LETTER TO THE EDITOR

Genetic susceptibility to therapy-related leukemia after Hodgkin lymphoma or non-Hodgkin lymphoma: role of drug metabolism, apoptosis and DNA repair

Blood Cancer Journal (2012) 2, e58; doi:10.1038/bcj.2012.4; published online 2 March 2012

Therapy-related myelodysplasia or acute myeloid leukemia (t-MDS/AML) is a major cause of non-relapse mortality in patients treated for Hodgkin lymphoma (HL) or non-Hodgkin lymphoma (NHL). t-MDS/AML is associated with exposure to alkylating agents and topoisomerase II inhibitors. The DNA-damaging events caused by these agents initiate apoptosis required for antineoplastic activity; occasionally, imperfect repair of chromosomal damage results in chromosomal aberrations, leading to leukogenesis.

The inter-individual variability in the risk of t-MDS/AML for any given exposure to genotoxic agents suggests the role of genetic susceptibility. In addition, t-MDS/AML shares morphological and genetic similarity with de novo MDS/AML suggesting that therapy-related and de novo MDS/AML may share genetic susceptibility loci. Previous studies have been largely inconclusive, primarily because of the focus on single genes. In the few studies where multiple genes were examined simultaneously, individuals with more than one risk variant were at higher risk, possibly because of the focus on single genes. In the few studies where multiple genes were examined simultaneously, individuals with more than one risk variant were at higher risk, possibly because of the focus on single genes. In the few studies where multiple genes were examined simultaneously, individuals with more than one risk variant were at higher risk, possibly because of the focus on single genes.

We genotyped 29 SNPs and 2 deletion polymorphisms for 23 candidate genes (Supplementary Table 3). Association between individual polymorphisms and t-MDS/AML was analyzed using exact conditional logistic regression, adjusted for gender, treatment modality (conventional therapy vs aHCT) and cumulative exposure to alkylating agents and topoisomerase II inhibitors. We observed a higher risk for t-MDS/AML among patients with deletion of GSTM1, the Pro allele of P72R in TP53, the T allele of CYP1A1*2A, and the T allele of rs6030469 of PTPTPT (Table 1). None of these associations withstood Bonferroni adjustment for multiple comparisons.

TP53 modulates DNA repair and apoptosis upon DNA damage. A common germline polymorphism of TP53, P72R, produces a proline to arginine change that enhances apoptotic activity. We used likelihood ratio tests with permutations implemented in UNPHASED to explore unadjusted gene-gene interaction between P72R in TP53 and all polymorphisms in other candidate genes. A significant interaction between P72R and C677T, a coding SNP in MTHFR, was detected and remained significant after correction for multiple testing using 10,000 permutations (adjusted Pinteraction = 0.0048). This interaction was confirmed after adjustment for therapeutic exposures (Table 1). The homozygous T allele of C677T increased the risk 71-fold (P = 0.0059) when combined with the Pro carrier of P72R (conferring decreased apoptotic activity) compared with its combination with homozygous Arg. We also detected an interaction between P72R and another coding SNP, A1298C, in MTHFR (Table 1). The homozygous A allele of A1298C increased the risk 23-fold (P = 0.0005) when combined with the Pro carrier of P72R compared with its combination with homozygous Arg in the adjusted analysis.
We then examined expression levels of the 23 candidate genes using Affymetrix HG U133 plus 2.0 array data procured from an independent study that studied gene-expression profiles in CD34<sup>+</sup> HSCs<sup>11</sup> to detect differential expression between cases and controls, adjusted for age at aHCT, gender and therapeutic exposures, using a general linear model implemented in R. In total, 10 genes were differentially expressed between cases and controls (Supplementary Table 4). This represents a significant enrichment (\(P = 0.0025\)) compared with a random draw of 23 genes from a whole genome of 20,722 genes interrogated on the U133 Plus 2 array. Signals (\(P < 0.05\)) were detected for TP53 and genes involved in drug metabolism (CYP3A4, GSTM1, GSTP1, and GSTT1), DNA repair (XRCC1, XRCC2, and MTHFR), and de novo AML (NMNAT2 and LAMC2). Expression level for TP53 was much lower in cases. Also, as hypothesized, expression of genes was observed at much lower level for the three detoxification...
tion enzymes, GSTM1, GSTP1 and GSTT1, whereas expression of a drug activation gene, CYP3A4, was significantly higher in cases. Relative expression levels measured by microarray were evaluated using TaqMan-based reverse transcribed quantitative PCR (RT-qPCR) gene-expression assays (Applied Biosystems) in 17 subjects for GSTM1 (Hs01683722_gH), TP53 (Hs1034249_m1) and MTHFR (Hs0015560_m1) referenced on ACTB (Hs99999903_m1); correlations were found to be statistically significant for GSTM1 ($R^2 = 0.50$, $P = 0.0015$) and TP53 ($R^2 = 0.24$, $P = 0.044$). Gene-expression levels of GSTM1 and GSTT1 were significantly correlated with depletion polymorphisms ($P < 0.0031$) in the respective genes after adjusting for multiple comparisons.

We observed associations between t-MDS/AML and genes responsible for drug metabolism supported by both genotyping and expression studies, underscoring the mechanistic relationship between t-MDS/AML and therapeutic exposures. Results were compatible with increased genotoxic stress resulting from enhanced drug activation and disrupted drug clearance among cases. Previous meta-analyses of case-control studies have demonstrated a modest increase in risk of de novo MDS/AML associated with the null genotypes of drug-detoxification genes, GSTM1 and GSTT1, and a trend for overrepresentation for 105Val allele of GSTP1.12 Thus, we confirmed an increased risk for t-MDS/AML associated with GSTM1 homozygous deletion, and detected significantly lower gene-expression level in cases for all three genes (GSTM1, GSTT1 and GSTP1). CYP3A4 encodes a hepatic phase I P450 protein involved in activation of cyclophosphamide, and some anthracycline agents. In the current study, CYP3A4 expression in the CD34+ cells was significantly higher in cases. We observed a higher risk for t-MDS/AML among Pro carriers of 72R in the genotyping study accompanied by a significantly lower expression level of TP53 in cases. More importantly, we detected a synergistic effect between TP53 and MTHFR. MTHFR directs 5, 10-methylene tetrahydrofolate toward methionine synthesis and conversion to the universal methyl donor, S-adenosylmethionine, at the expense of pyrimidine synthesis that is required for DNA synthesis and repair. Two polymorphisms, C677T and A1298C, have been associated with reduced enzyme activity.13 Both SNPs were found to interact with P72R in increasing the risk of t-MDS/AML. MTHFR has been extensively investigated for susceptibility to cancer because of its key role in intracellular folate homeostasis and metabolism that are fundamental to DNA synthesis, repair and methylation. Meta-analyses has shown that the T allele of C677T polymorphism and the A allele of A1298C are associated with gastric and colorectal cancers, respectively.14 Expression of both TP53 and MTHFR was significantly lower in cases compared with controls, supporting their role in t-MDS/AML development. We proposed a model to explain the interaction between TP53 and MTHFR (Supplementary Figure 1). Reduced MTHFR activity is associated with chromosomal aberrations during DNA repair.15 When combined with higher TP53 activity, it would normally result in efficient clearance of damaged cells through apoptosis. However, when combined with less-efficient TP53, it would result in accumulation of progenitor cells with chromosomal damage and increase the risk of t-MDS/AML. On the other hand, with normal MTHFR activity to support DNA repair, allele variants of TP53 do not have an impact on t-MDS/AML development, because efficient DNA repair would maximize DNA recovery and minimize the risk of chromosomal aberrations. The current study is the first to report a synergistic impact of SNPs in MTHFR and TP53 on t-MDS/AML, however, the observation requires confirmation.

The study was limited by a relatively small sample size; the rarity of t-MDS/AML precluded a large-scale study. However, the study was strengthened by the combined use of genotyping and gene-expression analyses with detailed information regarding therapeutic exposures, allowing for the more robust (and hence clinically relevant) observations to emerge. Supporting evidence from both genotyping and expression analyses for GSTM1, TP53 and MTHFR suggests their contribution to t-MDS/AML as germ-line genetic factors. In summary, we demonstrate that the risk of t-MDS/AML is related to the individual capacity of drug metabolism, apoptosis and DNA synthesis. These observations not only will further our understanding of the pathogenesis of t-MDS/AML but also, when confirmed in independent studies, will help identify those at the highest risk, setting the stage for targeted surveillance and/or interventions.

CONFLICT OF INTEREST
Dr Smita Bhatia’s work has been funded by the NIH and Leukemia/Lymphoma Society. Drs Ravi Bhatia and Forman’s works have been funded by the NIH. Dr Ravi Bhatia is in Advisory board/Honoraria for Novartis and Bristol Meyer Squibb. All the other authors declare no conflict of interest.

ACKNOWLEDGEMENTS
Supported in part by the Leukemia/Lymphoma Society (S Bhatia), R01 HL083050 (R Bhatia), R01 CA139633 (S Bhatia) and 1 P50 CA107399 (Forman).

Y Ding1, C-L Sun1, L Li2, M Li3, L Francisco1, M Sabado1,5, B Hahn1,6, J Gyorffy1,7, J Nee1, GP Larson4, SJ Forman2, R Bhatia2, S Bhatia1,8
1Population Sciences, City of Hope, Duarte, CA, USA; 2Hematology and Hematopoietic Cell Transplantation, City of Hope, Duarte, CA, USA; 3Information Sciences, City of Hope, Duarte, CA, USA; 4Molecular Medicine, City of Hope, Duarte, CA, USA E-mail: sbhatia@coh.org
5Current address: Claremont Graduate University, Claremont, CA, USA
6Current address: University of California, Hastings College of the Law, San Francisco, CA, USA
7Current address: Uniformed Services University of the Health Sciences, Bethesda, MD, USA
8Current address: City of Hope, Duarte, CA, USA

REFERENCES
1 Krishnan A, Bhatia S, Slovak ML, Arber DA, Niland JC, Nademanee A et al. Predictors of therapy-related leukemia and myelodysplasia following autologous transplantation for lymphoma: an assessment of risk factors. Blood 2000; 95: 1588–1593.
2 Pedersen-Bjergaard J, Andersen MK, Andersen MT, Christiansen DH. Genetics of therapy-related myelodysplasia and acute myeloid leukemia. Leukemia 2008; 22: 240–248.
3 Naoe T, Takeyama K, Yokozawa T, Kiyoi H, Seto M, Uike N et al. Analysis of genetic polymorphism in NQO1, GST-M1, GST-T1, and CYP3A4 in 469 Japanese patients with therapy-related leukemia/myelodysplastic syndrome and de novo acute myeloid leukemia. Clin Cancer Res 2000; 6: 4091–4095.
4 Woo MH, Shuster JJ, Chen C, Bash RO, Behrm FG, Camitta B et al. Glutathione S-transferase genotypes in children who develop treatment-related acute myeloid malignancies. Leukemia 2000; 14: 232–237.
5 Seedhouse C, Faulkner R, Ashraf N, Das-Gupta E, Russell N. Polymorphisms in genes involved in homologous recombination repair interact to increase the risk of developing acute myeloid leukemia. Clin Cancer Res 2004; 10: 2675–2680.
6 Ellis NA, Hua D, Yildiz O, Worroll JJ, Banejee M, Le Beau MM et al. MDM2 SNP309 and TP53 Arg72Pro interact to alter therapy-related acute myeloid leukemia susceptibility. Blood 2008; 112: 741–749.
7 Przychodzen BP, Jankowska AM, Smieszek SP, Mohan SR, Tiu RV, Jasek M et al. Investigations of genetic risk factors in MDS and AML using high-density 6.0 Polymethia arrays. 2008; San Francisco, CA, USA, p 638.
8 Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics 2000; 155: 945–959.
9 Kosoy R, Nassir R, Tian C, White PA, Butler LM, Silva G et al. Ancestry informative marker sets for determining continental origin and admixture proportions in common populations in America. Hum Mutat 2009; 30: 69–78.
10 Dudbridge E. UNPHASED User Guide: MRC Biostatistics Unit. Cambridge, 2006.
11 Li L, Li M, Sun C, Francisco L, Chakraborty S, Sabado M et al. Altered hematopoietic cell gene expression precedes development of therapy-related myelodysplasia/acute myeloid leukemia and identifies patients at risk. Cancer Cell 2011; 20: 591 - 605.

12 Ye Z, Song H. Glutathione s-transferase polymorphisms (GSTM1, GSTP1 and GSTT1) and the risk of acute leukaemia: a systematic review and meta-analysis. Eur J Cancer 2005; 41: 980 - 989.

13 Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab 1998; 64: 169 - 172.

14 Dong LM, Potter JD, White E, Ulrich CM, Cardon LR, Peters U. Genetic susceptibility to cancer: the role of polymorphisms in candidate genes. Jama 2008; 299: 2423 - 2436.

15 Robien K, Ulrich CM. 5,10-Methylenetetrahydrofolate reductase polymorphisms and leukemia risk: a HuGE minireview. Am J Epidemiol 2003; 157: 571 - 582.

This work is licensed under the Creative Commons Attribution-NonCommercial-No Derivative Works 3.0 Unported License. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-nd/3.0/

Supplementary Information accompanies the paper on Blood Cancer Journal website (http://www.nature.com/bcj)