original report

Neoplasm Risk Among Individuals With a Pathogenic Germline Variant in *DICER1*

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abstract

PURPOSE *DICER1* syndrome is an autosomal-dominant, pleiotropic tumor-predisposition disorder caused by pathogenic germline variants in *DICER1*. We sought to quantify risk, hazard rates, and the probability of neoplasm incidence accounting for competing risks ("cumulative incidence") of neoplasms (benign and malignant) and standardized incidence ratios for malignant tumors in individuals with *DICER1* pathogenic variation.

PATIENTS AND METHODS We combined data from three large cohorts of patients who carry germline pathogenic variation in *DICER1*. To reduce ascertainment bias, we distinguished probands from nonprobands. Neoplasm diagnoses were confirmed by review of pathology reports and/or central review of surgical pathology materials. Standardized cancer incidence ratios were determined relative to the SEER program, which does not capture all *DICER1*-associated neoplasms. For all malignancies and benign tumors ("neoplasms," excluding type Ir pleuropulmonary blastoma and thyroid nodules), we used the Kaplan-Meier method and nonparametric cumulative incidence curves to estimate neoplasm-free survival.

RESULTS We calculated the age at first neoplasm diagnosis (systematically ascertained cancers plus *DICER1*-associated neoplasms pleuropulmonary blastoma, cystic nephroma, and nasal chondromesenchymal hamartoma) in 102 female and male nonproband *DICER1* carriers. By age 10 years, 5.3% (95% CI, 0.6% to 9.7%) of nonproband *DICER1* carriers had developed a neoplasm (females, 4.0%; males, 6.6%). By age 50 years, 19.3% (95% CI, 8.4% to 29.0%) of nonprobands had developed a neoplasm (females, 26.5%; males, 10.2%). After age 10 years, female risk was elevated compared with male risk. Standardized cancer incidence ratio analysis of 102 nonproband *DICER1* carriers, which represented 3,344 person-years of observation, showed significant cancer excesses overall, particularly of gynecologic and thyroid cancers.

CONCLUSION This work provides the first quantitative analysis of site-specific neoplasm risk and excess malignancy risk in 102 systematically characterized nonproband *DICER1* carriers. Our findings inform *DICER1* syndrome phenotype, natural history, and genetic counseling.

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INTRODUCTION

DICER1 syndrome is an autosomal-dominant, familial pleiotropic tumor-predisposition disorder[1] caused by pathogenic germline variants in *DICER1*, an essential component of the microRNA processing pathway.[2] The hallmark neoplasm of *DICER1* syndrome is pleuropulmonary blastoma (PPB), a pediatric dysembryonic sarcoma of the lung and pleura.[3] Several other distinctive neoplasms, which often present in childhood, have been reported among *DICER1* pathogenic variant carriers, including cystic nephroma (CN), anaplastic renal sarcoma, Wilms tumor, Sertoli-Leydig cell tumor (SLCT) and gynandroblastoma, differentiated thyroid carcinoma, nasal chondromesenchymal hamartoma (NCMH), ciliary body medulloepithelioma (CBME), embryonal rhabdomyosarcoma (RMS), and primary brain tumors, including intracranial sarcoma, pituitary blastoma, and pineoblastoma.[4] *DICER1*-associated neoplasms are believed to evolve via an unusual form of Knudson's two-hit hypothesis. Instead of a second *DICER1* somatic loss-of-function mutation, they arise after acquisition of a trans-somatic missense *DICER1* mutation in an individual who harbors a pathogenic germline *DICER1* variant.[5-7] Critically, the somatic missense mutations in *DICER1*-associated neoplasms target one of five hotspot codons and result in hypomorphic *DICER1* RNase IIIb function.[8-10] Incomplete penetrance, especially for cancer phenotypes, and variable expressivity were recognized from early analyses of familial PPB pedigrees. The apparent requirement for somatic mutation in one of five specific codons may explain, in part, the incomplete penetrance noted in families.

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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The International PPB/*DICER1* Registry was established in 1988, shortly after the first clinicopathologic report of PPB to establish this neoplasm as a specific entity.[3] Although the registry is not population based, it nevertheless captures 68% to 84% of PPB in SEER-18 catchment areas,[11] increasing confidence in the generalizability of registry findings. Before 2016, all enrolled individuals had a documented *DICER1*-related condition, such as PPB. Since December 2016, *DICER1* carriers without a tumor history have also been enrolled. In 2011, the International Ovarian and Testicular Stromal Tumor Registry was founded as a parallel registry and applies similar strategies to study these rare ovarian and testicular neoplasms, including the link between *DICER1* and sex cord-stromal tumors. The *DICER1* study at the National Cancer Institute (NCI) also opened in 2011 with a goal to comprehensively study the phenotype of proband and nonproband *DICER1* carriers. Together, these three studies have contributed to the delineation of the phenotypic spectrum and natural history of *DICER1*-associated tumors and have elucidated treatment-related outcomes in PPB.[11]

To date, no published analyses have quantified risk, hazard rates, and probability of neoplasm incidence accounting for competing risks (hereafter, "cumulative incidence") of neoplasms (benign and malignant) in individuals with germline *DICER1* pathogenic variation (hereafter, "*DICER1* carriers"). This is especially important as germline pathogenic *DICER1* variation is estimated to occur in the general population in approximately one in 10,600 people.[12] To address these knowledge gaps, we determined the cumulative neoplasm incidence in probands and nonproband (proband relatives) *DICER1* carriers. We quantified the standardized incidence ratios of malignancy for SEER Program–tracked cancers in nonprobands and probands. Lastly, in *DICER1* carriers with one or more neoplasm, we determined pathologic type of neoplasm, age at onset, and frequency of multiple primary neoplasms.

PATIENTS AND METHODS

Study Participants

Individuals were enrolled in the International PPB/*DICER1* Registry (ClinicalTrials.gov identifier: NCT01464606), the NCI Natural History of *DICER1* Syndrome study (ClinicalTrials.gov identifier: NCT01247597), and/or the International Ovarian and Testicular Stromal Tumor Registry (ClinicalTrials.gov identifier: NCT01970696). Eligibility criteria included a history of *DICER1*-associated neoplasm or documentation of a pathogenic germline *DICER1* variant. In the NCI Natural History of *DICER1* Syndrome study (hereafter, the NCI *DICER1* study), the proband—the enrolled individual through whom each family was ascertained—and his or her biologic relatives were recruited and tested for *DICER1* pathogenic variation. Nonproband *DICER1* carriers are biologic relatives of an enrolled proband harboring the familial *DICER1* variant. Medical and family history, relationship to proband, germline *DICER1* testing results, and vital status were recorded for all participants. Medical records and questionnaires from participants (probands and nonprobands) in the three studies were systematically reviewed for neoplasm (benign and malignant) diagnoses. We conducted follow-ups at regular intervals with participants to document new diagnoses. Whenever possible, neoplasm diagnoses were confirmed by review of surgical pathology reports and/or central review of pathology materials. A subset of families was invited to a comprehensive, 3-day NIH Clinical Center evaluation, including imaging and physical examination. We considered individuals with a *DICER1*-associated neoplasm and no pathogenic germline *DICER1* variant (mosaic/sporadics) separately. As enrollment in more than one of these studies was common, we removed duplicate entries. All protocols were approved by the relevant institutional human subjects' committees, and informed written consent/assent was obtained for each participant.

Standardized incidence ratios (SIRs) for malignancy (collectively, cancers, which excluded nonmelanoma skin cancers) were determined assuming a Poisson distribution for the observed case counts. We compared the observed number of cancers in *DICER1* carriers with expected frequencies from the SEER program[13] on the basis of age-, sex-, race-, and birth cohort–specific cancer incidence data. We calculated the ratio of observed-to-expected cancers for the cohort. We described continuous variables using medians and ranges, testing for differences using the Wilcoxon rank-sum test. We assessed differences in proportions using the χ^2 test or an exact test when limited by infrequent observations ($n < 5$). Analyses were stratified by gender and/or adulthood (age 18 years and older). SEER analyses used SAS (SAS/STAT User's Guide, Version 9.3; SAS Institute, Cary, NC) and Surveillance Research Program, NCI SEER*Stat software (version 8.3.4; http://www.seer.cancer.gov/seerstat).

Analyses of survival, probability of neoplasm incidence accounting for competing risks (cumulative incidence), age at onset, and counts of multiple primaries included all malignancies except nonmelanoma skin cancer, plus NCMH and CN (collectively, "neoplasms"). We excluded type Ir PPB (lung cysts without a primitive malignant component) and thyroid nodules, previously reported,[14] from primary analyses. We excluded neoplasm recurrence and metastasis end points in our analysis, but included metachronous and synchronous neoplasms when this distinction could be determined confidently. The Kaplan-Meier method provided neoplasm-free survival estimates, with spline functions generating annual hazard of neoplasm diagnosis smoothed estimates[15,16] for NCI cohort male and female nonproband carriers. We estimated nonparametric cumulative incidence curves for competing first neoplasm diagnoses (types I to III PPB, CN, SLCT, and all other neoplasm types) using all NCI cohort

participants.[17] Onset dates for first observed neoplasms within all three cohorts were plotted by site. Neoplasms in individuals with multiple neoplastic diagnoses were tabulated, and pairwise counts from the most common neoplasm diagnoses were plotted in an undirected graph. We estimated neoplasm-free survival and hazard of neoplasm using MATLAB (version R2017a; MathWorks, Natick, MA). All other analyses were conducted using R (version 3.3.1)[18] with survival package (version 2.38).[19,20]

RESULTS

Table 1 lists the clinical data pooled from the registries and the NCI *DICER1* study (all participants; N = 207), the NCI *DICER1* study only (n = 148), and the NCI nonproband *DICER1* carriers (n = 102). The majority of participants were non-Hispanic whites. To evaluate survival bias, we considered the relationships of the nonprobands to the probands (Appendix Table A1, online only). Of note, of the 17 nonproband participants who were diagnosed with a neoplasm, six (19%) of the 32 parents and one (10%) of the 10 grandparents had an observed neoplasm versus four (14%) of 28 siblings and one (20%) of five offspring. Appendix Table A2 (online only) lists the number and neoplasm types observed in all *DICER1* carriers and in only nonprobands from the NCI *DICER1* study. Appendix Table A3 (online only) describes the 26 participants without a detectable pathogenic germline *DICER1* variant but with a history of at least one *DICER1*-associated neoplasm. These were either sporadic neoplasms or neoplasms arising in participants with suspected mosaicism. Appendix Table A4 (online only) lists the 31 neoplasms in the 26 mosaic/sporadic participants.

To better understand the full spectrum of malignancy in *DICER1* carriers, we conducted SIR analyses of 207 *DICER1* carriers (probands and nonprobands) in the entire cohort (Appendix Table A5, online only). Given that many study participants were ascertained because they had a *DICER1*-associated neoplasm, there were greatly increased, significant risks for known syndrome-associated neoplasms, including gynandroblastoma, PPB, RMS, SLCT, and thyroid carcinoma. We observed one neuroblastoma. SIR analysis of 148 *DICER1* carriers—probands and nonprobands (Appendix Table A6, online only)—from the NCI *DICER1* study cohort demonstrated a neoplasm profile that was similar to the *DICER1* carriers in the entire cohort.

To quantify the excess risk of cancer in the cohort while minimizing potential bias, we performed SIR analysis on 102 nonproband *DICER1* carriers from the NCI *DICER1* study, which represented 3,344 person-years of observation (Table 2). We observed evidence for elevated malignancy risk on the basis of SIR calculations. In addition to the malignancies listed in Table 2, we also observed one type I PPB, two CBMEs, two CNs, and one NCMH in the 102 nonproband *DICER1* carriers; however, as CBME, CN, NCMH, and type I PPB are not tracked by SEER, risk estimates could not be calculated.

To better understand the natural history of PPB, we quantified the number of type Ir PPB (regressed or nonprogressed type I PPB) in the 102 nonproband *DICER1* carriers. There were 28 type Ir PPBs in 28 participants (27%; female, n = 15; male, n = 13; $P > .05$). Of these, five were resected and pathologically confirmed and 23 were detected using imaging in individuals above the typical age of progression.

Figure 1 compares the age at first neoplasm diagnosis (systematically ascertained cancers plus *DICER1*-associated neoplasms type I PPB, CN, and NCMH) in the NCI study in

TABLE 1. Demographics, Neoplasm Burden, and Mortality in *DICER1* Carriers From the NCI *DICER1* Study, International Pleuropulmonary Blastoma/*DICER1* Registry, and Ovarian and Testicular Stromal Tumor Registry

Variable	All Participants	NCI *DICER1* Study	NCI *DICER1* Nonprobands
No. of *DICER1* carriers	207	148	102
No. of families	101	46	35
Median age at last follow-up (IQR), years	15.6 (29.5)	24.3 (33.2)	33.1 (35.2)
Sex, No. (% female)	113 (55)	81 (55)	53 (52)
Person-years of follow-up	4,737	3,942	3,337
Total No. of neoplasms	151	76	18
Total No. of malignant neoplasms	128	61	15
Mean neoplasms per *DICER1* carrier	0.73	0.51	0.18
No. (%) of *DICER1* carriers with one or more neoplasms	116 (56)	61 (41)	17 (17)
No. of deceased participants	7	0	0

NOTE. Among the 207 *DICER1* carriers, seven (three females, four males) were deceased, three had been diagnosed with a type II pleuropulmonary blastoma (PPB), one with a type II/III PPB, and three with a type III PPB. Of the neoplasms listed in the Appendix Table A8 (online only), all were considered malignant, except nasal chondromesenchymal hamartoma and cystic nephroma. See the Study Flow diagram (Appendix Fig. A2, online only).

Abbreviations: IQR, interquartile range; NCI, National Cancer Institute.

all female and male nonproband *DICER1* carriers to age 20 (Fig 1A) and 60 years (Fig 1B), respectively. By age 10 years, 5.3% (95% CI, 0.6% to 9.7%) of nonproband *DICER1* carriers had developed a neoplasm (females, 4.0%; males, 6.6%). Penetrance by decade for nonproband *DICER1* carriers age 10 to 60 years is shown in Appendix Table A7 (online only). Care should be taken in the interpretation of cumulative incidence in the older age ranges as the small number of individuals observed at those ages leads to reduced precision in estimation. The hazards of a neoplasm diagnosis (percent-per-year risk for cancers plus *DICER1*-associated neoplasms CN and NCMH) in nonproband *DICER1* carriers to age 20 years and age 60 years are shown in Figures 1C and 1D, respectively.

We systematically ascertained neoplasms in the 148 probands and nonprobands in the NCI *DICER1* study. Figure 2 shows the early childhood and lifetime cumulative incidence of types I, II, and III PPB, all PPB, CN, SLCT, other neoplasms, and all neoplasms combined, as a first malignancy event, in this cohort. Cumulative incidence is calculated with each PPB type, CN, SLCT, and other neoplasms as competing first malignancy events. As a result of the small numbers, deaths were censored rather than considered as a competing risk.

Finally, we plotted the age at neoplasm diagnosis and neoplasm type for the pooled 116 *DICER1* carriers with one or more neoplasms (proband and nonproband) in early childhood (Fig 3A) and to age 60 years (Fig 3B). Three individuals were diagnosed with CN in adolescence, older than the typically cited age risk of 4 years or younger. The wide age-at-onset range for SLCT was notable. Risk for thyroid carcinoma begins at younger than age 10 years and continues into adulthood, as we have previous reported.[14] NCMH was observed in children and young adults.[21] Of 116 *DICER1* carriers with a neoplasm, 26 (22%) developed multiple neoplasms over time. The most frequent co-occurrences involved known *DICER1*-associated neoplasms (eg, PPB, CN, NCMH, SLCT, or thyroid carcinoma; Appendix Fig. A1, online only). Among females with a *DICER1*-related ovarian neoplasm, we observed three (13%) of 24 with a contralateral metachronous SLCT and one (17%) of six with multiple primary RMS (Appendix Fig. A1).

DISCUSSION

This report provides the first quantitative analysis of site-specific neoplasm risk and excess malignancy risk in a large cohort of systematically characterized nonproband *DICER1* carriers. Follow-up of participants allowed for the prospective documentation of additional incident neoplasms over time. In nonprobands, we observed the spectrum of *DICER1*-associated neoplasms (thyroid carcinoma, type I PPB, CN, CBME, and gynecologic tumors) and, for the first time, quantified neoplasm penetrance and demonstrated significantly increased risks for all cancers combined. Of note, there were no deaths observed in nonproband *DICER1* carriers. Our findings inform *DICER1* syndrome phenotype, natural history, and genetic counseling.

Among all *DICER1* carriers, the most common neoplasms were PPB; gynecologic tumors, especially SLCT and RMS; and CN. Thyroid carcinoma was the most common neoplasm in nonproband *DICER1* carriers. Other less common but established *DICER1*-associated cancers, such as pineoblastoma, pituitary blastoma, and Wilms tumor, were not observed in this study. The six RMSs arose exclusively from the gynecologic tract. Case reports describe neuroblastoma in children with *DICER1* syndrome,[4] but we

TABLE 2. Standardized Incidence Ratios of 11 Cancers Observed in a Cohort of 102 Nonproband Carriers (3,344 person-years of risk) With a Germline *DICER1* Pathogenic Variant From the National Cancer Institute *DICER1* Study Only

Tumor Site	Observed No. of Cases	Expected No. of Cases	Standardized Incidence Ratio (observed/expected)	95% CI	Mean Age at Event, Years*
All sites, except nonmelanoma skin cancers	11	5.32	**2.1**	1.0 to 3.7	41
Gynandroblastoma	1	0.00	**1.0×10^5**	2.6×10^3 to 5.8×10^5	16
Breast cancer†	1	0.90	1.1	0.03 to 6.2	30
Melanoma	1	0.32	3.2	0.08 to 18	60
Prostate cancer	3	0.69	4.4	0.9 to 13	58
Sertoli-Leydig cell tumor	1	0.00	**2.7×10^3**	68 to 1.5×10^4	60
Thyroid cancer	4	0.21	**19**	5.1 to 48	29

NOTE. Bolded observed/expected ratio denotes $P < .05$.

*For privacy age rounded to nearest 5-year interval for tumor types affecting one person only.

†Breast cancer was observed in one female younger than age 30 years with poorly differentiated invasive ductal carcinoma (estrogen receptor/progesterone receptor negative, human epidermal growth factor receptor 2 positive). She underwent neoadjuvant chemotherapy. Lumpectomy was planned; however, the discovery of an additional right ductal carcinoma in situ prompted a mastectomy. There has been no recurrence. She had no family history of breast cancer and negative breast cancer panel genetic testing.

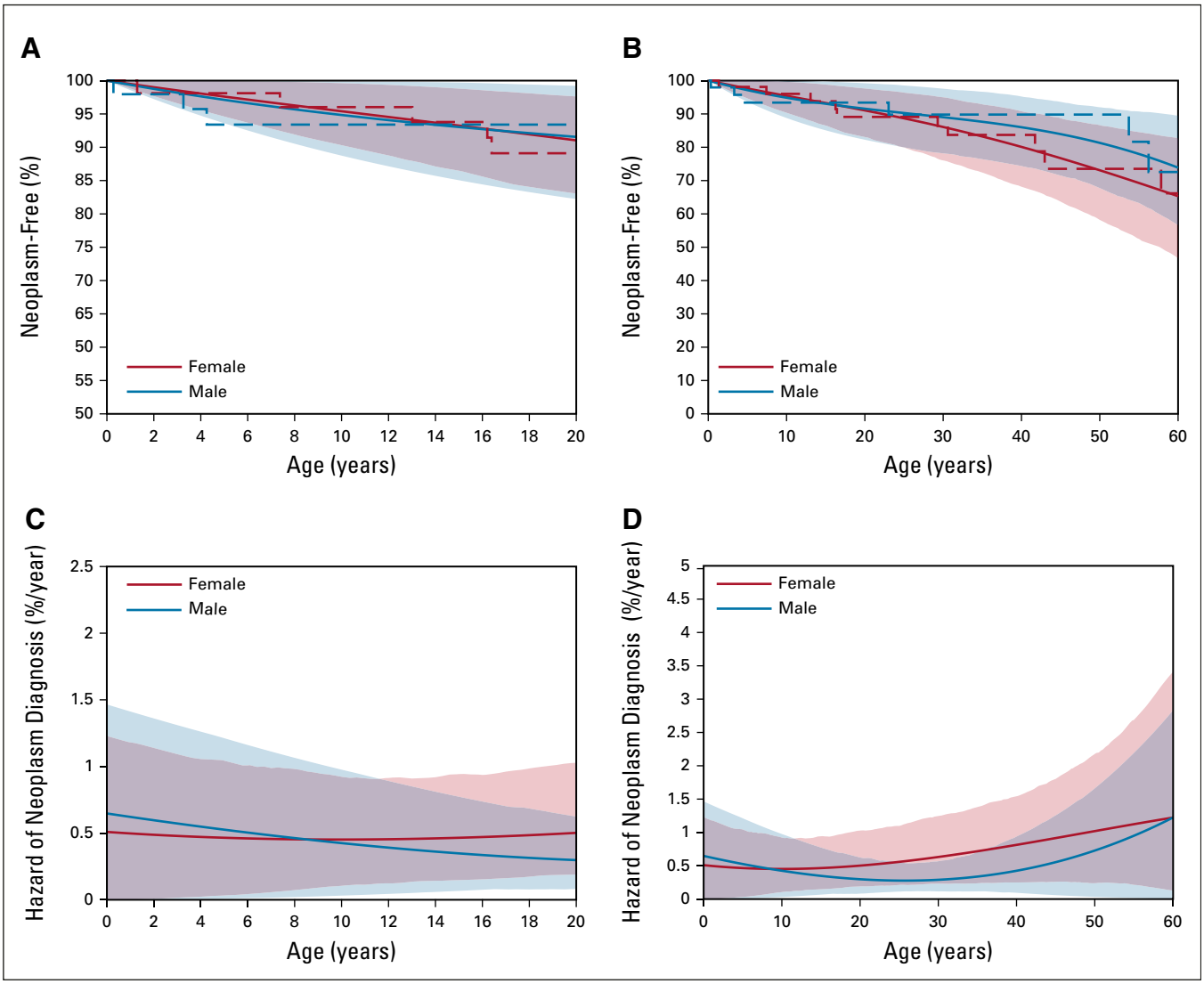

FIG 1. Estimated neoplasm-free survival and hazard of first neoplasm—cancers plus *DICER1*-associated neoplasms cystic nephroma and nasal chondromesenchymal hamartoma—incidence among nonproband *DICER1* carriers of the National Cancer Institute *DICER1* study. (A and B) Estimated neoplasm-free survival among these individuals. See Appendix Table A7 (online only) for penetrance of neoplasm diagnosis by decade. (C and D) Estimated hazard of first neoplasm diagnosis. Female participants are denoted in red and male in blue. Dashed lines indicate nonparametric estimates (Kaplan-Meier curves). Solid lines indicate spline-estimated cumulative incidence and hazard. Left panels display curves to age 20 years and right panels to age 60 years. Common vertical axis markers of 0.5% event-free survival and 0.025 hazard are indicated by horizontal dashed lines and bold axis markers, and age 20 years is similarly marked on the horizontal axis.

observed no significant neuroblastoma excess and no participant developed testicular cancer, which is consistent with previous reports.[22,23] Among all participants, we observed individual examples of rare cancers not previously reported in *DICER1* carriers (thymoma, spindle cell carcinoma of the kidney, and teratoma). The significantly elevated SIRs were driven by the exceedingly small (essentially zero) expected case numbers. The significant excess of malignant peripheral nerve sheath tumors in our SIR analysis is likely explained by the coexistence of neurofibromatosis type I in the two affected children. There were nonsignificant excesses of common adult tumors (melanoma, prostate, and breast cancer).

Most individuals with pathogenic germline *DICER1* variation live generally healthy lives. Although risks of malignancy are elevated, we note that many *DICER1*-related cancers, including PPB, SLCT, and gynandroblastoma, are most curable when found at an early stage. Neoplastic risk increases with age, especially in girls and women, predominantly because of gynecologic cancers. Ultrasound represents a feasible surveillance strategy for these tumors, which are largely curable with surgery alone when found before rupture or capsular invasion. With CN, a histologically benign condition, early diagnosis may be nephron sparing and resection may prevent progression to renal sarcoma.

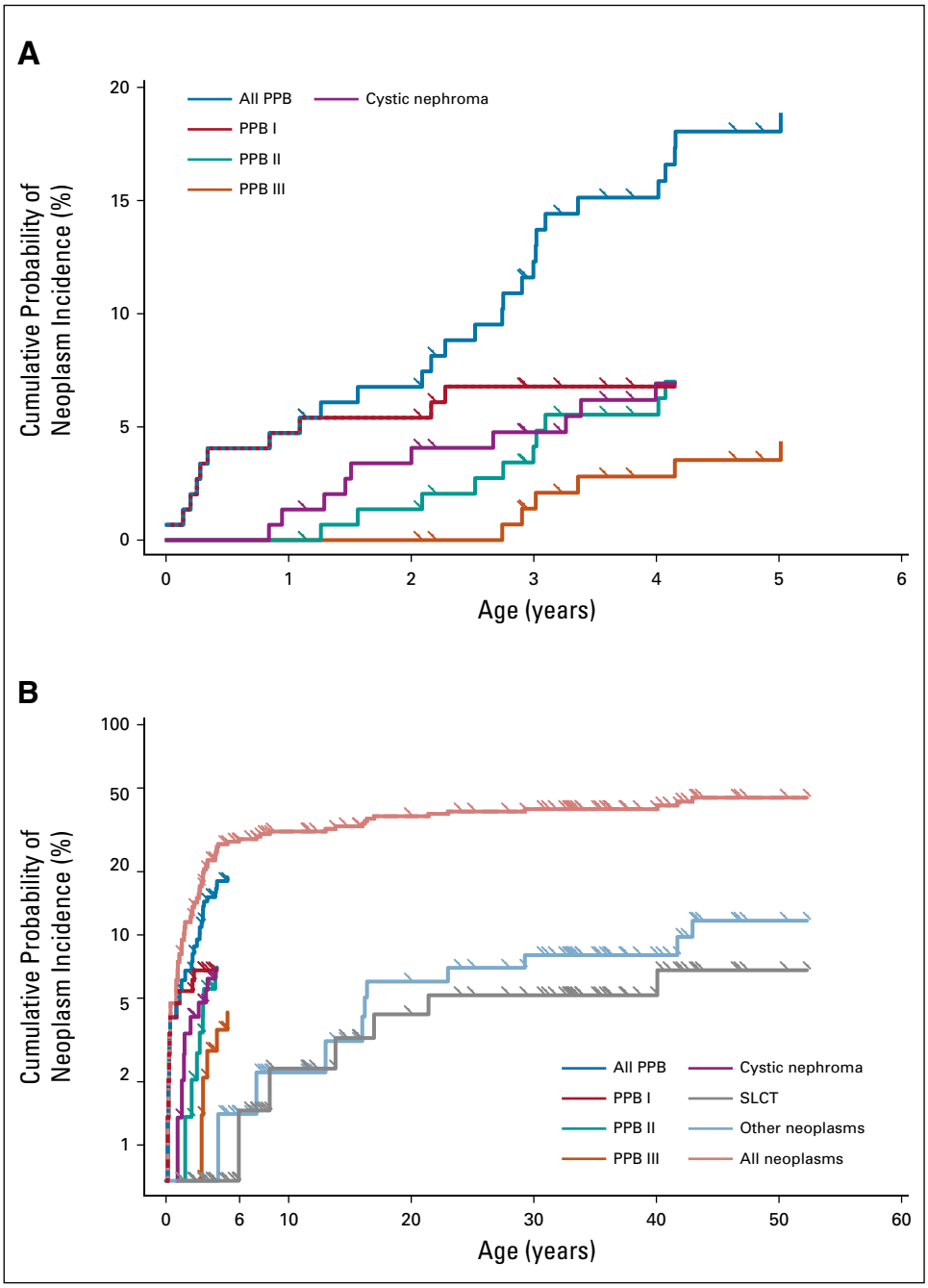

FIG 2. Estimated probability of neoplasm development accounting for competing risks—cumulative incidence—of specific *DICER1*-associated neoplasms among probands and nonprobands from the National Cancer Institute *DICER1* study: type I pleuropulmonary blastoma (PPB; red), type II PPB (green), type III PPB (orange), cystic nephroma (purple), and Sertoli-Leydig cell tumor (SLCT; gray). Hatched multicolor lines denote overlap of multiple neoplasm cumulative risks. Cumulative incidence to (A) age 6 years and (B) age 60 years. Tick marks denote censoring times.

With that in mind, we recommend familial cascade *DICER1* testing to identify additional family members who are at risk for *DICER1*-related neoplasms. We emphasize that, given the moderate penetrance in the *DICER1* syndrome, a negative family history should not dissuade an individual from genetic testing when an individual presents with a condition known to be associated with *DICER1*, including PPB, CN, ovarian SLCT, or gynecologic sarcoma.

Surveillance strategies to manage risk in *DICER1* carriers have been recently published.[24,25] We recommend a surveillance chest computed tomography scan in the first year of life and, if normal, again at age 2 years, shortly before the peak incidence of types II and III PPB. Intermittent chest radiography during childhood is also recommended. The risk of sedation, which may be needed for cross-sectional imaging, and radiation exposure with computed tomography scan or chest radiographs must be balanced with the dramatic increase in survival and the decreased need for intensive therapies when PPB is found as the purely cystic type I (5-year overall survival of 91% compared with that of type II or type III [5-year overall survival, 74% and 53%, respectively]).[11] Given the markedly increased risk for

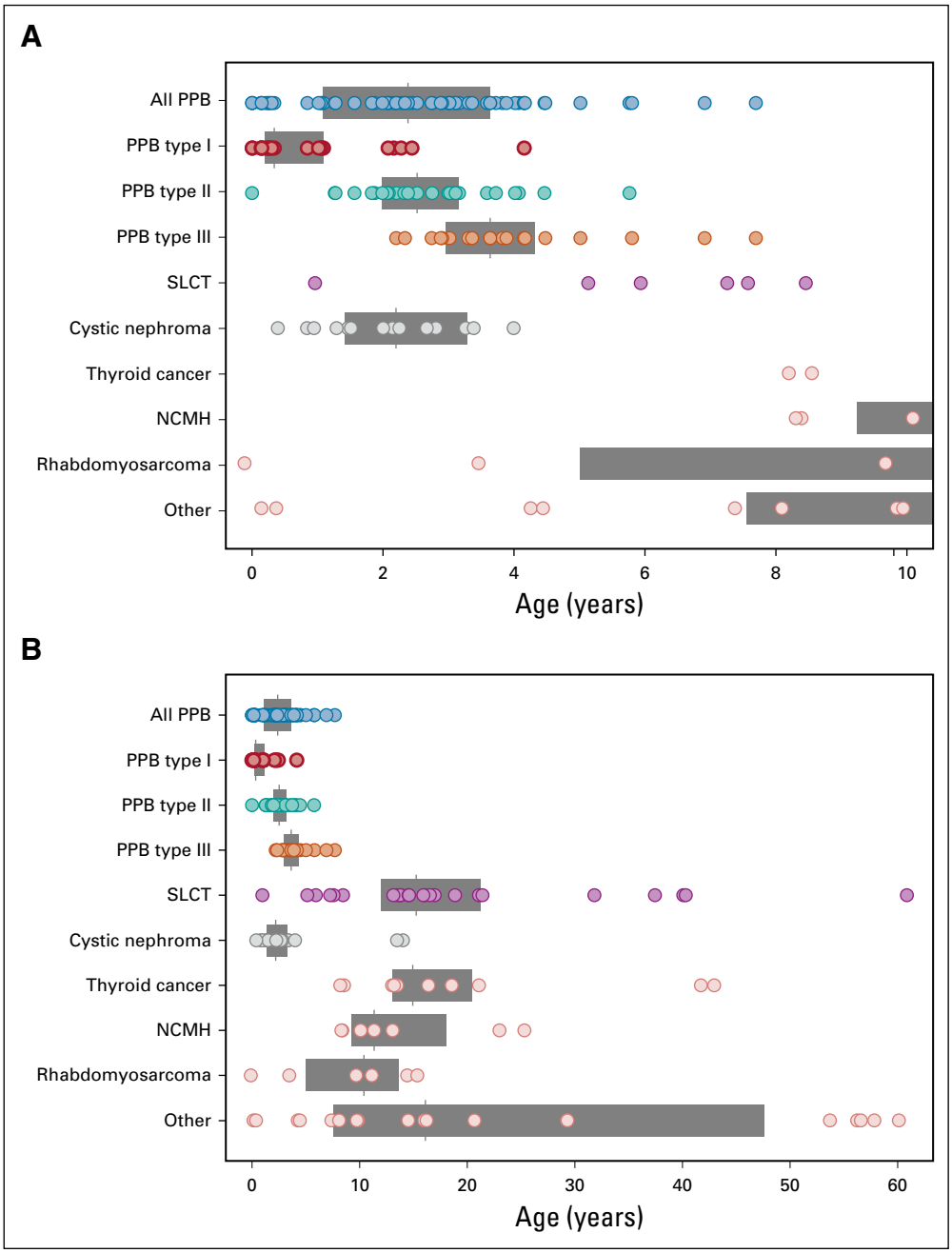

FIG 3. Age of neoplasm diagnosis and neoplasm type for the 116 *DICER1* carriers (151 neoplasms; proband and nonproband) from all three studies. Specific *DICER1*-associated neoplasms are highlighted in color: type I pleuropulmonary blastoma (PPB; red), type II PPB (green), type III PPB (orange), Sertoli-Leydig cell tumor (SLCT; purple), and cystic nephroma (gray). Diagnoses to (A) age 10 years and (B) age 60 years. Gray boxes span from the first to the third quartile of observed diagnoses, and vertical bars in the boxes indicate median diagnosis age. NCMH, nasal chondromesenchymal hamartoma.

ovarian sex cord-stromal tumors associated with a favorable prognosis if found at stage IA, we also recommend surveillance pelvic ultrasounds beginning in childhood and extending through adulthood. If an ovarian tumor is found, surgical resection should include appropriate staging. Thyroid evaluation is recommended every 3 years unless nodules are detected. When nodules are found, age-appropriate guidelines for the evaluation of thyroid nodules from established endocrine societies should be followed. To place these results and surveillance recommendations in context, the cumulative risk of breast cancer in *BRCA1* or *BRCA2* carriers is approximately 4% between the age of 21 and 30 years, increasing thereafter.[26] For this risk, annual surveillance breast magnetic resonance imaging is recommended from age 25 to 29 years.[27]

In *DICER1* carriers, penetrance for less serious conditions, such as thyroid nodules, is substantially higher than that for malignant conditions, such as types II or III PPB or SLCT. Although penetrance for benign thyroid nodules is high, only a small percentage progress to thyroid carcinoma.[14] We observed a similar natural history in PPB in this study. In the 102 nonprobands, we observed one type I PPB and 28 type Ir PPB. The latter are type I PPB that either experienced regression or, more likely, never experienced disease progression beyond a simple lung cyst and harbor

little-to-no malignant potential. The etiology and mechanism of this regression/nonprogression is unknown. Thus, in the 102 nonproband *DICER1* carriers, there was radiographic or histologic evidence of type I or Ir PPB in 29 individuals (28%). Our findings suggest that cystic PPB is common in *DICER1* carriers and that only a minority experience progression to type II or III PPB, given the rarity of these tumors. Factors that contribute to this transformation merit investigation, and additional studies are underway to determine what methods will best predict progression and/or detect progression at the earliest possible time point.

Strengths of this analysis include the large cohort of *DICER1* carriers that was pooled from multiple studies and detailed follow-up information from study participants. More heavily affected, multigenerational families may have a greater likelihood of undergoing *DICER1* genetic testing and may therefore inflate the risk of neoplasm estimates. SIR results are descriptive of those included in the NCI cohort and must be interpreted by accounting for the operative convenience sampling associated with study participation. SIR analysis is likely an underestimate as it is constrained to consider only cancers ascertained or precisely identified by the SEER system, which does not capture type I PPB, CBME, CN, or NCMH, all clinically relevant neoplasms observed in these participants. In families in which the original proband died before enrollment or did not enroll in the NCI study, the first ascertained enrolled individual was considered the proband for the purpose of proband/nonproband analysis. If deceased individuals who were enrolled only in a registry study had been included in the proband/nonproband analyses, at least one additional case of type I PPB in a nonproband would have been noted in this analysis. Survival bias is also reflected in higher neoplasm counts among younger relatives (ie, siblings and offspring) compared with older relatives (ie, parents and grandparents). Our study population was overwhelmingly non-Hispanic white. There are no racial groups with a known increased risk for *DICER1*-associated neoplasms, although this merits additional study. We acknowledge the limited ethnic and racial heterogeneity of the three studies and believe it reflects known, long-standing biases in biomedical research recruitment. Although this analysis represents the largest available cohort, the sample size confers relatively wide CIs.

We are now refining *DICER1* syndrome prevalence and penetrance estimates from large-scale exome sequencing data. Additional clinical strategies are needed to identify the subset of *DICER1* carriers who are at highest risk of the most aggressive disease phenotypes, particularly type II and III PPB and gynecologic tumors.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Neoplasm Risk Among Individuals With a Pathogenic Germline Variant in *DICER1*

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APPENDIX

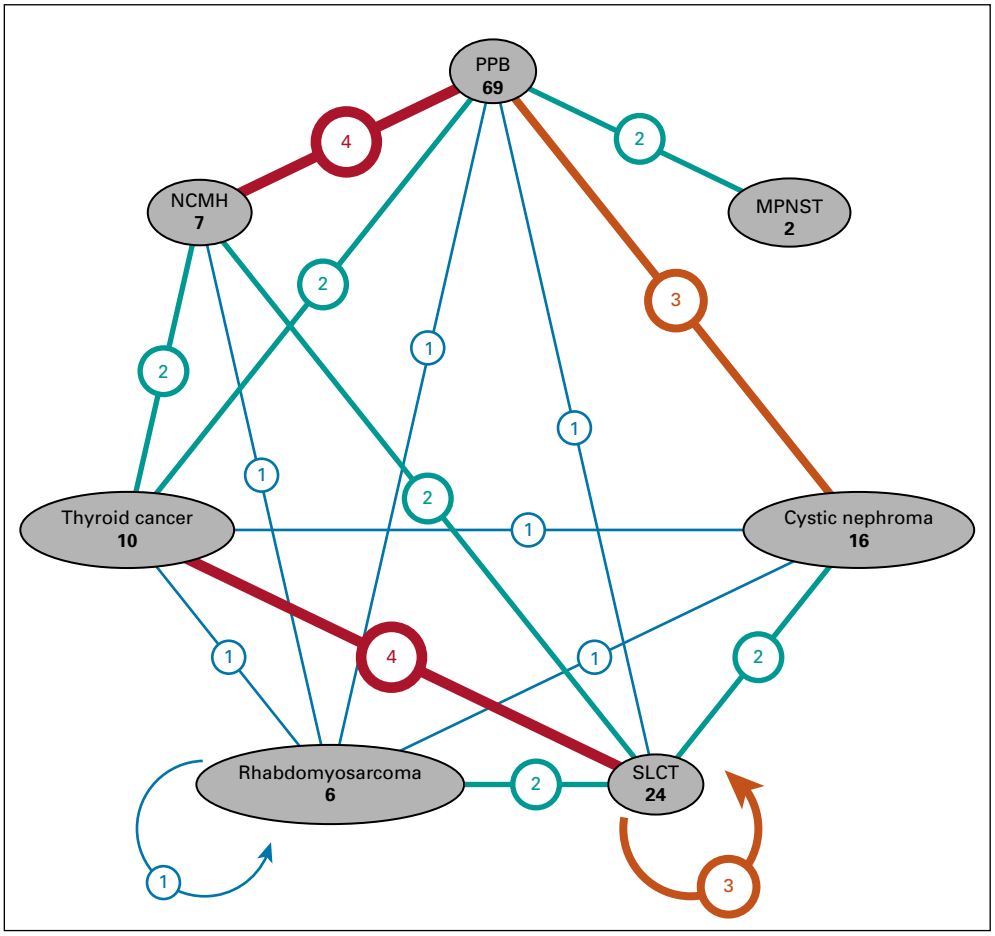

FIG A1. Diagram of counts of multiple primary *DICER1*-associated neoplasms: Pleuropulmonary blastoma (PPB, all types, except type Ir), malignant peripheral nerve sheath tumor (MPNST), nasal chondromesenchymal hamartoma (NCMH), Sertoli-Leydig cell tumor (SLCT), rhabdomyosarcoma, cystic nephroma, and thyroid cancer. Each neoplasm type is displayed as a vertex; for each pair of neoplasm, a line between their corresponding vertices indicates the number of individuals with at least one primary neoplasm of each type. The weight and color of the line, along with the number printed in a bubble on each line, indicate the number of individuals. Vertices with no shared line (eg, cystic nephroma and MPNST) indicate that no participants had a primary neoplasm of each type. One participant had multiple primary rhabdomyosarcomas, and three participants had multiple primary SLCTs. Data are also presented in Appendix Table A8 (online only).

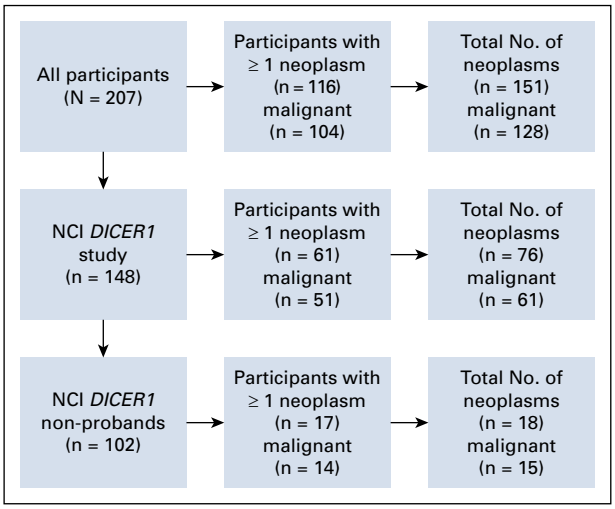

FIG A2. Study flow diagram detailing participant, neoplasm, and malignancy counts in the study (excluding type Ir PPB and thyroid nodules). NCI, National Cancer Institute; PPB, pleuropulmonary blastoma.

TABLE A1. Non-Proband NCI *DICER1* Study Participants, by Relation to Proband, With Corresponding Numbers of Neoplasms in Participants With One or More Neoplasms

Relation to Proband	No. of Non-Proband Participants	No. of Non-Proband Participants With Neoplasms
Parent	32	6
Sibling	28	4
Offspring	5	1
Grandparent	10	1
Aunt/uncle	12	3
Niece/nephew	5	1
Half-sibling	1	0
Cousin	7	0
Great aunt/uncle	2	1
Totals	102	17

TABLE A2. Counts of Observed Neoplasms by Type, Sex, and Participant Type in All *DICER1*-Carriers Participants and in Non-Proband *DICER1* Carriers From the NCI *DICER1* Study

	Females		Males		All	
Neoplasm Type	**All Participants**	**Non-Proband Carriers (NCI)**	**All Participants**	**Non-Proband Carriers (NCI)**	**All Participants**	**Non-Proband Carriers (NCI)**
Breast cancer	1	1	0	0	1	1
CBME	1	1	1	1	2	2
Cystic nephroma	7	1	9	1	16	2
Gynandroblastoma	2	1	0	0	2	1
Melanoma	2	1	0	0	2	1
MPNST	0	0	2	0	2	0
NCMH	4	0	3	1	7	1
Neuroblastoma	0	0	1	0	1	0
Non-melanoma skin	1	0	1	1	2	1
PPB type I	8	0	14	1	22	1
PPB type II	15	0	10	0	25	0
PPB type II-III	1	0	2	0	3	0
PPB type III	10	0	9	0	19	0
Prostate Cancer	–	–	3	3	3	3
Rhabdomyosarcoma	6	0	0	0	6	0
SLCT	24	1	–	–	24	1
Spindle cell carcinoma	1	0	0	0	1	0
Teratoma	1	0	0	0	1	0
Thymoma	1	0	0	0	1	0
Thyroid cancer	9	4	1	0	10	4
Undifferentiated ovarian sarcoma	1	0	–	–	1	0
Total observed neoplasms	95	10	56	8	151	18
Number of participants with						
At least one observed neoplasm	69	10	47	7	116	17
No neoplasms	44	43	47	42	91	85
Totals	113	53	94	49	207	102

Abbreviations: CBME, ciliary body medulloepithelioma; MPNST, malignant peripheral nerve sheath tumor; NCI, National Cancer Institute; NCMH, nasal chondromesenchymal hamartoma; PPB, pleuropulmonary blastoma; SLCT, Sertoli-Leydig cell tumor.

TABLE A3. Demographics, Neoplasm Burden, and Mortality in *DICER1*-Mosaic/Sporadic Participants From the NCI *DICER1* Study, International Pleuropulmonary Blastoma Registry, and Ovarian and Testicular Stromal Tumor Registry

Variable	All Participants	NCI *DICER1* Study
No. of mosaic/sporadic participants	26	21
No. of families	26	21
Median age at last follow-up, years (IQR)	12 (11.4)	11.6 (11)
Sex (No. % female)	16 (61.5)	12 (57.1)
Person-years of follow-up	370	302
Total No. of neoplasms	31	20
Total No. of malignant neoplasms	28	19
Mean neoplasms per mosaic/sporadic participant	1.19	0.95
No. of mosaic/sporadic participants with ≥ 1 neoplasms (%)	24 (92.3)	19 (90.5)
No. of deceased participants	1	1

NOTE. There were no mosaic/sporadic participants in both the NCI *DICER1* study and the registries. Two mosaic/sporadic participants had type Ir PPB (lung cysts) only and no tumors.

Abbreviations: IQR, interquartile range; NCI, National Cancer Institute; PPB, pleuropulmonary blastoma.

TABLE A4. Neoplasm Counts Among *DICER1*-Mosaic/Sporadic Participants

	Observed No. of Neoplasms	
Neoplasm Type	All Participants	NCI *DICER1* Study
PPB type I	7	6
PPB type II	5	5
PPB type III	4	2
SLCT	9	6
Cystic nephroma	3	1
Thyroid cancer	2	0
Primitive abdominal neoplasm, primary site unknown	1	0

Abbreviations: NCI, National Cancer Institute; PPB, pleuropulmonary blastoma; SLCT, Sertoli-Leydig cell tumor.

TABLE A5. SIR of 102 Cancers Observed in Entire Cohort of 207 *DICER1* Carriers (probands and non-probands; 4,747 person-years of risk)

Tumor Site	Observed No. of Cases	Expected No. of Cases	SIR (observed/expected)	95% Confidence Interval (lower)	95% Confidence Interval (upper)	Mean Age at Event (years)*
All sites (excluding non-melanoma skin cancers)	102	5.73	**18**	15	22	12
Thymoma	1	0	**547**	14	3.0×10^3	10
Gynandroblastoma	2	0.00	**1.4×10^5**	1.7×10^4	4.9×10^5	16
Breast cancer	1	0.93	1.08	0.03	6.0	30
MPNST	2	0	**398**	48	1.4×10^3	10
Melanoma	2	0.35	5.8	0.7	21	44
Neuroblastoma	1	0.03	31	0.78	174	0
Pleuropulmonary blastoma (non-type I)	47	0	**3.2×10^6**	2.4×10^6	4.3×10^6	3
Prostate cancer	3	0.69	4.3	0.89	13	58
Rhabdomyosarcoma	6	0.01	**403**	148	878	10
Sertoli-Leydig cell tumor	24	0	**4.2×10^4**	2.7×10^4	6.3×10^4	19
Spindle cell carcinoma	1	0	**821**	21	4.6×10^3	10
Teratoma	1	0	**350**	9	1.9×10^3	0
Thyroid cancer	10	0.25	**39**	19	72	20
Undifferentiated ovarian sarcoma	1	0	**6.8×10^4**	1.7×10^3	3.8×10^5	10

NOTE. Bolded observed/expected SIR ratio denotes $P < .05$. Type I pleuropulmonary blastoma rates are not tracked by SEER. The MPNST were observed in two children with concomitant neurofibromatosis type 1, a monogenic disorder associated with MPNST. The low-grade, spindle cell kidney carcinoma and malignant teratoma occurred as independent primaries in a female with a truncating *DICER1* germline variant, who was also diagnosed with an NCMH, embryonal rhabdomyosarcoma of the vagina and papillary thyroid cancer. Although this is an unusually large number of independent primary cancers in a single *DICER1* carrier, up to six primaries have been reported in the literature.[7] Her family history was unremarkable for an excess of cancers. Exome germline sequencing did not reveal other pathogenic or likely pathogenic alleles in other genes (eg, *TP53*).

Abbreviations: MPNST, malignant peripheral nerve sheath tumors; NCMH, nasal chondromesenchymal hamartoma; SIR, standardized incidence ratios.

*For privacy age rounded to nearest 5-year interval for tumor types affecting one person only.

TABLE A6. SIR of 45 Cancers Observed in Cohort of 148 *DICER1* Carriers (probands and non-probands; 3,951 person-years of risk) From the NCI *DICER1* Study Only

Tumor Site	Observed No. of Cases	Expected No. of Cases	SIR (observed/expected)	95% Confidence Interval (lower)	95% Confidence Interval (upper)	Mean Age at Event (years)*
All sites (excluding non-melanoma skin cancers)	45	5.50	**8.1**	6.0	11	17
Gynandroblastoma	2	0	**1.7×10^5**	2.1×10^4	6.1×10^5	16
Breast cancer	1	0.91	1.1	0.03	6.1	30
Melanoma	2	0.33	6.07	0.74	22	44
Pleuropulmonary blastoma (non-type I)	17	0	**3.4×10^6**	2.0×10^6	5.4×10^6	3
Prostate cancer	3	0.69	4.4	0.9	13	58
Rhabdomyosarcoma	2	0.01	**175**	21	631	8
Sertoli-Leydig cell tumor	10	0	**2.2×10^4**	1.0×10^4	3.9×10^4	22
Spindle cell carcinoma	1	0	**840**	21	4.7×10^3	10
Teratoma	1	0	**488**	12	2.8×10^3	0
Thyroid cancer	6	0.23	**25**	9.3	55	24

NOTE. Bolded observed/expected SIR ratio denotes $P < .05$. Type I pleuropulmonary blastoma rates are not tracked by SEER.

Abbreviations: NCI, National Cancer Institute; SIR, standardized incidence ratios.

*For privacy, age is rounded to nearest 5-year interval for tumor types affecting one person only.

TABLE A7. Non-Proband *DICER1*-Carrier Cumulative Age-Specific Neoplasm Risk (cancers plus *DICER1*-associated neoplasms CN and NCMH), Per Decade

Variable	Cumulative Neoplasm Risk (95% CI)					
Age (years)	10	20	30	40	50	60
Females	4.0 (0 to 9.2)	10.9 (1.4 to 19.5)	13.6 (2.7 to 23.2)	16.3 (4.3 to 26.7)	26.5 (8.0 to 41.2)	33.8 (10.2 to 51.2)
Males	6.6 (0 to 13.6)	6.6 (0 to 13.6)	10.2 (0 to 19.5)	10.2 (0 to 19.5)	10.2 (0 to 19.5)	27.4 (0.4 to 47.1)
Overall	5.3 (0.6 to 9.7)	9.4 (2.9 to 15.5)	12.4 (4.8 to 19.4)	14.0 (5.8 to 21.4)	19.3 (8.4 to 29.0)	31.5 (14.2 to 45.3)

NOTE. See also Figures 1A and 1B.

TABLE A8. Table of *DICER1*-Carriers With More Than One Reported Neoplasm Diagnosis

Proband	Neoplasm Diagnosis No. 1	Neoplasm Diagnosis No. 2	Neoplasm Diagnosis No. 3	Neoplasm Diagnosis No. 4	Neoplasm Diagnosis No. 5
No	SLCT	Thyroid cancer	Cystic nephroma	–	–
No	Prostate cancer	Prostate cancer	–	–	
Yes	Teratoma	Rhabdomyosarcoma	Spindle cell carcinoma	Thyroid cancer	NCMH
Yes	PPB type III	Thyroid cancer	NCMH	SLCT	–
Yes	Rhabdomyosarcoma	SLCT	Rhabdomyosarcoma	–	–
Yes	Rhabdomyosarcoma	Cystic nephroma	SLCT	–	–
Yes	SLCT	Thyroid cancer	SLCT	–	–
Yes	PPB type I	Cystic nephroma	–	–	–
Yes	PPB type III	Cystic nephroma	–	–	–
Yes	PPB type II	Melanoma	–	–	–
Yes	PPB type II	MPNST	–	–	–
Yes	PPB type III	MPNST	–	–	–
Yes	PPB type II	NCMH	–	–	–
Yes	PPB type III	NCMH	–	–	–
Yes	PPB type III	NCMH	–	–	–
Yes	SLCT	NCMH	–	–	–
Yes	PPB type II	Non-melanoma skin	–	–	–
Yes	Cystic nephroma	PPB type I	–	–	–
Yes	Neuroblastoma	PPB type I	–	–	–
Yes	PPB type III	Rhabdomyosarcoma	–	–	–
Yes	SLCT	SLCT	–	–	–
Yes	SLCT	SLCT	–	–	–
Yes	Undifferentiated ovarian sarcoma	SLCT	–	–	–
Yes	PPB type II	Thymoma	–	–	–
Yes	PPB type II	Thyroid cancer	–	–	–
Yes	SLCT	Thyroid cancer	–	–	–

NOTE. Two non-probands carriers with multiple neoplasm are listed at the top of the table. There were 27 second neoplasms in the full cohort, four of which were fully concurrent with the first neoplasm, with an additional two that were nearly concurrent (0.005 and 0.05 years apart). There was a median gap of 4.1 years between the first and second neoplasm, with an interquartile range of 6.7 years. There were six third neoplasms, one of which was concurrent with the first and second neoplasm in that individual. Data are also presented in Appendix Figure A1.

Abbreviations: MPNST, malignant peripheral nerve sheath tumor; NCMH, nasal chondromesenchymal hamartoma; PPB, pleuropulmonary blastoma; SLCT, Sertoli-Leydig cell tumor.