

Inherited Mutations in Cancer Susceptibility Genes Are Common Among Survivors of Breast Cancer Who Develop Therapy-Related Leukemia

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BACKGROUND: Risk factors for the development of therapy-related leukemia (TRL), an often lethal late complication of cytotoxic therapy, remain poorly understood and may differ for survivors of different malignancies. Survivors of breast cancer (BC) now account for the majority of TRL cases, making the study of TRL risk factors in this population a priority. **METHODS:** Subjects with TRL after cytotoxic therapy for a primary BC were identified from the TRL registry at The University of Chicago. Those with an available germline DNA sample were screened with a comprehensive gene panel covering known inherited BC susceptibility genes. Clinical and TRL characteristics of all subjects and those with identified germline mutations were described. **RESULTS:** Nineteen of 88 survivors of BC with TRL (22%) had an additional primary cancer and 40 of the 70 survivors with an available family history (57%) had a close relative with breast, ovarian, or pancreatic cancer. Of the 47 subjects with available DNA, 10 (21%) were found to carry a deleterious inherited mutation in *BRCA1* (3 subjects; 6%), *BRCA2* (2 subjects; 4%), *TP53* (tumor protein p53) (3 subjects; 6%), *CHEK2* (checkpoint kinase 2) (1 subject; 2%), and *PALB2* (partner and localizer of *BRCA2*) (1 subject; 2%). **CONCLUSIONS:** Survivors of BC with TRL have personal and family histories suggestive of inherited cancer susceptibility and frequently carry germline mutations in BC susceptibility genes. The data from the current study support the role of these genes in TRL risk and suggest that long-term follow-up studies of women with germline mutations who are treated for BC and functional studies of the effects of heterozygous mutations in these genes on bone marrow function after cytotoxic exposures are warranted. *Cancer* 2016;122:304-11. © 2015 American Cancer Society.

KEYWORDS: breast cancer, inherited, leukemia, therapy-related.

INTRODUCTION

Therapy-related leukemias (TRLs), including therapy-related myeloid neoplasms (t-MNs) and therapy-related acute lymphoblastic leukemia (t-ALL), are an often lethal, late complication of prior cytotoxic therapy for survivors of a first cancer.¹⁻⁴ With increases in cancer survivorship,⁵ the number of cases of TRL is expected to rise. Thus, efforts to understand and prevent this complication are essential.

TRLs currently are believed to be the direct consequence of mutational events induced by prior cytotoxic exposures, but to our knowledge, the exact mechanisms and risk factors remain unclear. Associations between specific exposures and the phenotype of the TRL that develops support a key role for the exposures in the genesis of TRL. For example, exposure to topoisomerase II inhibitors is associated with TRL characterized by clonal cytogenetic abnormalities involving *KMT2A/MLL* on chromosome band 11q23 with a short latency of 2 to 3 years after exposure. In contrast, exposure to alkylating agents or radiation is associated with TRL with abnormalities of chromosomes 5 and/or 7, which more often occur with a latency of 5 to 7 years.⁶ Furthermore, the incidence of TRL is reported to be increased in breast cancer (BC)

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adjuvant trials using higher chemotherapy dose intensity, concomitant use of radiation, and/or the use of hematopoietic growth factors.^{7,8}

However, the observation of acute myeloid leukemia (AML) and ALL cases occurring in patients after they have undergone surgery only for a primary malignancy^{1-3,9} raises the possibility that some TRLs may be independent second primary cancers unrelated to prior cytotoxic exposures. Individuals with inherited cancer syndromes such as Li-Fraumeni syndrome or dyskeratosis congenita, which predispose affected individuals to both leukemias and solid tumors, could explain some of these cases and present clinically in a manner similar to TRLs. Another possibility is that individuals who carry an inherited mutation in a cancer susceptibility gene could be at a higher risk of developing TRL after DNA-damaging exposures compared with other patients.

Because survivors of BC now account for the largest number of TRL cases,^{2,10} and the genes responsible for inherited susceptibility to BC are well characterized, patients who develop TRL after BC represent an ideal population in which to examine the role of inherited cancer susceptibility in the etiology of TRL. However, to the best of our knowledge, a comprehensive assessment of all currently known moderate-penetrance to high-penetrance BC susceptibility genes in patients with TRL after BC has not been performed to date. Herein we present the clinical and TRL characteristics of 88 well-annotated survivors of BC with TRL and the results of a comprehensive screen for inherited mutations in known BC susceptibility genes.

MATERIALS AND METHODS

Study Population

Cases were drawn from the TRL registry at The University of Chicago, which contains data regarding all consented patients with a history of cytotoxic exposures for a prior malignant or nonmalignant condition who subsequently developed myelodysplastic syndrome or an acute leukemia and were evaluated at The University of Chicago between 1972 and 2012. Additional clinical data were abstracted by individual chart review. Family histories consisted of physician documentation at the time of initial consultation. Formal pedigrees were available for 8 subjects who had a prior cancer risk evaluation. The current study was approved by The University of Chicago Institutional Review Board in accordance with the Declaration of Helsinki.

Definitions

Latency was defined as the time from the first cytotoxic exposure to the first bone marrow examination diagnostic of a TRL. The mechanism of action of chemotherapeutic agents was categorized as previously defined.⁴ Cytogenetic abnormalities were detailed according to the International System for Human Cytogenetic Nomenclature.¹¹

Tissue Sources

Constitutional DNA sources included Epstein-Barr virus-transformed lymphoblastoid cell lines generated at the time of complete remission; buccal swabs, peripheral blood, or bone marrow obtained at the time of complete remission; and cultured skin fibroblasts. A leukemia sample was used if it was the only sample available with sufficient DNA.

BC Susceptibility Gene Sequencing

BROCA targeted genomic capture (an openly available, targeted capture and genomic sequencing approach) and next-generation sequencing (NGS) were performed as previously described (see Supporting Information Table 1).¹² Single-nucleotide variants, small insertions and deletions (indels), and large genomic rearrangements were identified as previously described.^{12,13} Deleterious mutations (defined as nonsense and frameshift mutations, large genomic rearrangements, and missense mutations with experimental evidence supporting their deleterious nature) were validated by independent polymerase chain reaction (PCR) amplification and Sanger sequencing or by real-time PCR using TaqMan probes (Life Technologies, Carlsbad, Calif). Variants were only considered germline if they were confirmed in a constitutional DNA source.

Acquired Mutation Sequencing

OncoPlex targeted genomic capture and NGS was performed as previously described (see Supporting Information Table 2).¹⁴ All variants with data supporting a role in leukemia were validated by independent PCR amplification and Sanger sequencing. Constitutional DNAs were used to confirm the somatic nature of identified variants when available.

Statistical Analysis

Kaplan-Meier curves were used to calculate overall survival (OS). Stata statistical software was used for all analyses (version 12.1; StataCorp; College Station, Tex).

RESULTS

Clinical Characteristics of Survivors of BC Who Developed TRL

In total, 88 female survivors of BC were identified (Table 1). The median age at the time of diagnosis of primary BC was 52 years (range, 23-83 years). Nineteen subjects (22%) had an additional primary cancer diagnosis. A family cancer history was available for 70 subjects (80%), of whom 40 (57%) reported at least 1 first-degree or second-degree relative with breast, ovarian, or pancreatic cancer. Of those subjects for whom prior cytotoxic exposure data were available (86 patients; 98%), chemotherapy was a component of the exposures for 67 subjects (78%). All but 1 patient received a multiagent regimen. Regimens incorporating both doxorubicin and cyclophosphamide were the most common (37 subjects; 56%). Radiation exposure was reported for 68 subjects (79%). Four subjects (5%) had undergone a prior autologous stem cell transplantation and 11 (13%) had received myeloid growth factors.

TRL Characteristics in Survivors of BC

The majority of survivors of BC developed t-MN (81 patients; 92%), but 7 cases of t-ALL (8%) were also observed (Table 1). The median latency from the time of first cytotoxic exposure to TRL diagnosis among the 86 patients for whom latency was available was 58 months (interquartile range, 28-105 months). Clonal cytogenetic abnormalities were observed in 77 of the 84 subjects with an available karyotype (92%). Among these, abnormalities of chromosomes 5 and/or 7 and recurring balanced translocations were both common, occurring in 51% (43 patients) and 35% (29 patients) of subjects, respectively. Rearrangements involving *KMT2A/MLL* on chromosome band 11q23 were the most common (11 of 84 subjects; 13%), followed by t(15;17) (6 of 84 subjects; 7%) and those involving 21q22 (5 of 84 subjects; 6%) (see Supporting Information Table 3). Greater than 25% of the observed recurring balanced translocations were t(9;11)(p22;q23) (8 of 29 subjects; 28%). OS after a diagnosis of TRL was poor (median, 13 months; interquartile range, 5-22 months).

Inherited Mutation Detection and Distribution

BROCA targeted capture and NGS of the 47 subjects for whom DNAs were available resulted in >500-fold median coverage with 97% and 99.5% of bases covered at least 50-fold and 10-fold, respectively. The clinical characteristics of sequenced subjects did not differ from the 41 subjects without available DNA

TABLE 1. Clinical Characteristics, Prior Cytotoxic Exposures, and TRL Characteristics of 88 Survivors of BC

Characteristic	No. (%)
Age at diagnosis of BC, y	
≤35	9 (10)
36–45	14 (16)
46–55	29 (33)
≥56	34 (39)
Unknown	2 (2)
Race/ethnicity	
White (non-AJ)	65 (74)
White (AJ)	3 (3)
African American	5 (6)
Other/unknown	15 (17)
Additional cancer diagnoses (n = 19) ^a	
Second primary BC	7 (8)
Ovarian cancer	3 (3)
Other	12 (14)
Family history of cancer in a first-degree or second-degree relative (n = 70)	
BC	33 (47)
Breast, ovarian, or pancreatic cancer	40 (57)
Prior therapy	
Chemotherapy plus RT	49 (56) ^b
Chemotherapy only	18 (20) ^b
RT only	19 (22)
Unknown	2 (2)
Chemotherapy class exposures	
Topoisomerase II inhibitor	40 (45)
Alkylating agent	58 (66)
Unknown	8 (9)
Type of TRL	
t-MN	81 (92)
t-ALL	7 (8)
Median latency (IQR), mo ^c	58 (28–105)
Cytogenetics ^d	
Normal karyotype	7 (8)
Abnormal karyotype	77 (88)
Abnormalities of chromosome 5 and/or 7	43 (49)
Recurring balanced translocations	29 (33)
Other clonal abnormality	7 (8)
Unknown	4 (5)
Median OS (IQR), mo ^e	
From BC diagnosis ^e	102 (60–173)
From TRL diagnosis	13 (5–22)

Abbreviations: AJ, Ashkenazi Jewish; BC, breast cancer; IQR, interquartile range; OS, overall survival; RT, radiotherapy; t-ALL, therapy-related acute lymphoblastic leukemia; t-MN, therapy-related myeloid neoplasm; TRL, therapy-related leukemia.

^aIncluded 3 subjects with multiple primary tumors; other cancers included uterine (2 subjects), melanoma (2 subjects), lung (2 subjects), non-Hodgkin lymphoma (2 subjects), osteosarcoma (1 subject), bladder (1 subject), cervical (1 subject), and multiple myeloma (1 subject).

^bSpecific agents were unknown for 4 subjects in the chemotherapy plus RT group and 2 subjects in the chemotherapy-only group.

^cLatency was unknown for 3 subjects.

^dTwo subjects had abnormalities of both chromosomes 5 and/or 7 and a recurring balanced translocation (t(15;17) and t(9;22)).

^eOS was unknown for 3 subjects.

samples (see Supporting Information Table 4). Overall, 10 survivors of BC (21%) who developed TRL carried a deleterious inherited mutation, distributed among *BRCA1* (3 subjects; 6%), *TP53* (tumor protein p53) (3

TABLE 2. Clinical and Cytogenetic Characteristics by Germline Mutation Status Among 47 Sequenced Subjects^a

	No Mutation (n = 37) ^b	<i>BRCA1</i> or <i>BRCA2</i> (n = 5)	<i>TP53</i> (n = 3)	<i>PALB2</i> (n = 1)	<i>CHEK2</i> (n = 1)
Age at primary diagnosis (range), y	53 (31–79)	50 (33–53)	23 (23–24)	51	42
Median latency (range), mo	53 (11–792)	133 (30–408)	48 (30–81)	90	21
Therapy-related leukemia type					
t-MN	35 (95)	5 (100)	1 (33)	1 (100)	1 (100)
t-ALL	2 (5)	0	2 (67)	0	0
Cytogenetics, no. (%)					
Normal karyotype	4 (11)	2 (40)	0	0	0
Clonal abnormality	30 (81)	3 (60)	3 (100)	1 (100)	1 (100) ^c
Balanced translocations ^d	14 (38)	1 (20)	1 (33)	0	1 (100) ^c
Chromosome 5 and/or 7 abnormalities ^{d,e}	15 (41)	2 (40)	1 (33)	1 (100)	0
Complex ^e	11 (30)	1 (20)	3 (100)	1 (100)	0
Unknown	3 (8)	0	0	0	0
Median survival from TRL diagnosis (IQR), mo	13 (7–27)	14	29	14	52

Abbreviations: *CHEK2* indicates checkpoint kinase 2; IQR, interquartile range; *PALB2*, partner and localizer of *BRCA2*; t-ALL, therapy-related acute lymphoblastic leukemia; t-MN, therapy-related myeloid neoplasm; *TP53*, tumor protein p53; TRL, therapy-related leukemia.

^aTissue sources used for sequencing included lymphoblastoid cell lines (24 subjects), buccal swabs (8 subjects), peripheral blood or bone marrow in remission (6 subjects), skin fibroblasts (1 subject), and peripheral blood or bone marrow samples with leukemia (8 subjects).

^bOne subject's age at diagnosis and latency were unknown.

^cThis subject had fluorescence in situ hybridization studies only.

^dTwo subjects had both a balanced translocation (t(15;17) and t(9;22)) and an abnormality of chromosome 5 and/or 7.

^eComplex karyotype as defined in Dohner et al.³³ Eleven of 15 subjects with chromosome 5 and/or 7 abnormalities with no inherited mutation had a complex karyotype as well as 1 of 2 subjects with *BRCA1/BRCA2*, 1 subject with a *TP53*, and the 1 subject with a *PALB2* mutation.

subjects; 6%), *BRCA2* (2 subjects; 4%), *CHEK2* (checkpoint kinase 2) (1 subject; 2%), and *PALB2* (partner and localizer of *BRCA2*) (1 subject; 2%) (Fig. 1). By TRL subtype, 8 of 43 subjects (19%) with t-MN had an inherited mutation, which were distributed among all 5 of these genes. Of the cases of t-ALL, 2 of 4 subjects (50%) had a mutation, both in *TP53*.

Observed patterns among those subjects with specific inherited mutations included (Table 2)¹⁵: 1) those with germline *TP53* mutations were the only subjects with an inherited mutation to develop t-ALL (2 of 3 subjects [67%] vs 0 of 7 subjects [0%] with inherited mutations in other genes), and all 3 developed TRL with complex karyotypes; and 2) those with a *BRCA1* or *BRCA2* mutation had an especially long latency to the development of TRL (median of 133 months vs 53 months in those without an inherited mutation), and the majority developed TRL featuring a normal karyotype (2 of 5 subjects; 40%) or a single karyotypic abnormality (2 of 5 subjects; 40%). Six of the 10 subjects (60%) with an inherited mutation had a family history of cancer and 2 (20%) did not; for the remaining 2 subjects (20%), the family history was unknown (Table 3).

Additional Informative Cases

We identified 3 additional subjects who did not fit our original study population who had previously identified

germline *BRCA1* mutations. We included them in the current study for descriptive purposes (see Supporting Information Table 5): 1) 1 subject who developed chronic myeloid leukemia (CML) after BC who was treated with surgery only; 2) 1 subject who developed CML 33 months before a diagnosis of BC; and 3) 1 survivor of ovarian cancer who developed a t-MN with a t(9;11) after cytotoxic chemotherapy.

Somatic Mutations in TRL After BC

To identify somatic mutations that contribute to TRL after BC, we sequenced leukemia samples available from 9 subjects using OncoPlex. Somatic mutations were identified in 8 of the 9 subjects (Table 4). These mutations were distributed among 17 genes (see Supporting Information Table 6). The median number of somatic mutations per sample was 2 (range, 0-9 somatic mutations). *FLT3* and *TET2* were the genes most commonly mutated, with each mutated in 3 of 9 subjects (33%). Mutations in *ASXL1*, *NRAS*, and *WT1* were observed in 2 of 9 subjects (22%). Combinations observed in *de novo* AML, including a *KIT* exon 17 mutation in a t(8;21) t-MN and a *FLT3* mutation in a t(15;17) t-MN, were identified. The leukemia sample from subject UPIN12, who developed a t-MN with a complex karyotype in the setting of a germline *BRCA1* mutation, had somatic mutations in *TET2*, *NRAS*, and *TP53*.

TABLE 3. Detailed Clinical Characteristics of the 10 Survivors of BC Who Carried Inherited BC Susceptibility Gene Mutations and Developed TRL

Patient ID	Gene	Mutation	Age at BC Diagnosis, Years	Chemotherapy/ RT	Other Tumors	Family Cancer History	TRL Type	TRL Latency, Months	TRL OS, Months	Sample Used for Sequencing	Karyotype at TRL Diagnosis
UPIN12	BRCA1	Exon 13 duplication	33	Yes/yes	Ovary, second breast	Breast, prostate	t-MN	408	3	BM leukemia; confirmed in buccal DNA	45,XX,add(4)(q23),del(5)(q13.3q33),del(6)(p21.3p23),-7,+8,-16[20]
UPIN19	BRCA1	Exon 13-15 deletion	53	Yes/yes	Second breast, NHL	Breast, ovary, colon, melanoma, uterine	t-MN	133	59 ^a	Skin fibroblasts	46,XX[30]
UPIN81	BRCA1	187delAG	50	Yes/yes	NHL	Thyroid, lymphoma, head and neck	t-MN	30	6 ^a	PB in CR	46,XX[30]
UPIN49	BRCA2	5301insA	48	Yes/no		Unknown	t-MN	216	14	LBL	46,XX,t(3;21)(q26.2;q22.3)[30]/46,XX[1]
UPIN52	BRCA2	8138del5	51	Yes/yes		Unknown	t-MN	63	7	LBL	45,XX,-7[27]/46,XX[3]
UPIN04	TP53	E339X	24	Yes/no		Leukemia, brain, lung	t-ALL	30	66	LBL; confirmed in BM in CR	96,XXXX,+X,+1,+2,-4,-5,+6,-9,+10,+12,+14,-15,-16,-17,+18,+21,+22[7]/97,idem,+mar[10]/46,XX[1]/Two related non-clonal abnormal cells
UPIN60	TP53	G245S	23	Yes/yes	Sarcoma in RT field	Sarcoma	t-MN	81	29	LBL	46,XX,der(15)del(15)(q23q24)t(15;17)(q24.1;q21.1),der(17)t(15;17)(q24.1;q21.1)[24]/48,XX,idem,+8,+8[12]/46,XX[4]
UPIN09	TP53	I232T	23	Yes/yes		None	t-ALL	48	28	BM in CR	54,XX,+X,+4,+6,+14,+17,+18,+21,+21[3]/46,XX[23]
UPIN53	PALB2	Y1183X	51	No/yes		Breast	t-MN	90	14	LBL	45,XX,-2,der(3)t(2;3)(p11.2;p11.1),der(5)del(5)(q15q33.3)t(5;12)(q33.3;q22),del(7)(q11.2q36),+8,add(9)(q34),der(12)t(5;12)(q33.3;q22),der(12)t(3;12)(p11.2;p13),-18[15]/46,idem,+mar[3]/46,XX[2]
UPIN70	CHEK2	1100delC	42	Yes/yes		None	t-MN	21	52	Buccal	FISH only: +9,inv(16),+21

Abbreviations: BC, breast cancer; BM, bone marrow; CHEK2 indicates checkpoint kinase 2; CR, complete remission; FISH, fluorescence in situ hybridization; LBL, lymphoblastoid cell line; NHL, non-Hodgkin lymphoma; OS, overall survival; PALB2, partner and localizer of BRCA2; PB, peripheral blood; RT, radiotherapy; t-ALL, therapy-related acute lymphoblastic leukemia; t-MN, therapy-related myeloid neoplasm; TP53, tumor protein p53; TRL, therapy-related leukemia.
^aStill in follow-up.

TABLE 4. Somatic Mutations in 9 TRL Cases After BC

		Complex With Abnormalities of Chromosome 5 and/or 7		t(6;9)	t(9;11)		t(15;17)		t(8;21)	
		UPIN17	UPIN12 ^a	UPIN13	UPIN16	UPIN37	UPIN18	UPIN35	UPIN21	UPIN14
<i>FLT3</i>	33%			■	■			■		
<i>TET2</i>	33%	■	■	■						
<i>ASXL1</i>	22%	■								■
<i>NRAS</i>	22%		■		■					
<i>WT1</i>	22%			■					■	
<i>RUNX1</i>	11%	▤▤▤▤▤▤▤▤▤▤								
<i>AKT1</i>	11%	■								
<i>CDH1</i>	11%	■								
<i>KRAS</i>	11%	■								
<i>NF1</i>	11%	■								
<i>NOTCH2</i>	11%	■								
<i>PTPN11</i>	11%	■								
<i>TP53</i>	11%		■							
<i>ZRSR2</i>	11%			■						
<i>DNMT3A</i>	11%					■				
<i>RB1</i>	11%									
<i>KIT</i>	11%									■

Abbreviations: BC, breast cancer; black, frameshift, small insertions/deletions, or nonsense mutations; gray, missense mutation; striped, splice site mutation; TRL, therapy-related leukemia.

^a Patient UPIN12 carried an inherited *BRCA1* mutation.

DISCUSSION

Through a comprehensive screen of inherited BC susceptibility genes, we found that 1 in 5 of the survivors of BC with TRL in the current series carried a deleterious inherited mutation. These mutations were distributed among 5 genes, all with key roles in DNA repair and/or DNA damage-sensing pathways. In addition, many of the well-annotated survivors of BC with TRL in the current series had a personal history of additional malignancies and/or a family history of cancer in close relatives, suggesting a cancer-prone population. The data from the current study support a role for inherited cancer susceptibility in TRL after BC.

TRLs have typically been considered a direct and stochastic consequence of cytotoxic therapies. However, investigations have provided evidence in support of the role of underlying cancer susceptibility, particularly among survivors of BC. Using Surveillance, Epidemiology, and End Results data, Martin et al demonstrated that young women with BC had the highest risk of developing t-MN (relative risk of 4.14) and that the age-dependent risk of TRL among these young women mirrored the risk of developing a second BC or an ovarian cancer, suggesting a shared underlying genetic risk factor.¹⁶ Two other small series also added support. In the first, sequencing of *BRCA1*, *BRCA2*, *TP53*, and *CHEK2* 1100delC identified deleterious germline mutations in 3 of 14 unselected

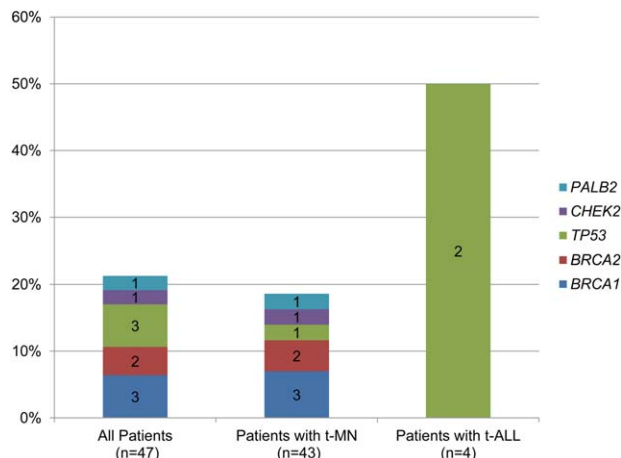


Figure 1. Inherited mutations in breast cancer susceptibility genes among 47 subjects with therapy-related leukemia. CHEK2 indicates checkpoint kinase 2; PALB2, partner and localizer of BRCA2; t-ALL, therapy-related acute lymphoblastic leukemia; t-MN, therapy-related myeloid neoplasms; TP53, tumor protein p53.

patients with BC with TRL (21%).¹⁷ In the second series, sequencing of *BRCA1* and *BRCA2* in 13 women with TRL after early-onset BC identified germline *BRCA2* mutations among 2 women (15%).¹⁸ The current study data add to the spectrum of genes involved and confirm the high yield of genetic testing in this population. The findings of the current study support a recommendation

for genetic testing for all women who develop TRL after BC to allow primary prevention in at-risk close relatives and those who survive their TRL.

All of the BC susceptibility genes with mutations identified in the current series function to sense or repair DNA damage and the majority are closely tied to leukemia risk. *PALB2* and *BRCA2*, key components of the Fanconi anemia (FA) DNA repair pathway, cause FA, which is an inherited bone marrow failure syndrome featuring an 800-fold increased risk of myelodysplastic syndrome/AML, when mutations in both alleles are inherited.^{19,20} Reduced expression of *BRCA1*, a gene also involved in the FA pathway, has been demonstrated in t-MN cases,²¹ and an increased risk of leukemia has been reported in an epidemiologic study in relatives of *BRCA1* mutation carriers.²² Inherited mutations in *TP53* cause Li-Fraumeni syndrome, in which 3% to 5% of the tumors that develop are leukemias.^{23,24} *TP53* is also somatically mutated in 2% of de novo AML cases²⁵ and 11% to 38% of t-MN cases.^{26,27} To the best of our knowledge, data for *CHEK2* involvement in leukemia are limited, but leukemias have been reported in kindreds with inherited *CHEK2* mutations.²⁸

Observations from the current study provide additional evidence that some cases of TRL are more likely independent secondary primary cancers, whereas others are more clearly linked to the cytotoxic exposures. For example, patient UPIN49 was found to carry an inherited *BRCA2* mutation and developed a t-MN with a t(3;21) 18 years after treatment of BC. This timeframe is well beyond the expected 2 to 3 years for t-MN with translocations involving 21q22,⁶ suggesting a possible independent event. Our previous report of 2 cases of acute promyelocytic leukemia in women with BC with *BRCA2* mutations who were treated with surgery only²⁹ and the 2 cases of CML occurring either before BC or after BC that were treated with surgery alone in *BRCA1* mutation carriers reported herein also support this idea and suggest that inherited heterozygous mutations in *BRCA1* and *BRCA2* may contribute to leukemia risk.

In contrast, TRL with t(9;11), a chromosomal translocation which was observed in 10% of the patients in the current study and in 3 other series of patients with BC, suggests that survivors of BC are uniquely predisposed to TRL with this specific cytogenetic abnormality. Chandra et al reported that 62% of the t-MN cases with t(9;11) at their institution occurred within the setting of a prior BC.³⁰ A t(9;11) was identified in 3 of 36 t-MN cases (8%) in a recent series of survivors of BC⁹ and was overrepresented among t-MN cases (20 of 182 cases; 11%) versus de novo AML (35 of 2381 cases; 1%) in a study in

which survivors of BC accounted for 37% of t-MN cases.² Further study of the nonhomologous end-joining repair mechanism implicated in the t(9;11) translocation in survivors of BC with TRL is warranted.

Finally, we observed 7 cases of t-ALL among the 88 survivors of BC with TRL in the current study (8%). We identified deleterious mutations, both occurring in *TP53*, among 2 of 4 cases (50%) studied. Both of these mutation carriers developed BC before age 30 years, a clinical phenotype that in and of itself should prompt genetic testing. However, inherited mutations in *BRCA1* and *BRCA2* would be expected to account for the majority of mutations identified in patients with early-onset BC, with *TP53* mutations expected in approximately 4% of those with a diagnosis of BC at age 30 years or younger.³¹ The data from the current study suggest that when BC is followed by t-ALL, the likelihood of a *TP53* mutation is higher.

The current study has limitations. First, this was a small series, which limited our ability to assess for differences among different groups of mutation carriers. Second, to the best of our knowledge, it is unknown how the percentage of mutation carriers identified in the current study population compares with a similar population of patients with BC who did not develop TRL. It took several decades to obtain the number of cases presented in the current study, making it difficult to ascertain a control group of similarly treated patients with BC with a similar length of follow-up who did not develop TRL with which to compare our group. In addition, studies of *BRCA1* and *BRCA2* among unselected patients with BC suggest that approximately 5% carry a deleterious mutation,^{32,33} but to our knowledge, comprehensive panel-based genetic testing as used herein has not yet been applied to a large, population-based group of patients with BC. Thus, the true frequency of mutations in all of the genes studied herein among a general population of patients with BC is unknown and deserves further study.

The results of the current study demonstrated that 1 in 5 survivors of BC who develop TRL carry an inherited mutation in a BC susceptibility gene. The mutations involve 5 genes, which all function to maintain DNA integrity, thereby suggesting a role for these pathways in leukemia risk in the setting of cytotoxic exposures or, for some, regardless of exposures. The data from the current study suggest that a long-term prospective trial following similarly treated women with BC for whom germline mutation status is known for the development of TRL as well as functional testing of the role of these genes in bone marrow dysfunction after cytotoxic exposures are warranted.

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CONFLICT OF INTEREST DISCLOSURES

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