies of an admixed cohort.

Candidate gene analysis		Genes	Major allele	Minor allele	Cases MAF	Controls MAF	<i>P</i> -value	Odds Ratio	95% Confidence Interval
Rs ID									
rs861539 [23]		<i>XRCC3</i>	G	A	0.45	0.29	0.006	2.04	1.2–3.46
rs4646903 [24]		<i>CYP1A1</i>	A	G	0.07	0.16	0.06	0.36	0.13–1.02
rs1903863 [25]		<i>CTNNA3</i>	G	T	0.32	0.42	0.09	0.65	0.37–1.13
rs11801299 [26]		<i>MDM4</i>	G	A	0.26	0.17	0.1	1.69	0.92–3.09
rs7091927 [25]		<i>CTNNA3</i>	T	C	0.18	0.30	0.11	0.51	0.26–1
eQTL analysis									
Rs ID		Genes	Major allele	Minor allele	Cases MAF	Controls MAF	<i>P</i>-value	Odds Ratio	95% Confidence Interval
rs17036651		<i>MSH2</i>	T	G	0.05	0.18	0.008	0.26	0.09–0.81
rs9612498		<i>GSTT1</i>	C	T	0.53	0.36	0.009	2.02	1.19–3.41
rs2236619		<i>GSTT1</i>	T	C	0.53	0.36	0.009	2.02	1.19–3.41
rs41475048		<i>LAMC2</i>	A	G	0.30	0.17	0.01	2.13	1.19–3.81
rs9608213		<i>GSTT1</i>	G	A	0.53	0.37	0.011	1.98	1.17–3.35

Table 5

Publications evaluating SMN in neuroblastoma survivors.

Reference	Population	Patients (n)	Years evaluated	Include <5 year survivors	SMN (n)	AML (n)	Cumulative incidence of SMN	Years from dx
Present study	INRG database, all risk groups	5,987	1990–2010	Yes	43	8	1.8% ^a	10
<i>Garaventa et al.</i> [27]	Single center, MIBG treated	119	1984–2001	Yes	5	2	20%	15
<i>Rubino et al.</i> [5]	Multicenter, all risk groups	544	1948–1986	No	12	1	8.9%	30
<i>Laverdiere et al.</i> [4]	Multicenter, all risk groups	954	1970–1986	No	13	2	7.0%	30
<i>Kushner et al.</i> [28]	Single center, high-risk	184	1990–2006	Yes	8 ^b	7	7.1%	5
<i>Martin et al.</i> [29]	Single center, high-risk	87	1991–2011	Yes	10	6	34.2%	15
<i>Federico et al.</i> [30]	Single institution, all risks	646	1961–2005	Yes	21	4	4.6%	30
<i>Applebaum et al.</i> [6]	SEER database	2,801	1973–2006	Yes	34	6	10.4% ^a	30
<i>Huibregtse et al.</i> [31]	Multicenter, MIBG treated	644	1984–2014	Yes	19	11	14.3%	10

^aHigh-risk cohort only

^bOnly secondary leukemia was evaluated in this study