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ARTICLE

Therapy-related myeloid neoplasms with different latencies: a detailed clinicopathologic analysis

Yen-Chun Liu[1,2]✉, Gwendolyn M. Illar[1], Raniah Al Amri[1], Briana C. Canady[1], Bryan Rea[1], Svetlana A. Yatsenko[1] and Julia T. Geyer[3]

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Therapy-related myeloid neoplasm (t-MN) arising in patients with prior cytotoxic treatments is considered a distinct entity due to its unfavorable prognosis. Latencies between the initial cytotoxic therapy and the occurrence of t-MNs vary but usually fall between 1 and 10 years. t-MNs with unusually short or long latencies are not well characterized. It is unclear if they are biologically similar to the ones with ordinary latencies and should be kept in the t-MN entity. We compiled a cohort of t-MN cases including short (<1 year), ordinary (1–10 years), and extended (>10 years) latencies from two tertiary medical centers. Both the t-MNs with ordinary and extended latencies showed high likelihood of high-risk genetic abnormalities and demonstrated no significant survival differences. But the t-MNs with extended latencies were more likely associated with history of multiple cancers ($p = 0.007$) and were younger at the time of cytotoxic treatments ($p < 0.001$) when compared to the t-MNs with ordinary latencies. The t-MN with short latencies appears to be a very rare and highly heterogeneous group. In summary, the genetic composition appears similar in the t-MNs with ordinary and extended latencies. However, the association between the t-MN with extended latencies and history of multiple cancers raises a possibility that cancer predisposition may contribute to the accumulation of genetic abnormalities in these patients. Investigation into potential germline mutations in the t-MN patients with extended latencies may provide important information for related family members.

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INTRODUCTION

Based on the current WHO classification, therapy-related myeloid neoplasms (t-MN) encompass myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), and myelodysplastic/myeloproliferative neoplasm (MDS/MPN) arising in patients with prior histories of cytoreductive therapy (either chemotherapy or radiation therapy) for prior neoplastic or non-neoplastic disorders[1]. t-MNs demonstrate relatively poor prognosis when compared to *de novo* lesions and are thus deemed a distinct entity[2–6].

Prior studies suggest two main subsets of t-MN with corresponding prior treatments[3–5,7,8]. The more common subset associated with alkylating agents and/or radiation therapy tends to occur 5–10 years after exposure and demonstrates chromosome abnormalities involving chromosome 5 and/or 7, complex karyotypes, and *TP53* aberrations. The second subset associated with topoisomerase II inhibitors tends to occur with a shorter latency (1–5 years) and demonstrates translocations involving *KMT2A* at 11q23.3 and *RUNX1* at 21q22.1. In reality, it may be difficult to classify patients receiving treatments comprising a mixture of agents and/or radiation into these two specific subsets. Utilizing advanced molecular tools, many studies have shown that t-MN tends to demonstrate adverse genetic abnormalities similar to those seen in other high-risk myeloid neoplasms[9–15].

The incidence of t-MN varies in patients of different types of prior cancers. A population-based study has suggested pronounced relative risk of developing therapy-related acute myeloid leukemia (t-AML) for patients with malignancies of soft tissue, ovary, lung, Hodgkin lymphoma (HL) and myeloma[16]. However, probably due to the disease prevalence, roughly half of the t-AML patients showed histories of breast cancer and non-Hodgkin lymphoma (NHL). Interestingly, the study also identified variation in risks developing t-AML within the same primary cancer type during the decades of time covered by this population study, which was felt to reflect the changing treatments. The finding also highlights the association between the cytotoxic treatments and the development of t-MNs.

Although t-MN has long been thought to be a complication of the DNA damage caused by the cytoreductive therapy, the observation that not all the patients receiving cytoreductive treatments developed t-MN challenges the postulation that cytotoxic therapy is the only cause for t-MN. The current model of t-MN development highlights the interplay between the intrinsic and extrinsic factors, integrating components such as inherited cancer predisposition, exposure to cytotoxic therapy, clonal selection of cells with somatic mutations (clonal hematopoiesis of indeterminate potential), and damage to the bone marrow stroma niche[17].

By definition, any myeloid neoplasm (excluding MPN) occurring in a patient with prior cytotoxic therapy can be considered as t-MN, regardless of the latencies after the exposures to cytoreductive treatments. Although most of the t-MNs appear to develop 1–10 years after the exposure[3–5,7,8], some patients with t-MNs demonstrate either very short (<1 year) or extended (>10 years)

[1]Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. [2]Department of Pathology, St. Jude Children's Research Hospital, Memphis, TN, USA. [3]Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, NY, USA. ✉email: yen-chun.liu@stjude.org

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latencies. It is unclear if these t-MNs with unusual latencies are phenotypically and genetically similar to the remaining t-MNs. In this study, utilizing the t-MN cohorts from two tertiary medical centers, we sought to characterize the clinicopathologic features of t-MNs with unusual latencies and compare them with the t-MNs with ordinary latencies that fall between 1–10 years.

MATERIALS AND METHODS

Case selection

t-MN cases were retrieved from the pathology archives at the University of Pittsburgh Medical Center and Weill Cornell Medicine between 2011 and 2020. A review of the clinical records and pathology material was performed. This study was approved by the Institutional Review Boards at both institutions.

Cytogenetic studies

Conventional cytogenetic analysis was performed on G-banded metaphase cells prepared from BM aspirate unstimulated cultures according to the standard protocol[18]. Twenty metaphases were analyzed, and the results were reported using the ISCN nomenclature[19]. A complex karyotype was defined as ≥3 chromosomal abnormalities.

Mutation analysis

A polymerase chain reaction (PCR)-based Amplicon target enrichment sequencing assay as well as *CEBPA* mutation analysis by fragment length analysis were performed as described in a prior study[20]. The mutation hotspots of the following genes were interrogated: *ABL1, ASXL1, BCOR, BCORL1, BRAF, CALR, CBL, CDKN2A, CEBPA, CSF3R, DNMT3A, ETV6, EZH2, FBXW7, FLT3, GATA2, HRAS, IDH1, IDH2, JAK2, KIT, KRAS, MPL, MYD88, NPM1, NRAS, PHF6, PTEN, PTPN11, RUNX1, SETBP1, SF3B1, SRSF2, TET2, TP53, U2AF1, WT1*, and *ZRSR2*. A subset of cases underwent updated sequencing assays with expanded panels, including hotspot regions of additional genes such as *ATM, CBLB, KDM6A, KMT2A, NF1, PPM1D, RAD21*, and *STAG2*.

Statistical analysis

Continuous variables were analyzed using standard parametric statistical assays such as the *t*-test and non-parametric tests such as the Mann–Whitney test as appropriate. Categorical variables were analyzed using the Fisher exact test and the Chi-squared test. Logistic regression analysis was used as appropriate. Survival analysis was performed by the Kaplan–Meier method, and the log-rank test was used to compare survival curves. The time at diagnosis of t-MN was used as the start time point to calculate survival. Statistical analyses were performed using Stata version 15.1 (StataCorp) and GraphPad Prism version 9.0.2 (GraphPad Software).

RESULTS

One hundred and ninety-six cases of t-MN were identified between 2011 and 2020. Our t-MN cohort included one pediatric patient (12 years old) and four young adults (defined as <30 years old). The mean age of the patients at diagnoses was 67 years old (median: 68 years old). The male:female ratio was 1:1.08. Among them, 167/196 patients had histories of chemotherapy, whereas 95/196 patients underwent radiation therapy; 66/196 of the t-MN patients received both chemotherapy and radiation therapy. The morphologic findings classified 108/196 cases as t-MDS or t-MDS/MPN based on the 2017 WHO classification, whereas 88/196 cases were classified as t-AML. Seventy-three of the 108 t-MDS or t-MDS/MPN patients had follow-up bone marrow examinations after the diagnosis of t-MN; 36/73 t-MN patients developed progression into t-AML. Among the cases with documented prior cytoreductive treatments (194/196), the latencies between the patient's initial cytoreductive therapies and the diagnoses of t-MNs ranged from less than a year to 36 years (Fig. 1). The mean latency was 8.5 years, whereas the median was 6 years (the 5th–95th percentile: 1–26.35 years). In our cohort, a latency of 10 years was estimated to be at the 70th percentile of the distribution.

The t-MN cases with documented prior cytotoxic treatments were classified as t-MN-short (with a latency of less than a year), t-MN-ordinary (with a latency over 1–10 years), and t-MN-long (with an extended latency of more than 10 years) based on the common notion that most of the t-MNs occur within 1–10 years after the initial exposure to the cytotoxic treatments. Laboratory and demographic data from each group were summarized in Table 1. Statistical analyses were performed to compare t-MNs with ordinary and long latencies; t-MN-short group was not included in the analyses considering the low number of cases in the group ($n = 4$). There were no significant differences in the parameters such as male/female ratio, incidence of disease progression as well as history of chemotherapy and/or radiation exposure between the t-MNs with ordinary and extended latencies. The t-MN patients with extended latencies appeared to be older at diagnosis when compared to the patients with ordinary latencies ($p = 0.036$). Cases of t-MN with short latencies appeared to have higher platelet counts.

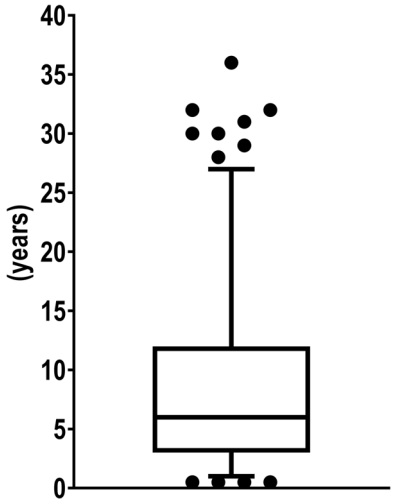

Fig. 1 Box–Whisker plot of latencies between the initial cytoreductive treatments and the diagnoses of t-MNs in the cohort. The box covers values from the 1st quartile to the 3rd quartile. The whiskers cover values outside the box extending to the 5th percentile and the 95th percentile on each end. A latency of 1 year was estimated to be at the 5th percentile, whereas a latency of 10 years was estimated to be at the 70th percentile.

In our cohort, complex karyotypes (51%, 98/191) as well as deletions of the long arm/whole chromosome of chromosome 5 and 7 (48%, 84/176) were identified in high percentage of t-MN cases. Other alterations such as chromosome 17p loss (20%, 37/189), translocations involving *KMT2A/MLL* at 11q23.3 (8%, 14/175), and translocations involving *RUNX1* at 21q22.1 (3%, 4/149) were also identified. Similar to that reported in other studies, recurrent chromosome abnormalities associated with favorable prognosis such as t(8;21)(q22;q22); *RUNX1-RUNX1T1*, inv(16)(p13.1q22); *CBFB-MYH11*, and t(15;17)(q24;q21); *PML-RARA* were only identified in low percentage of t-MN cases (1%, 2%, and 2%, respectively). Mutation analyses, using amplicon target enrichment of coding and non-coding regions of the genes recurrently mutated in myeloid neoplasms, demonstrated *TP53* mutation as the most common mutation in the cohort (48%, 72/149). *NPM1* mutation was seen in only 3% of the t-MN cases (4/149).

Data from the cytogenetic and mutation analysis in t-MNs with different latencies were shown in Table 2 and Fig. 2. In our study, the small number of t-MNs with short latencies appeared to be more likely to show normal karyotypes (3/4) when compared to t-MNs with ordinary latencies (14/133) and extended latencies (9/54). t-MN with ordinary and extended latencies did not demonstrate significant differences in the incidence of cytogenetic abnormalities examined by the chromosome analysis and

Table 1. Clinicopathologic features of therapy-related myeloid neoplasms with different latencies.

	t-MN-short (t-MN with latencies < 1 year) (*N* = 4)	t-MN-ordinary (t-MN with latencies of 1–10 years) (*N* = 136)	t-MN-long (t-MN with latencies > 10 years) (*N* = 56)	*p* values
t-AML: t-MDS (or t-MDS/MPN)	2:2	64:72	22:34	
t-MDS (or t-MDS/MPN) progression to t-AML	0/1	21/49	15/23	
Male:Female	2:2	67:69	25:31	
Age at diagnosis of t-MN (y/o)	70.8 (65–76)	65.0 (12–88)*	70.0 (27–92)*	*$p = 0.036$
Hgb (g/dl) at diagnosis	11.1 (8.0–14.5)	9.1 (5.9–13.1)	9.2 (5.9–13.4)	
MCV (fl) at diagnosis	93.5 (88–99)	94.0 (64–116)	95.9 (74–117)	
WBC (K/ul) at diagnosis	9.9 (1.8–22.4)	8.1 (0.7–93.0)	5.9 (0.6–57.1)	
Plt (K/ul) at diagnosis	234.8 (22–619)	73.8 (7–367)	72.1 (14–313)	
History of CT	2/4	117/136	48/56	
History of RT	2/4	62/136	31/56	
History of CT and RT	0/4	43/136	23/56	

CT chemotherapy, *RT* radiation therapy.

Table 2. Genetic characterization of therapy-related myeloid neoplasms with different latencies.

	t-MN-short (t-MN with latencies of <1 year) (*N* = 4)	t-MN-ordinary (t-MN with latencies of 1–10 years) (*N* = 136)	t-MN-long (t-MN with latencies of >10 years) (*N* = 56)	*p* values
Normal karyotype	3/4	14/133	9/54	
del(5q)/-5 or del(7q)/-7	1/4	60/124	23/48	
Complex karyotype	1/4	69/133	28/54	
t(8;21)(q22;q22); *RUNX1-RUNX1T1*	0/4	2/123	0/48	
inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*	0/4	4/123	0/48	
t(15;17)(q24;q21); *PML-RARA*	0/4	4/122	0/48	
t(11q23.3); *KMT2A*	0/4	13/123	1/48	
t(21q22.1); *RUNX1*	0/4	2/122	1/48	
17p loss	0/4	23/131	14/54	
Mutation in *TP53*	0/2	50/101	22/46	
Mutation in *NPM1*	1/2	2/101	1/46	

mutations characterized by the next-generation sequencing assay targeting genes recurrently mutated in myeloid neoplasms.

The median survival of the whole t-MN cohort was 11.88 months. In consideration of the limited numbers of t-MNs with short latencies ($n = 4$), these cases were excluded from the survival analysis shown in Fig. 3. There was no statistically significant difference in overall survival between the t-MNs with ordinary and extended latencies. Excluding the pediatric and young adult patients from the analysis did not alter the results. Though it was unclear if the survival data from the four t-MN patients with short latencies were representative, the 4 t-MN patients with short latencies were all alive at the last follow-up (with a follow-up time ranging from 8 to 60 months). Despite the retrospective nature of the study, there were no statistically significant differences in the percentages of patients receiving induction therapy or bone marrow/stem cell transplant among groups of t-MN with different latencies.

Intriguingly, we found that the patients who had t-MNs with extended latencies were more likely to have histories of multiple cancers (other than the t-MN) (15/56) (vs. 14/136 in t-MNs with ordinary latencies, $p = 0.007$), more likely to have histories of HL (6/56) (vs. 2/134 in t-MNs with ordinary latencies, $p = 0.008$) and were likely to be younger at the time of initial cytoreductive treatment (vs. t-MNs with ordinary latencies, $p < 0.001$) (Table 3). t-MN with extended latencies did not show statistically significant associations with prior histories of carcinoma, NHL and sarcoma when compared to the other t-MN groups. However, in the multivariable logistic regression analysis, t-MNs with extended latencies were only associated with history of multiple cancers and younger age at initial exposure, not history of HL.

In the t-MN patients with histories of multiple cancers, the latencies for development of t-MN ranged from 1 to 32 years with the mean being 12.5 years (median: 12 years). The latency for t-MN in patients with multiple cancers was significantly longer than that seen in the t-MN patients without multiple malignancies (mean: 7.8 years, median: 6 year; $p < 0.05$ for both parametric and non-parametric tests) (Fig. 4A). When comparing t-MNs undergoing genetic characterization in the patients with and without multiple malignancies, t-MNs in the patients with multiple cancers were more likely to demonstrate *BCOR* mutations though they were present only in 3 out of 21 cases (vs. 2/123 in patients without multiple cancers; $p = 0.02$). There were no statistically significant differences in the incidence of other cytogenetic abnormalities and mutations characterized by the target-panel next-generation sequencing assay (Fig. 3). A high percentage of the t-MN patients with histories of multiple cancers had carcinoma (25/29), which was a significantly higher percentage than that in the t-MN patients without multiple cancers (vs. 91/167; $p = 0.001$).

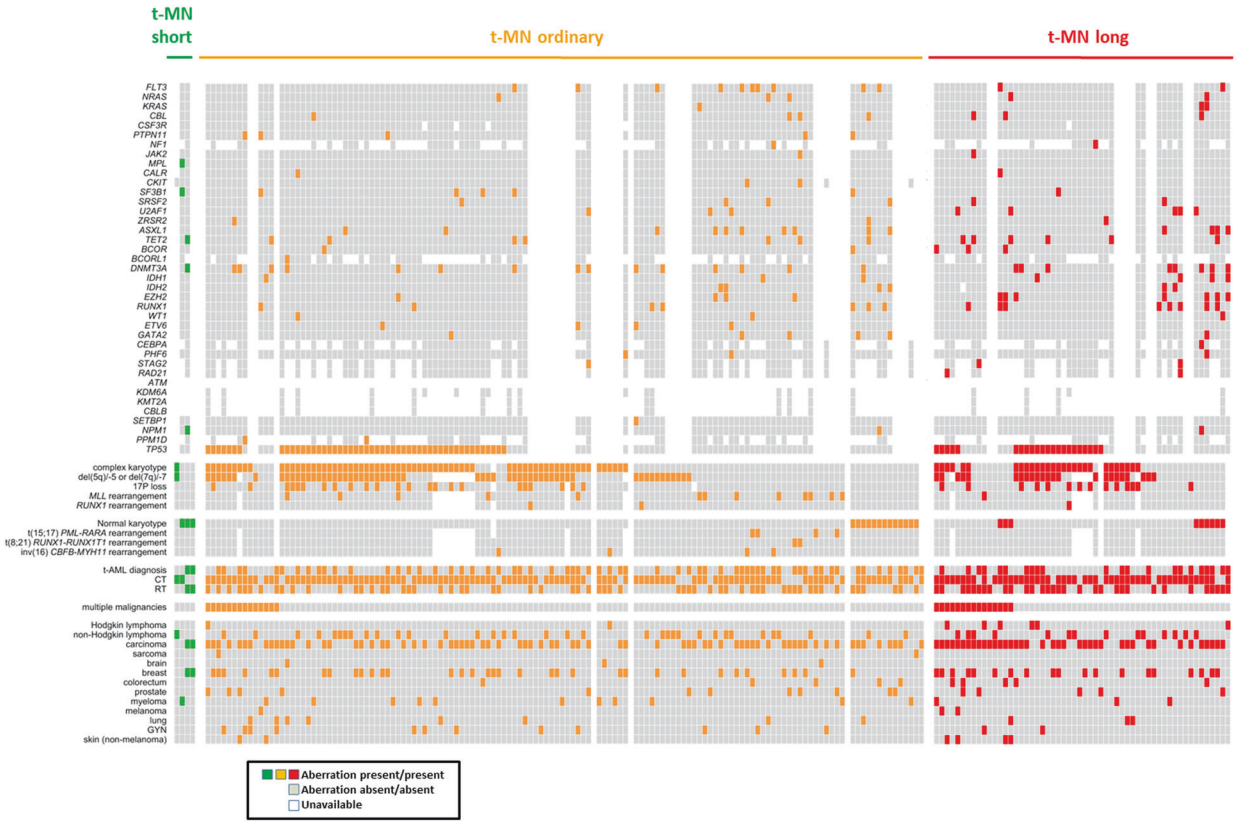

Fig. 2 Heat map summarizing the clinicopathologic features of t-MNs with different latencies. Colored squares indicate the presence of the specified chromosome abnormality, mutation, or the history of disease/treatment. Different colors are used in different t-MN groups (green: t-MN short; orange: t-MN ordinary; red: t-MN long). Gray squares indicate the absence of the specified aberration or history. White squares indicate that information is not available.

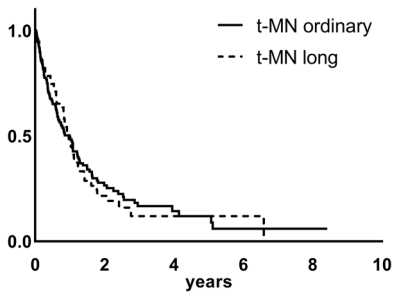

Fig. 3 There were no significant differences in overall survival between t-MNs with ordinary and extended latencies. Cases of t-MN with short latencies were not shown in the figure in consideration of the small sample size.

Among the t-MN patients with multiple malignancies, 13/29 patients had histories of breast cancer, 5/29 patients had histories of gynecologic cancers, 3/29 had melanoma and 6/29 patients had non-melanoma skin cancers (Fig. 4B). Regarding the history of cytoreductive treatments, higher percentage of t-MN patients with multiple cancers had histories of both chemotherapy and radiation therapy (15/29), when compared to the patients without multiple cancers (vs. 51/167, $p = 0.026$). There were no statistically significant differences in the percentage of patients with histories of chemotherapy only or radiation therapy only between the t-MN groups with and without multiple cancers (11/29 vs. 90/167 for CT only; 3/29 vs. 26/167 for RT only).

Among the four cases with t-MNs diagnosed within 1 year of the cytoreductive treatment, one case was an AML with mutated *NPM1*. One case was a myeloid neoplasm with myeloproliferative and myelodysplastic features showing *MPL* and *SF3B1* mutation. One case with no prior bone marrow examination was diagnosed as t-MN with a complex karyotype. The remaining t-MN case with short latency presented with normal karyotype but 5% blasts at diagnosis. Follow-up bone marrows of this patient demonstrated clonal evolution showing acquired del(7q) and the subsequent presence of a concurrent separate clone with trisomy 1 and der(1;15)(q10;q10).

In an attempt to further characterize the t-MN group with short latencies, we extended the analysis for t-MN-short to include all the cases with latencies less than 2 years ($N = 23$). The definition of the t-MN-ordinary was modified as t-MNs with latencies of 2–10 years accordingly ($N = 117$). All the findings described above regarding t-MN-ordinary and t-MN-long groups remain unchanged except for the difference in the age at diagnosis of t-MN, which was no longer statistically significant. Comparing the now expanded short latency group to the remaining t-MNs revealed a new statistically significant association with increased presence of inversion 16 in the expanded t-MN-short group. The number of t-MN cases with inversion 16 was 3/22 (14%) in the expanded t-MN-short group, compared with 1/105 in the t-MN-ordinary group ($p = 0.016$) and 0/48 in the t-MN-long group ($p = 0.028$). There were no significant survival differences among the 3 t-MN groups when using 2 and 10 years, instead of 1 and 10 years, to define the t-MNs with short and long latencies (Supplementary Fig. 1). The findings from the re-analysis are summarized as Supplementary Tables 1–3.

DISCUSSION

t-MN is considered a distinct entity based on the WHO classification due to its overall poor prognosis when compared to the *de novo* lesions[2–6]. Population studies suggested the

Table 3. t-MN patients with extended latencies tend to be younger when they received the initial cytoreductive therapy and are more likely to have multiple malignancies.

	t-MN-short (t-MN with latencies of <1 year) (*N* = 4)	t-MN-ordinary (t-MN with latencies of 1–10 years) (*N* = 136)	t-MN-long (t-MN with latencies of > 10 years) (*N* = 56)	*p* values
Age at the time of initial cytoreduction treatment (y/o)	70.0 (64–76)	59.4 (5–87)*	50.8 (12–74)*	*$p < 0.001$
History of multiple malignancies	0/4	14/136*	15/56*	*$p = 0.007$
History of Hodgkin lymphoma	0/4	2/134*	6/56*	*$p = 0.008$
History of non-Hodgkin lymphoma	1/4	35/136	14/56	
History of carcinoma	2/4	75/136	39/56	
History of sarcoma	0/4	2/136	0/56	

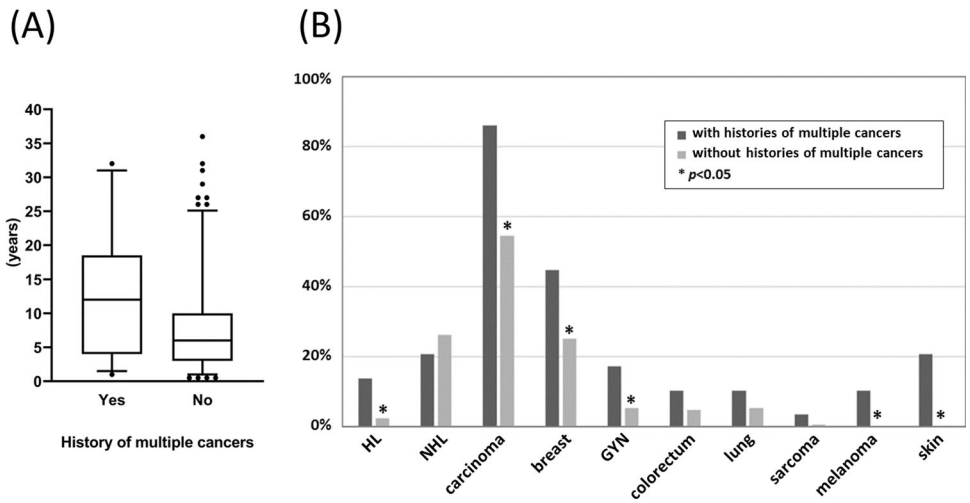

Fig. 4 Comparisons between t-MNs in patients with and without multiple cancers. A t-MNs in patients with multiple cancers showed longer latencies when compared to t-MNs in patients without multiple cancers. **B** Profiles of primary cancer types in t-MN patients with multiple cancers appeared different from those seen in t-MN patients without multiple malignancies.

majority of the t-MNs occurred within 10 years after the diagnoses of prior malignancies, though patients with extended latencies were also observed[11]. A prior study utilizing Surveillance, Epidemiology, and End Results data estimated the risk of developing t-MN in a patient with solid tumor peaked at 2 years but returned to the baseline in 10–15 years, while the risk of t-MN in patients with prior hematologic malignancies showed a different time course[21]. It was hypothesized that the t-MNs with extended latencies might represent random second cancers or second cancers arising in patients with cancer predisposition.

The interesting association between t-MN with extended latencies and history of multiple cancers we identified highlights the potential role of cancer predisposition in the development of these t-MNs. It is known that cancer patients were more likely to have inherited mutations in cancer-associated genes when compared to people without cancer[22–24]. Pathogenic germline mutations in DNA repair genes were identified in a significant portion of the t-MN patients[25–27] and reportedly increased the risk for subsequent tumors in the patients with childhood malignancies[28]. Many of the germline mutations identified in the t-MN patients involve DNA damage-sensing, DNA repair, and drug metabolism[25,29–32], which presumably make these patients more susceptible to the DNA damage caused by the cytoreductive treatments. We found a study describing a cohort of 47 patients with t-MN or therapy-related acute lymphoblastic leukemia after breast cancer; the study showed extended latencies between the diagnosis of breast cancer and therapy-related hematologic malignancies in patients with germline *BRCA1* or *BRCA2* mutations (median 133 months, 30–408 months)[25]. The finding, together with our results, supports the hypothesis that increased cancer susceptibility contributes to the development of t-MNs with long latencies. Our finding also warrants the investigation for possible germline cancer predisposition in the t-MN patients with long latencies, which can provide important information for the related family members. However, since a similarly extended latency was not observed in the small number of patients with germline mutations in *TP53, PALB2,* and *CHEK2* in the prior study of breast cancer patients with therapy-related hematologic malignancies, the development of t-MNs likely involves various mechanisms in different genetic backgrounds.

t-MN with long latencies, despite their association with multiple cancers, showed comparable genetic features and survival when compared to the t-MNs with ordinary latencies. The similar genetic composition and lack of survival difference did not particularly support the notion to exclude t-MNs with long latencies from the t-MN entity. t-MN, like other malignancies, is the result of a clonal selection process[17]. Irrespective of the mechanisms generating the genetic aberrations, such as aging, exposure to cytotoxic therapies, and impaired DNA damage response related to cancer predisposition, the genotype in the final selected clone presumptively is one of the most important parameters dictating the prognosis and outcome. Disregarding latencies, it has been reported that although the history of prior cytotoxic treatment disadvantageously impacts the survival in cases with favorable cytogenetic abnormalities[6], the negative impact of prior cytotoxic therapy on survival is less pronounced in older patients and in

patients with adverse cytogenetics[11]. The finding likely reflects the fact that both t-MNs and the myeloid neoplasms in the elderly frequently demonstrate high-risk genotypes[33,34].

Another interesting finding in our study was that t-MN patients with extended latencies were younger at the time of cytotoxic treatments. Younger age at exposure was implicated in increasing the risk of t-MNs[16,35], although confirming a true susceptibility in young individuals is challenging based on the population studies alone, since it is difficult to ascertain that the increased risk is not just a reflection of the lower background incidence of myeloid neoplasms in younger people. The fact that the association between t-MNs with long latencies and HL was no longer significant in the multivariable logistic regression analysis suggested HL may be related to cancer predisposition or the younger age at the diagnosis and treatment for the primary cancers. HL had peak incidence among young individuals and HL was also recognized as one of the malignancies with greater familial risk. For example, the risk of HL with a diseased sibling was higher than the risk of NHL in a similar scenario[36,37]. In addition, the risk of HL increased if the HL relative was diagnosed at a younger age. This observation, not seen in NHL families, implies a possibly stronger association with cancer predisposition in HL[37].

When focusing on t-MN patients with multiple cancers, they showed variable but overall longer latencies than those without multiple malignancies. They were also more likely to have *BCOR* mutations though these mutations were only identified in a small portion of cases showing different cytogenetic abnormalities and co-mutations. BCOR is a component of the PRC1.1 complex, one of the 6 mammalian Polycomb Repressive Complex-1 catalyzing repressive histone modifications and participating in stemness and differentiation[38]. It is intriguing to hypothesize that *BCOR* aberrations are associated with specific cancer predisposition mutations and thus were only identified in a subset of t-MN cases with histories of multiple cancers. The finding that higher percentage of the t-MN patients with multiple cancers had histories of both chemotherapy and radiation therapy may potentially reflect the treatment modalities adopted for different cancers in these patients; but further studies are required to elucidate its potential biologic significance.

Our observations in the t-MN cases with short latency likely highlight the heterogeneity in this rare group of patients. The presence of strong driver mutations (i.e., mutated *NPM1*, concurrent mutations in *MPL* and *SF3B1* or inversion 16) suggested some genetic similarities to the *de novo* cases in a subset of t-MN patients with short latencies. In the case presenting with a complex karyotype, the possibility that a pre-existent t-MN stayed undiagnosed until the worsening cytopenia caused by treatment for concurrent malignancy cannot be excluded. The t-MN case that demonstrated clonal evolution after the initial diagnosis may suggest that the t-MN diagnosis was made at a relatively early stage of the disease course, which may be a potential explanation for the short latency in this case. Larger studies are desirable to confirm the findings.

Regarding the limitations of the study, we acknowledge that it is difficult to precisely determine the latencies in t-MNs since the disease may precede the diagnosis. The latencies recorded in our cohort reflected the time these lesions caught clinical attention. However, even without explicit latencies, the observations in our study still offer useful guidance for daily practice considering the information was collected from a comparable clinical setting. Target-panel mutation analysis covering genes recurrently mutated in myeloid neoplasms was used to characterize the t-MN cases in our study. With high-risk genetic abnormalities identified in the majority of the t-MNs, it is less likely that a significant portion of these t-MN cases will show additional critical somatic driver mutations. Future studies with more extensive genetic characterization in t-MNs with different latencies will help uncover potential germline or somatic mutations outside the panel to further elucidate the mechanisms of t-MN development.

In summary, our study demonstrated t-MNs with ordinary and extended latencies showed similar genetic features common in high-risk genotypes. The findings, in conjunction with the absence of significant survival differences, did not support the argument to exclude the t-MNs with long latencies from the t-MN classification. However, the association between the t-MNs with extended latencies and history of multiple cancers highlighted the possibility that increased cancer susceptibility contributed to the genetic abnormalities seen in these lesions. t-MN with short latency likely represents a heterogeneous group with a subset showing features more similar to the *de novo* lesions.

DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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AUTHOR CONTRIBUTIONS

Y.L. and J.G. performed study concept and design; Y.L. and J.G. performed writing, review, and revision of the paper; Y.L., G.I., R.A., B.C., B.R., and J.G. performed acquisition of data; Y.L. performed analysis and interpretation of the data as well as statistical analysis. S.Y. performed the interpretation of the cytogenetic data. All authors read and approved the final paper.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Institutional Review Boards at University of Pittsburgh and Weill Cornell Medicine with a waiver of consent. It was performed in accordance with the Declaration of Helsinki.

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Correspondence and requests for materials should be addressed to Yen-Chun Liu.

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