Inherited cancer predisposing mutations in patients with therapy-related myeloid neoplasms

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Funding information
American Cancer Society; Cancer Research Foundation; National Institutes of Health, Grant/Award Number: HD0433871, PO1CA040046 and R01CA231880

Summary
Some patients with therapy-related myeloid neoplasms (t-MN) may have unsuspected inherited cancer predisposition syndrome (CPS). We propose a set of clinical criteria to identify t-MN patients with high risk of CPS (HR-CPS). Among 225 t-MN patients with an antecedent non-myeloid malignancy, our clinical criteria identified 52 (23%) HR-CPS patients. Germline whole-exome sequencing identified pathogenic or likely pathogenic variants in 10 of 27 HR-CPS patients compared to 0 of 9 low-risk CPS patients (37% vs. 0%, p = 0.04). These simple clinical criteria identify t-MN patients most likely to benefit from genetic testing for inherited CPS.

KEYWORDS
cancer predisposition, germline, leukaemia, therapy-related myeloid neoplasm, whole-exome sequencing

INTRODUCTION

Therapy-related acute myeloid leukaemia (AML) and therapy-related myelodysplastic syndrome (MDS; collectively referred to as therapy-related myeloid neoplasms (t-MN)) are frequently fatal conditions resulting from prior exposure to chemotherapy, radiation therapy, and/or immunosuppressive agents. The incidence of t-MN is influenced by a number of factors, but is most affected by the agent(s) to which a patient is exposed, and the dosage and schedule of treatment. Two mechanisms have been proposed for the aetiology of t-MN: (i) direct damage to DNA in normal haematopoietic stem cells...
by mutagenic agents; and (2) selection and expansion of pre-existing mutant haematopoietic clones.

As many t-MN patients have had more than one primary cancer, some may have an unsuspected cancer predisposition syndrome (CPS). Indeed, one of the very first successes of whole-genome sequencing identified a deleterious germ-line \( TP53 \) deletion in a patient with t-MN.\(^2 \) Furthermore, germline mutations in CPS genes such as \( BRCA1 \), \( BRCA2 \), and \( BARD1 \), as well as \( TP53 \), have been reported in t-MN patients.\(^3,4 \)

To investigate the occurrence of CPS in t-MN patients, we analysed an unselected series of 226 patients who developed t-MN following exposure to chemotherapy and/or radiation for an antecedent non-myeloid malignancy (Table 1; see Appendix S1 for details on the analysis). The most common primary malignancies were: breast cancer (\( n = 72; 32\%) \), non-Hodgkin-lymphoma (NHL; \( n = 38; 17\%) \), Hodgkin lymphoma (HL; \( n = 26; 12\%) \), and acute and chronic lymphoid leukaemias (\( n = 17; 8\%) \).

Because family history data were missing or incomplete for almost all t-MN patients, we employed criteria used routinely by clinical cancer geneticists to identify high-risk CPS (HR-CPS) patients: (1) the diagnosis of breast or colon cancer under age 50; (2) the diagnosis of sarcoma under age 46 (meeting revised Chompret criteria for Li–Fraumeni syndrome); and (3) the diagnosis of two or more primary cancers other than non-melanoma skin cancer in addition to the diagnosis of a t-MN. Because of the known biology of t-MN, we also included the following two criteria: (4) latency to t-MN of more than 15 years following initial exposure to cytotoxic therapy—since most t-MN cases occur with a latency of 10 years or less, long-latency t-MN may in fact represent a second primary cancer; and (5) a latency to t-MN diagnosis of no more than one year following exposure to cytotoxic therapy and a t-MN karyotype with either abnormalities of chromosome 5 and/or 7 or a complex tumour karyotype (\( \geq 3 \) unrelated cytogenetic abnormalities), since this subset of t-MN very rarely occurs with a latency of less than three

**Table 1** Demographic and clinical characteristics of all 226 t-MN patients stratified as high-risk or low-risk cancer predisposition syndromes (HR-CPS or LR-CPS), and of the 27 HR-CPS patients and nine LR-CPS patients analysed via whole-exome sequencing (WES). For individual-level data on these patients, see Table S3. Note that some HR-CPS patients met multiple HR-CPS criteria.
years. All other patients were considered low-risk for CPS (LR-CPS).

We identified 52/226 patients (23%) meeting one or more HR-CPS criteria, suggesting that an unexpectedly high proportion of t-MN patients may have an undiagnosed CPS (Table 1). Of these, 25 were diagnosed with breast cancer at less than 50 years of age; 11 had multiple primary cancers; eight had latencies longer than 15 years; seven had cytogenetic abnormalities of chromosomes 5/7 or complex karyotypes with latencies of no more than one year; and three had sarcomas. Two patients met more than one criterion. HR-CPS patients had an earlier median age of diagnosis for both their primary malignancy and t-MN as compared to LR-CPS patients (43 versus 53.2 years of age for primary malignancy ($p = 3.4 \times 10^{-7}$), and 49.5 versus 59 years of age for t-MN ($p = 0.011$)).

We performed whole-exome sequencing (WES) on all HR-CPS patients with available germline DNA (27/52), as well as nine LR-CPS patients with available germline DNA (see Table 1 and Table S1). Following WES and quality control, the average exome coverage at 10× for the HR-CPS and LR-CPS patients was 95% and 85% respectively, with an average read depth of 70× and 101× respectively (Table S2). After filtering out non-coding variants and those leading to synonymous amino acid changes, an average of 8767 rare germline variants [minor allele frequency (MAF) < 0.01] were identified in each sample.

To identify high/moderate penetrance CPS variants in t-MN patients, we searched Ambry Genetics’ (Aliso Viejo, CA, USA) clinical database for variants in the 52 genes associated with hereditary cancer on the CancerNext-Expanded hereditary cancer gene panel (Table S3). Ambry Genetics is a genetic testing laboratory, and the clinical validity of all gene–disease associations are thoroughly assessed using a weighted scoring system guided by ClinGen gene curation criteria (https://clinicalgenome.org/curation-activities/gene-disease-validity/).

We identified eight t-MN patients with a germline pathogenic variant or likely pathogenic variant (VLP) in two tumour suppressor genes: TP53 ($n = 6$), and CHEK2 ($n = 2$). We also identified one t-MN patient (2A) with a variant of uncertain significance (VUS) in TP53 who also had a co-occurring pathogenic TP53 variant (Table 2). All variants were heterozygous with allele fractions of approximately 50%. One identical TP53 pathogenic variant was identified in patients 2A and 15A. Variants were confirmed by Sanger sequencing, based on DNA availability (Table S4). See supporting evidence for each variant in the Appendix S1.

Because criteria for clinically actionable CPS variants are very stringent, we hypothesized that there may be other patients in our series with CPS variants not identified by our clinical pipeline. Consequently, we performed an in-silico bioinformatics analysis to identify rare potentially deleterious variants in the CPS genes identified by our clinical analysis, TP53 and CHEK2. Variants meeting the following criteria were characterized as bioinformatically defined CPS variants: (1) MAF less than 0.01 in both the 1000 Genomes Project and the NHLBI Exome Sequencing Project; (2) CADD Phred$^9$ scores of 20 or higher or GERP RS$^10$ scores of 4 or higher, both of which are highly sensitive for identifying pathogenic variants in the ClinVar database; and (3) not classified as benign or very likely benign by Ambry. We successfully rediscovered 8/9 variants in HR-CPS patients (Table 2); only patient 104A (TP53 pathogenic variant) was not identified because his variant did not meet CADD Phred or GERP RS score thresholds (16.5/3.5). Additionally, we identified two patients with variants predicted to be deleterious, both of whom are HR-CPS patients, and both of whom

<table>
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<th>Patient ID</th>
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<th>Primary malignancy</th>
<th>Age at primary cancer diagnosis (yrs)</th>
<th>Latency to t-MN (yrs)</th>
<th>Gene</th>
<th>Variant</th>
<th>Variant classification</th>
<th>Discovered by clinical analysis</th>
<th>Discovered by bioinformatics analysis</th>
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<td>32</td>
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have variants in CHEK2. This in-silico analysis replicates and potentially extends the results of our clinical analysis, though the pathogenicity of these biinformatically predicted deleterious variants should be validated in additional studies. Combining our clinical and bioinformatic analyses, all individuals with pathogenic variants, VLPs, or VUS were HR-CPS patients (10/27, 37%), versus 0/9 LR-CPS patients (Fisher's exact p = 0.04).

In conclusion, whereas only 5%-10% of all cancers are caused by deleterious germline mutations in CPS genes, we found evidence for CPS mutations in a large proportion of t-MN patients, all of whom met HR-CPS criteria. Based upon the combined results of our clinical and bioinformatic analysis, we identified putative CPS variants in 10/27 HR-CPS patients, and 0/9 LR-CPS patients, with all variants identified in either TP53 or CHEK2. However, given the small sample size here, the HR-CPS criteria should be tested in a larger cohort to determine their sensitivity and specificity for identifying patients with CPS. Another possible limitation of our results is that the CancerNext-Expanded gene panel does not include genes associated with predisposition to ‘pure’ haematological malignancy syndromes such as RUNX1 or CEBPA.

Wong and colleagues demonstrated that haematopoietic stem cell clones with somatic TP53 mutations can be selected for by exposure to chemotherapy and outcompete clones with wild-type TP53, thereby giving rise to t-MN. Our results complement these observations by suggesting that individuals with germline TP53 mutations are similarly at increased risk for t-MN.

Moreover, our results align with work by Singhal and colleagues suggesting that CPS are likely to be more common among t-MN patients and that clinical criteria are needed to guide CPS screening in t-MN patients. The simple HR-CPS criteria we employed, which do not require a family history, may provide a simple and effective means of identifying t-MN patients for CPS screening and should be studied further.

Currently, the vast majority of patients who could benefit from genetic testing for inherited CPS are not being offered genetic testing. Our findings provide both a powerful rationale for the importance of genetic testing in t-MN patients as well as a simple clinical algorithm to identify those t-MN patients who are most likely to benefit. These results may have significant diagnostic and clinical implications for both these patients and their family members.

**AUTHOR CONTRIBUTIONS**

Andrew J. Shih, Andrew D. Skol, Felicia Hernandez, Friedrich Stölzel, James M. Allan, and Kenan Onel designed the research; Andrew J. Shih, Andrew D. Skol, Friedrich Stölzel, James M. Allan, and Kenan Onel wrote the manuscript with substantial input from Felicia Hernandez; Andrew J. Shih, Tomi Jun, Andrew D. Skol, Riyue Bao, Lei Huang, Sapana Vora, Megan E. McNerney, Eric A. Hungate, Aaron Elliott, Robert Huether, and Felicia Hernandez performed the research and statistical analysis; Megan E. McNerney, Michelle M. Le Beau, and Richard A. Larson contributed critical reagents and clinical data; Kenan Onel directed the research.

**ACKNOWLEDGEMENTS**

This work was supported by grants from the National Institutes of Health (R01CA231880 to Megan E. McNerney; PO1CA40046 to Michelle M. Le Beau; and HD0433871 to Kenan Onel); the American Cancer Society (Megan E. McNerney); the American Cancer Society-Illinois Division (Kenan Onel); and the Cancer Research Foundation (Michelle M. Le Beau, Richard A. Larson, and Kenan Onel). The bioinformatics analysis was partially performed on The University of Chicago Center for Research Informatics high-performance computing clusters. We thank M. Jarsulic for the technical support of the clusters.

**CONFLICT OF INTERESTS**

All authors declare no conflicts of interest or competing financial interests.

**DATA AVAILABILITY STATEMENT**

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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**REFERENCES**


SUPPORTING INFORMATION
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