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MDM2 SNP309 and *TP53* Arg72Pro interact to alter therapy-related acute myeloid leukemia susceptibility

Nathan A. Ellis,[1,2] Dezheng Huo,[3] Ozlem Yildiz,[4] Lisa J. Worrillow,[5] Mekhala Banerjee,[1] Michelle M. Le Beau,[1,2] Richard A. Larson,[1,2] James M. Allan,[5] and Kenan Onel[2,4]

[1]Department of Medicine; [2]University of Chicago Cancer Research Center; [3]Department of Health Studies; and [4]Department of Pediatrics, University of Chicago, IL; and [5]Department of Biology, University of York, York, United Kingdom

The p53 tumor suppressor directs the cellular response to many mechanistically distinct DNA-damaging agents and is selected against during the pathogenesis of therapy-related acute myeloid leukemia (t-AML). We hypothesized that constitutional genetic variation in the p53 pathway would affect t-AML risk. Therefore, we tested associations between patients with t-AML (n = 171) and 2 common functional p53-pathway variants, the *MDM2* SNP309 and the *TP53* codon 72 polymorphism. Although neither polymorphism alone influenced the risk of t-AML, an interactive effect was detected such that *MDM2* TT *TP53* Arg/Arg double homozygotes, and individuals carrying both a *MDM2* G allele and a *TP53* Pro allele, were at increased risk of t-AML (*P* value for interaction is .009). This interactive effect was observed in patients previously treated with chemotherapy but not in patients treated with radiotherapy, and in patients with loss of chromosomes 5 and/or 7, acquired abnormalities associated with prior exposure to alkylator chemotherapy. In addition, there was a trend toward shorter latency to t-AML in *MDM2* GG versus TT homozygotes in females but not in males, and in younger but not older patients. These data indicate that the *MDM2* and *TP53* variants interact to modulate responses to genotoxic therapy and are determinants of risk for t-AML. (Blood. 2008;112:741-749)

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Introduction

As a result of improvements in therapy, many more individuals are being cured of cancer, and median survival times for patients with cancer have increased significantly in the past 20 years.[1,2] An unanticipated consequence of this success is that an increasing number of cancer survivors are developing second therapy-related cancers, including therapy-related acute myeloid leukemia (t-AML). As many as 10% of patients treated for a first cancer develop this fatal side effect of prior cytotoxic therapy; currently, it is estimated that t-AML comprises 10% to 20% of all AML. Clinically, t-AML is considered and treated as a single syndrome, although 2 distinct groups of patients have been described, dependent on prior treatment.[3] Comprising about 75% of cases, the most common subtype of t-AML occurs 3 to 10 years after exposure to alkylating agents or radiation, is often preceded by a therapy-related myelodysplastic syndrome (t-MDS, defined here as less than 30% bone marrow blast cells), and is often characterized by cytogenetic abnormalities involving the loss of all or part of chromosomes 5 or 7.[4-6] In a recent series of 306 consecutive patients with t-AML seen at the University of Chicago, 21% of the patients had abnormalities of chromosome 5, 28% had abnormalities of chromosome 7, and 21% had abnormalities of both chromosomes 5 and 7.[7] Loss of the p53 tumor suppressor gene (*TP53*) is also very common in this subgroup of patients, particularly in association with loss or deletion of chromosome 5.[8,9] Risk is related to the total cumulative dose of alkylating agents. In contrast, following treatment with topoisomerase II inhibitors, the time to the development of t-AML is shorter (1-3 years), antecedent MDS is rare, and balanced gene rearrangements involving *MLL* at 11q23 or *RUNX1/AML1* at 21q22 are common.[10,11] Risk is less clearly related to total cumulative dose, but is associated with dosing schedule.[12] Ominously, in some studies up to 12% of patients treated with epipodophyllotoxin-type topoisomerase II inhibitors develop t-AML.[13]

Therapy-induced DNA damage to nonneoplastic cells can result in mutational events that lead to malignant transformation. That only a subset of all patients treated with cytotoxic agents and/or radiation develops t-AML suggests that these individuals may be genetically predisposed toward t-AML. Constitutional genetic variation in components of DNA damage response pathways would be predicted to alter the efficiency by which cells repair promutagenic lesions induced by treatment. Indeed, previous candidate gene studies have implicated variants in a number of these pathways in the development of t-AML, including nucleotide excision repair,[14] mismatch repair,[15-17] recombination repair,[18] NADPH:quinone oxidoreductase,[19] and carcinogen detoxification[20] (reviewed in Seedhouse and Russell[21]).

The p53 tumor suppressor is a transcription factor that mediates cellular responses to DNA damage by regulating cell-cycle arrest, senescence, and apoptosis.[22] The high frequency with which *TP53* is lost or mutated in t-AML suggests that an intact p53 pathway is important in protecting against leukemic transformation following previous therapy and is selected against in the pathogenesis of this cancer.[4,7,9,23,24] If so, then constitutional polymorphic variation in *TP53* and genes encoding other components of this DNA damage

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response pathway may increase the risk of t-AML. A common single nucleotide polymorphism (SNP) in *TP53* at codon 72 that encodes either an arginine (Arg) or a proline (Pro) has been shown to alter the efficiency by which p53 induces apoptosis and suppresses malignant transformation[25,26]; specifically, the Arg72 form of p53 is more effective at inducing apoptosis,[25] whereas the Pro72 form is more effective at inducing cell-cycle arrest.[27] In numerous studies, the codon 72 polymorphism has been implicated in susceptibility to the development of various different cancers, but failure to find consistent associations has made it difficult to draw definitive conclusions.[28-33]

MDM2 is a ubiquitin E3 ligase that negatively regulates the stability of p53.[34] A SNP (SNP309) located in the core promoter of *MDM2* affects binding of the SP1 transcription factor.[35] Specifically, SP1 binds with higher affinity to the G allele relative to the T allele at SNP309, increasing the steady-state levels of MDM2, which in turn reduces the basal levels of p53.[35-37] Lower basal levels of p53 are postulated to affect cellular responses to DNA damage and risk of malignant transformation. Consistent with this model, several recent association studies have implicated *MDM2* SNP309 as a modifier of the age of onset of breast, colorectal, lung, and other cancers (reviewed by Bond and Levine[38]).

Given their roles in mediating cellular responses to DNA-damaging agents, we hypothesized that polymorphic variation in *TP53* and *MDM2* affects the risk of developing t-AML and the time to development of t-AML following therapeutic exposure. In order to test these hypotheses, we examined the *TP53* codon 72 and *MDM2* SNP309 polymorphisms in 2 separate, large, and well-characterized cohorts of patients with t-AML. This study is the largest yet undertaken to uncover genetic predispositions to t-AML. We found that neither SNP by itself is associated with t-AML, but importantly, they interact to modulate t-AML risk.

Table 1. Clinical characteristics of the patients with t-MDS/t-AML in the 2 study groups

	University of Chicago patients, no.	United Kingdom patients, no.
Patients with secondary disease	80	91
Sex, M/F	37/43	38/53
Primary cancer diagnosis	78	51
Hematologic malignancies	40	19
Leukemia	9	4
Hodgkin disease	14	11
Non-Hodgkin lymphoma	16	4
Myeloma	1	0
Solid tumors	38	32
Breast cancer	17	15
Other	21*	17†
Other disease	2‡	6§
Secondary leukemia		
Acute myeloid	62	91
Myelodysplastic syndrome	18	0
Treatment modality		
Radiation therapy only	11	40
Chemotherapy only	32	32
Combined modality therapy	37	19
Cytogenetic subtype		
Chromosome 5, 7, or both	51	22
Translocation/inversion	9	12
Other	10	13
Normal	8	24
No information	2	20
Mean age at t-MDS/t-AML, y (SD)	57.5 (15.8)	55.3 (13.4)

*Includes cancers of the cervix (2), esophagus, head and neck, lung, ovaries (4), prostate (3), rectum, stomach, testis, thyroid (2), and vulva; astrocytoma; a sarcoma; and a primary site unknown.

†Includes cancers of the bladder, cervix (2), colon, lung (2), prostate (2), testis, and uterus (4); basal cell carcinoma; malignant histiocytosis (2); and osteosarcoma.

‡Includes the diagnosis of Crohn disease (2).

§Includes diagnoses of goiter, rheumatoid arthritis, sarcoidosis, systemic vasculitis, retinal vasculitis, and tuberculosis.

Methods

Patients

The patients consisted of 2 groups of persons who developed either AML or MDS following previous chemotherapy and/or radiotherapy for another disorder (Table 1). The first group of patients (the University of Chicago [UC] patients; n = 80) was ascertained in the Section of Hematology/Oncology in the Department of Medicine at the University of Chicago from 1980 to 2002, representing a subset of the patients with t-MDS/t-AML identified in this period. The criterion for inclusion in the study was availability of an Epstein-Barr virus–transformed lymphoblastoid cell line derived from a blood sample for DNA preparation. There were 62 patients with t-AML and 18 patients with t-MDS. For 40 patients, the primary diagnosis was a hematologic malignancy (leukemia or lymphoma). The primary diagnosis for 38 patients was a solid tumor; breast cancer (n = 17) was the single most common cancer site. For the remaining 2 patients, the primary diagnosis was Crohn disease, a nonmalignant condition for which chemotherapeutic treatment had been prescribed. Information on treatment regimen was available for all 80 patients: 11 had radiation treatment only, 32 had chemotherapy only, and 37 had combined modality treatment. Cytogenetic analysis was performed for 78 patients: 51 patients had loss or deletion of chromosomes 5, 7, or both 5 and 7; 9 had a recurring reciprocal translocation [t(3;21), t(8;21), t(11q23), t(15;17)] or inversion [inv(16)]; 10 had other clonal cytogenetic abnormalities; and 8 had a normal karyotype. Many patients had received various chemotherapy agents, often including both alkylating agents and topoisomerase II inhibitors.[7] Date of initiation of treatment for the primary disorder was recorded for 79 of the 80 patients, and the time to development of t-MDS/t-AML (latency) was recorded in months.

The second group of patients (the UK patients; n = 91) was ascertained as previously described.[17] Briefly, 24 patients with t-AML were obtained as part of a United Kingdom population-based case-control study of adult acute leukemia that recruited between 1991 and 1996,[39] and the remaining 67 patients with t-AML were obtained via United Kingdom Medical Research Council clinical trials AML10, 11, and 12, which were recruited between 1988 and 2002. All 91 patients had pathologically confirmed AML; none had t-MDS. Information on the primary diagnosis was available for 57 patients, including 19 patients with hematologic malignancies, 32 patients with solid tumors—of which 15 were breast cancers—and 6 patients with nonmalignant disease. Prior exposure to cytotoxic therapy or radiotherapy, however, was confirmed for all patients. Information on treatment regimen was available for all 91 patients: 40 had radiation treatment only, 32 had chemotherapy only, and 19 had combined modality treatment. Cytogenetic analysis was available for 71 of the 91 patients: 22 had loss or deletion of chromosomes 5, 7, or both 5 and 7; 12 had a recurring reciprocal translocation [t(3;21), t(8;21), t(11q23), t(15;17)] or inversion [inv(16)]; 13 had other clonal cytogenetic abnormalities; and 24 had a normal karyotype. Year of primary diagnosis of cancer was recorded for 61 of the 91 patients. The latency in months was calculated assuming that the primary diagnosis occurred in the middle of the year of diagnosis. United Kingdom patients with de novo AML were obtained as part of a population-based case-control study of adult acute leukemia that recruited in the north and southwest of England between 1991 and 1996.[39]

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Table 2. Tests for associations between t-AML and *MDM2* or *TP53* genotypes

	University of Chicago			United Kingdom			Combined analysis
Genotype*	No. patients (%)	No. controls (%)	OR (95% CI)	No. patients (%)	No. controls (%)	OR (95% CI)	Site-adjusted OR (95% CI)
***MDM2* SNP309**							
TT	31 (40)	958 (42)	1.0 (Ref)	35 (39)	330 (46)	1.0 (Ref)	1.0 (Ref)
TG	34 (44)	1027 (45)	1.02 (0.62-1.68)	40 (45)	303 (42)	1.24 (0.77-2.01)	1.13 (0.80-1.60)
GG	13 (17)	286 (13)	1.40 (0.73-2.72)	14 (16)	88 (12)	1.50 (0.77-2.92)	1.46 (0.91-2.32)
P for trend			.41			.20	.13
***TP53* Arg72Pro**							
Arg/Arg	42 (52)	1255 (56)	1.0 (Ref)	53 (58)	459 (58)	1.0 (Ref)	1.0 (Ref)
Arg/Pro	35 (44)	838 (38)	1.25 (0.79-1.97)	31 (34)	289 (36)	0.93 (0.58-1.48)	1.08 (0.78-1.50)
Pro/Pro	3 (4)	131 (6)	0.68 (0.21-2.24)	7 (8)	50 (6)	1.21 (0.52-2.81)	0.98 (0.50-1.92)
P for trend			.80			.92	.80

Ref indicates reference.

**MDM2* SNP309 and *TP53* Arg72Pro genotypes. The *TP53* G allele encodes arginine and the C allele encodes proline.

Controls

Controls for the UC series came from the Nurses Health Study and the Nurses Health Study 2.[40] These controls consisted of 2392 women who were matched to patients with breast cancer on the basis of age, menopausal status, recent postmenopausal hormone use, and date of blood draw. These controls were used in the present study because the UC patients are drawn from a broad catchment area, and these controls represent a similarly broad sampling of Americans of European descent. United Kingdom controls were matched to patients with AML (described here) and were obtained as part of a population-based case-control study of adult acute leukemia that recruited in the north and southwest of England between 1991 and 1996.[39]

The allele frequencies of both the *TP53* and *MDM2* SNPs were virtually identical between women and men as determined by analysis of controls in the UK study (data not shown). The *TP53* C allele frequency was 0.24 in female and 0.25 in male controls. The *MDM2* G allele frequency was 0.34 in female and 0.33 in male controls. When compared against each other, genotype distributions did not differ between the UC and the UK control cohorts for either polymorphism examined, nor was there evidence for an interaction between the variants in one control cohort but not the other. Thus, it is unlikely that the 2 control cohorts differ significantly from each other, or that the use of either introduced an unanticipated bias into the analysis.

DNA extraction

Genomic DNA was extracted from lymphoblastoid cell lines (UC series) or from peripheral blood (UK series) using Qiagen QIAamp DNA mini kit (Qiagen, Valencia, CA). The samples were then quantified using the Pico Green dsDNA Quantitation kit per the manufacturer's instructions (Molecular Probes, Eugene, OR).

SNP genotyping

Allele frequencies were determined by allele-specific polymerase chain reaction (PCR) using either the 5′ nuclease allelic discrimination assay (Taqman; Applied Biosystems, Foster City, CA) or restriction fragment–length polymorphism (RFLP) analysis. For 5′ nuclease assays, primer and probe sequences were designed using Primer Express v.2 software (ABI PRISM; Applied Biosystems). They were manufactured as Assays-by-Design (Applied Biosystems), and performed according to the manufacturer's specifications. In brief, 10-μL reactions were set up in 96-well plates with 2 μL amplified template genomic DNA, and cycled under standard conditions: 50°C for 2 minutes, then a denaturation step at 95°C for 10 minutes, followed by 40 cycles of 92°C for 30 seconds, and 60°C for 1 minute. Endpoint reads were conducted on the ABI 7300 sequence detection system. Cluster analysis was conducted on the scatter plot of allele A versus allele B. Genotypic segregation was determined and displayed in the allelic plot with 4 clusters: no amplification (genotype not assigned), allele A, allele B, and heterozygous. These data were then exported for further analysis. For the *MDM2* SNP309, the amplification primers used were 5′-CGGGAGTTCAGGGTAAAGGT and 5′-GCGCAGCGTTCACACTAG. The T allele–specific probe was 5′-VIC-CTCCCGCGCCGAAG-TAM. The G allele–specific probe was 5′-FAM-TCCCGCGCCGCAG-TAM. For the p53 codon 72 SNP, the amplification primers used were 5′-ATGAAGCTCCCAGAATGC and 5′-GCCGGTGTAGGAGCT. The G allele–specific probe was 5′-FAM-CTGCTCCCCCCGTGGCCC-TAM. The C allele–specific probe was 5′-VIC-CTGCTCCCCGCGTGGCCC-TAM. The G allele encodes the arginine residue and the C allele the proline residue. For UK patients with t-AML, patients with de novo AML, and controls, *TP53* codon 72 status was determined using the RFLP assay, as previously described.[41] Taqman and RFLP assay accuracy was determined by direct sequencing of 30 independent samples. Concordance between assays was 100%.

Statistical analysis

The Hardy-Weinberg equilibrium of *MDM2* SNP309 and *TP53* codon 72 loci was tested separately in the patients and controls of the UC and UK study groups using the chi-square test. No deviation from Hardy-Weinberg equilibrium was detected in any group (all $P > .05$). Logistic regression models were used to examine whether the *MDM2* SNP309 and *TP53* codon 72 polymorphism were associated with leukemia. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using logistic regression. We also fit a logistic regression model with the genotype modeled as an ordinal variable, with levels 0, 1, and 2 representing the number of copies of the minor allele. This model is equivalent to the Cochran-Armitage trend test. We tested for interaction between the *MDM2* SNP309 and *TP53* codon 72 polymorphisms in the framework of a logistic regression model. Because the *MDM2* TT genotype results in the highest baseline levels of p53 expression, we first stratified the patients and controls by *MDM2* genotype (TT vs TG and GG), and then we compared the *TP53* genotypes based on the presence or absence of the C allele, which encodes a proline at residue 72 (GG vs CC and CG). The association analyses were conducted first for UC and UK study groups separately, followed by combined analysis adjusted for study site.

To test for association between each SNP and latency, we stratified the patients by genotype and compared the distribution of latency times in each group using the Cuzick nonparametric test for trend.[42] Latencies were recorded in months for the UC patient group and, where known, in years in the UK patient group, which we converted into months (see "Patients"). Association between latency and each SNP was tested in each study group separately, in the combined study group, and stratified by sex. Because the UC study group contained patients with t-MDS as well as t-AML, we conducted the analysis with and without the patients with t-MDS. Association between latency and cytogenetic abnormality was analyzed by the Kruskal-Wallis test. Association between SNP genotype and treatment for the primary cancer stratified by radiation, chemotherapy, and combined modality therapy was analyzed by polytomous logistic regression. Association between each SNP and abnormalities of chromosomes 5 or 7 was analyzed in the entire study group by logistic regression.

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Table 3. Interactive effect of *MDM2* and *TP53* genotypes

	University of Chicago			United Kingdom			Combined analysis
Effect	No. patients (%)	No. controls (%)	OR (95% CI)	No. patients (%)	No. controls (%)	OR (95% CI)	Site-adjusted OR (95% CI)
On development of t-AML (*MDM2 TP53* genotype*)							
MDM2 TT *TP53* Pro/Pro, Arg/Pro	9 (12)	406 (19)	1.0 (Ref)	11 (12)	134 (19)	1.0 (Ref)	1.0 (Ref)
MDM2 TT *TP53* Arg/Arg	22 (28)	488 (23)	2.03 (0.93-4.47)	24 (27)	186 (26)	1.57 (0.74-3.32)	1.78 (1.03-3.06)
MDM2 TG, GG *TP53* Pro/Pro, Arg/Pro	27 (35)	519 (25)	2.35 (1.09-5.05)	25 (28)	172 (24)	1.77 (0.84-3.73)	2.04 (1.20-3.48)
MDM2 TG, GG *TP53* Arg/Arg	20 (26)	705 (33)	1.28 (0.58-2.84)	29 (33)	211 (30)	1.67 (0.81-3.46)	1.48 (0.86-2.52)
P for interaction†			.009			.29	.009
On development of t-AML with any prior chemotherapy (*MDM2 TP53* genotype*)							
MDM2 TT *TP53* Pro/Pro, Arg/Pro	8 (12)	406 (19)	1.0 (Ref)	5 (10)	134 (19)	1.0 (Ref)	1.0 (Ref)
MDM2 TT *TP53* Arg/Arg	19 (28)	488 (23)	1.98 (0.86-4.56)	15 (30)	186 (26)	2.16 (0.77-6.09)	2.04 (1.07-3.92)
MDM2 TG, GG *TP53* Pro/Pro, Arg/Pro	22 (32)	519 (25)	2.15 (0.95-4.88)	12 (24)	172 (24)	1.87 (0.64-5.44)	2.05 (1.07-3.92)
MDM2 TG, GG *TP53* Arg/Arg	19 (28)	705 (33)	1.37 (0.59-3.15)	18 (36)	211 (30)	2.29 (0.83-6.30)	1.70 (0.89-3.23)
P for interaction†			.033			.38	.029
On development of t-AML with abnormalities of chromosome 5 and/or 7 (*MDM2 TP53* genotype*)							
MDM2 TT *TP53* Pro/Pro, Arg/Pro	6 (12)	406 (19)	1.0 (Ref)	3 (13)	134 (19)	1.0 (Ref)	1.0 (Ref)
MDM2 TT *TP53* Arg/Arg	13 (26)	488 (23)	1.80 (0.68-4.78)	6 (27)	186 (26)	1.44 (0.35-5.86)	1.68 (0.75-3.74)
MDM2 TG, GG *TP53* Pro/Pro, Arg/Pro	16 (32)	519 (25)	2.09 (0.81-5.38)	11 (50)	172 (24)	2.86 (0.78-10.44)	2.34 (1.09-5.02)
MDM2 TG, GG *TP53* Arg/Arg	19 (30)	705 (33)	1.44 (0.55-3.74)	2 (9)	211 (30)	0.42 (0.07-2.57)	1.12 (0.50-2.53)
P for interaction†			.12			.03	.015

Ref indicates reference.

**MDM2* SNP309 and *TP53* Arg72Pro genotypes. The *TP53* G allele encodes arginine and the C allele encodes proline.

†*P* values were calculated by logistic regression.

Results

Interaction between MDM2 SNP309 and TP53 codon 72 polymorphisms in t-AML

To determine whether the *MDM2* SNP309 and *TP53* codon 72 polymorphisms are associated with risk of t-AML, we compared the genotype frequencies of these SNPs in the 80 UC patients with t-AML and 2377 successfully genotyped controls from the Nurses Health Studies, in the 91 UK patients with t-AML and 816 UK controls, and in the 2 cohorts combined (Table 2). The genotype frequencies of the *MDM2* and the *TP53* polymorphisms were not statistically different in cases versus controls in any of these 3 analyses (Table 2). In testing for an interaction between the *MDM2* and *TP53* polymorphisms, however, we observed that in *MDM2* TT homozygotes there was a higher frequency of *TP53* GG (Arg/Arg) homozygotes relative to *TP53* Arg/Pro and Pro/Pro genotypes in patients with t-AML compared with controls (combined OR = 1.78, CI 1.03-3.06; Table 3). We also observed a higher frequency of carriers of *MDM2* SNP309 G alleles and *TP53* codon 72 Pro alleles in patients compared with controls (combined OR = 2.04, CI 1.20-3.48). The *MDM2-TP53* interactive effect was significant in the combined analysis (*P* value for interaction, .009). The interaction was observed in both the UC and UK case-control series as evidenced by the increased ORs in both cohorts, and achieved statistical significance in the UC series ($P = .009$). We conclude that whereas neither polymorphism on its own influences the development of t-AML, an interactive effect between the variants is observed that influences t-AML susceptibility. These data are consistent with 2 models, one in which individuals with either the *MDM2* TT *TP53* Arg/Arg doubly homozygous genotype or at least one *MDM2* SNP309 G allele and one *TP53* codon 72 Pro allele are at increased risk of developing t-AML, and the other in which the *TP53* Pro variant is protective against t-AML in individuals with the *MDM2* TT genotype.

MDM2 and TP53 genotypes and cancer treatment

We considered the possibility that the risk of t-AML might be modified by *MDM2* or *TP53* genotypes or an interaction between the 2 based on prior treatment. Consequently, we examined the distribution of *MDM2* and *TP53* genotypes stratified by treatment modalities (radiation only, chemotherapy only, and combined modality therapy). Because many patients had been previously treated with multiple agents, we were unable to stratify patients further by treatment for analysis (eg, alkylating agents only, topoisomerase-II inhibitors only). Likewise, because the number of patients is small, we tested for effects of the *MDM2* and *TP53* genotypes as a function of prior therapy in a combined analysis of all UC plus UK patients. An association between *MDM2* genotype and prior therapy was not observed. There was, however, a significant association between *TP53* Pro/Pro homozygotes and risk of t-AML following radiation treatment ($P = .04$); the OR for this effect was 2.71 (Table 4). There was no association between *TP53* genotype and either chemotherapy alone or combined modality therapy.

To test for an interactive effect between *MDM2* and *TP53* genotypes and prior therapy, we divided patients into those who had previously been exposed to any radiotherapy (n = 105), and those who had previously been exposed to any chemotherapy (n = 118). We observed a significant interaction between *MDM2* and *TP53* genotypes in the any chemotherapy group but not in the any radiotherapy group (Table 3 and Table S1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article). This effect was identical to that observed in the entire cohort, and it was found in both the UC and UK cohorts, as evidenced by their similarly trending ORs for all genotype combinations. The *MDM2-TP53* interactive

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Table 4. Tests for associations between *MDM2* or *TP53* genotypes and t-AML stratified by treatment modality

	University of Chicago, no. (%)				United Kingdom, no (%)				Combined analysis: site-adjusted OR (95% CI)		
Genotype*	RT	CT	CMT	Control	RT	CT	CMT	Control	RT vs control	CT vs control	CMT vs control
***MDM2* SNP309**											
TT	4 (40)	13 (42)	14 (38)	958 (42)	15 (38)	11 (35)	9 (47)	330 (46)	1.0	1.0	1.0
TG	5 (50)	12 (39)	17 (46)	1027 (45)	18 (46)	15 (48)	7 (37)	303 (42)	1.27 (0.68-2.36)	1.13 (0.65-1.98)	1.03 (0.58-1.83)
GG	1 (10)	6 (19)	6 (16)	286 (13)	6 (15)	5 (16)	3 (16)	88 (12)	1.34 (0.56-3.27)	1.62 (0.79-3.36)	1.36 (0.62-2.97)
P for trend†									.41	.23	.52
***TP53* Arg72Pro**											
Arg/Arg	4 (36)	17 (53)	21 (57)	1255 (56)	20 (50)	22 (69)	11 (58)	459 (58)	1.0	1.0	1.0
Arg/Pro	6 (55)	14 (44)	15 (41)	838 (38)	14 (35)	9 (28)	8 (42)	289 (36)	1.32 (0.72-2.42)	0.91 (0.54-1.54)	1.10 (0.64-1.89)
Pro/Pro	1 (9)	1 (3)	1 (3)	131 (6)	6 (15)	1 (3)	0 (0)	50 (6)	2.71 (1.13-6.50)	0.48 (0.12-2.02)	0.30 (0.04-2.17)
P for trend†									.041	.38	.58

**MDM2* SNP309 and *TP53* Arg72Pro genotypes. The *TP53* G allele encodes arginine and the C allele encodes proline.
†*P* values were calculated by polytomous logistic regression.

effect was significant in the combined analysis (P value for interaction, .03) and also achieved statistical significance in the UC series ($P = .03$).

Specific cytogenetic abnormalities associated with t-AML, such as loss or deletion of chromosomes 5 and/or 7, are associated with prior alkylating agent treatment, whereas others, such as recurring translocations involving t(3;21), t(8;21), 11q23, t(15;17), and inv(16), are associated with topoisomerase II inhibitors. As expected, patients with t-AML with abnormalities of chromosomes 5 and/or 7 were more common among those treated with chemotherapy or combined modality therapy, as compared with those treated with radiation therapy alone (data not shown), and they had a longer latency to t-AML compared with patients with translocations and inversions (Table S2). We tested for associations between *MDM2* and *TP53* genotypes and abnormalities of chromosome 5 and/or 7. Other abnormalities were not examined due to small sample sizes. We did not find any evidence for an association between *MDM2* and *TP53* genotypes and abnormalities of chromosome 5 or 7 when analyzed individually (Table S3); however, an interactive effect was observed in patients with these chromosomal abnormalities that again mirrored the effect identified in the entire cohort (Table 3; P value for interaction, .02). Taken together, these data demonstrate that whereas neither variant alone is associated with altered risk, an interactive effect is observed between the *MDM2* SNP309 and the *TP53* codon 72 polymorphism that influences t-AML susceptibility in patients previously treated with chemotherapy, and in patients with abnormalities of chromosomes 5 and/or 7, acquired abnormalities associated with prior exposure to alkylating chemotherapy. Two models may explain these observations, one in which the *MDM2* TT *TP53* Arg/Arg doubly homozygous genotype and genotypes with at least one *MDM2* SNP309 G allele and one *TP53* codon 72 Pro allele are associated with an increased risk for t-AML in these patients, and the other in which the *TP53* Pro variant is protective against t-AML in patients with the *MDM2* TT genotype who were treated with chemotherapy or who developed abnormalities of chromosomes 5 and/or 7.

MDM2 and TP53 genotypes and risk of de novo AML

We hypothesized that genetic variation in *MDM2* and *TP53* may also be associated with a predisposition to de novo AML. We tested for associations between *MDM2* and *TP53* genotypes and de novo AML in a series of 404 patients with de novo AML and 816 age- and sex-matched controls from the United Kingdom, and we also looked for possible interactions between *MDM2* and *TP53*. The presence of an *MDM2* SNP309 G allele was associated with a modest increase in the risk of de novo AML ($P = .10$; Table S4). *TP53* genotypes were not associated with risk of de novo AML, and no interactive effect was observed between *MDM2* and *TP53*. These data suggest that the interactive effect seen between *MDM2* and *TP53* is specific to prior therapy and risk of t-AML.

MDM2 SNP309 G allele and latency

The *MDM2* SNP309 G allele has been associated with a younger age of onset in breast, colorectal, and lung cancers,[38] and in each case the largest effect was observed in GG homozygotes. Consequently, we examined the association between *MDM2* genotype and time from cancer therapy exposure to the development of t-AML. In the UC patients with t-AML, the median latency to t-AML for the *MDM2* GG genotype was 32 months, whereas the median latency of the *MDM2* TG and TT genotypes were 72 and 65 months, respectively. These differences approached significance by the Cuzick nonparametric test for trend ($P = .06$; Table 5). The effect of *MDM2* genotype on latency was similar if we excluded the 18 patients who presented with t-MDS ($P = .05$; data not shown). In the UK patients with t-AML, the median latency for the *MDM2* GG genotype was 70 months, and for the *MDM2* TG and TT genotypes it was 71 and 72 months, respectively. In the combined analysis, the median latency for the *MDM2* GG genotype was 44 months, and for the *MDM2* TG and TT genotypes it was 72 and

Table 5. Distributions of latencies of patients with t-AML stratified by *MDM2* genotype

		Latency, mo		
Genotype	No. of patients	Median	IQR	*P*
UC patients with t-AML				.063
TT	31	65	45-125	
TG	33	72	25-105	
GG	13	32	22-63	
Total	77	63	34-114	
UK patients with t-AML				.94
TT	24	71	21-149	
TG	28	71	28-129	
GG	7	70	38-122	
Total	59	70	27-139	
Combined patients with t-AML				.21
TT	55	65	35-139	
TG	61	72	27-119	
GG	20	44	29-115	
Total	136	64	31-124	

IQR indicates interquartile range.

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65 months, respectively, but the difference was not significant ($P = .21$). We also assessed the contribution of *TP53* genotypes to latency in both the UC and UK series and found no effect in either cohort ($P > .80$; data not shown).

Recently, it was reported that the association between the *MDM2* SNP309 G allele and accelerated cancer development was observed specifically in premenopausal females.[36] Consequently, we examined the relationship between latency and *MDM2* genotypes in women and men separately and stratified by age in the combined UC and UK cohorts. The median latency to t-AML in women with the *MDM2* GG genotype was 41 months, whereas the median latency for women with the *MDM2* GT and TT genotypes was 66 and 79 months, respectively. In contrast, the median latency to t-AML in men with the *MDM2* GG genotype was 47 months, and 84 and 56 months for men with the GT and TT genotypes (Table 6). There was a trend toward a significant correlation between the number of *MDM2* SNP309 G alleles and latency in women ($P = .11$ by the Cuzick nonparametric test for trend) but not in men ($P = .96$), suggesting that the dosage of *MDM2* SNP309 G alleles may have a stronger effect on accelerating t-AML development in women compared with men. However, formal testing for interaction between sex and *MDM2* effect on latency using quartile regression could not rule out the possibility that the observed differential effects are due to chance ($P = .45$). Sex-specific latency to t-AML was not associated with the *TP53* genotype ($P > .83$; data not shown). Sex alone was not associated with study site, *MDM2* genotypes, or *TP53* genotypes.

We also observed a trend toward association with respect to *MDM2* genotype and age at which the prior therapy was administered. For patients with a primary diagnosis at younger than 50 years old, the median latency of the *MDM2* TT genotype was 118 months, whereas the median latency of the TG and GG genotypes was 84 and 66 months, respectively ($P = .07$; Table 7). We did not observe a significant difference in latency with respect to *MDM2* genotype for patients aged 50 years and older. Taken together, these sex and age data are consistent with a hypothesis that the *MDM2* TT genotype is protective against cancer in younger women.[38]

Discussion

The development of t-AML is driven by DNA damage induced by the cytotoxic treatment of a primary condition, often a malignant disease. This damage engages response pathways in hematopoietic stem and progenitor cells, leading to DNA repair or to cell death.

Table 6. Distributions of latencies of patients with t-AML in the combined UC and UK series stratified by *MDM2* genotype and sex

Genotype	No. of patients	Latency, mo		P
		Median	IQR	
Women				.11
TT	34	79	34-139	
TG	34	66	25-96	
GG	11	41	22-108	
Total	79	64	28-129	
Men				.96
TT	21	56	38-118	
TG	27	84	36-120	
GG	9	47	31-122	
Total	57	59	36-120	

IQR indicates interquartile range.

Cells that survive with acquired mutations due to incorrect repair are at risk for leukemic transformation. Genetic variation in pathways that mediate cellular responses to DNA damage can affect the risk of developing t-AML, presumably by influencing the chances that hematopoietic cells survive with leukemogenic mutations. The p53 pathway is a critical regulator of the choice between the processes of repair and survival versus apoptosis; hence, we studied 2 functionally important polymorphisms in this pathway—the *MDM2* SNP309 and *TP53* codon 72 polymorphisms—to determine the relationship between these polymorphisms and the development of t-AML, as well as the relationship between these SNPs and clinical variables such as the latency interval to t-AML and the risk of t-AML stratified by treatment modality. Using the resources of 2 large and well-characterized cohorts of patients with t-AML, we were able to undertake the largest study to date on genetic predisposition to t-AML.

In both the UC and UK patient series, neither the *MDM2* SNP309 nor the *TP53* codon 72 polymorphism alone was associated with the development of t-AML. We detected, however, an interactive effect between the 2 variants (Table 3; *P* value for interaction, .009), whereby *MDM2* TT *TP53* Arg/Arg double homozygotes exhibited an approximately 1.8-fold greater risk of t-AML relative to individuals with the *MDM2* TT *TP53* Arg/Pro and Pro/Pro genotypes. Similarly, individuals with an *MDM2* SNP309 G allele who were *TP53* codon 72 Pro allele carriers exhibited an approximately 2-fold greater risk of t-AML.

Loss of p53 in t-AML is associated with prior alkylating therapy. We hypothesized that the interactive effect between MDM2 and TP53 would be most apparent in patients previously treated with alkylating agents, but because many patients with t-AML had been exposed to multiple cytotoxic agents, we were unable to assess this directly. Thus, we reasoned instead that the interactive effect between *MDM2* and *TP53* would be evident in patients previously treated with chemotherapy. Indeed, this interactive effect was observed in these patients but not in patients previously exposed to radiotherapy (Table 3; *P* value for interaction, .03). Furthermore, because abnormalities of chromosomes 5 and/or 7 are associated with prior alkylating therapy, we tested for this interactive effect in patients whose t-AML exhibited abnormalities of chromosomes 5 and/or 7. Again, this interactive effect was observed (Table 3; *P* value for interaction, .02). These data indicate that the interaction between MDM2 and TP53 affects t-AML risk only in patients previously treated with chemotherapy. Although our findings are consistent with the hypothesis that this interaction affects t-AML risk in patients exposed to alkylating agents, it is not possible to determine from this analysis whether this effect is driven by a single agent or group of agents. We noted that a smaller fraction of patients were treated with chemotherapy or exhibited abnormalities of chromosomes 5 and/or 7 in the UK series compared with the UC series (50 of 91 vs 68 of 80 and 22 of 91 vs 51 of 80, respectively). Therefore, a possible explanation for why the *MDM2-TP53* interaction was less prominent in the entire UK series than in the entire UC series is that fewer patients were exposed to chemotherapy (Table 1).

Few studies have addressed the possibility that the *MDM2* SNP309 and *TP53* codon 72 polymorphisms might interact to affect cancer risk. Bougeard et al found that carrier status for the *MDM2* SNP309 G allele, when coinherited with the *TP53* codon 72 Arg allele, was associated with rapid cancer development in carriers of germline *TP53* mutations.[43] Hong et al observed that

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patients with the *MDM2* GG, *TP53* Pro/Pro genotype had an increased risk of esophageal squamous cell carcinoma compared with other genotype groups.[44] Cox et al noted evidence for an interaction in patients with breast cancer in the Nurses Health Studies, but found no consistent direction of interaction.[40] Our results differ from these studies in that the Arg72 form of p53 in *MDM2* TT homozygotes and the Pro72 form of p53 in *MDM2* SNP309 G allele carriers were both associated with greater risk of t-AML.

Biochemical studies have suggested that the Arg72 form of p53 is more effective at signaling apoptosis than the Pro72 form.[25] Moreover, the Arg72 form of p53 has a higher affinity for the MDM2 protein compared with the p53 Pro form.[25] Thus, we theorize that in individuals with lower levels of MDM2 protein (the TT genotype), the Arg72 form of p53 is degraded more efficiently than the Pro72 form, leading to less effective p53-mediated tumor suppression in individuals with the *TP53* Arg/Arg genotype. In contrast, in individuals with higher levels of MDM2 (the GT and GG genotypes), differences in the degradation of the 2 isoforms may be attenuated, and it is the Pro72 form that leads to less effective p53-mediated tumor suppression, because it is less efficient at apoptosis. In both cases, t-AML susceptibility is increased because the likelihood that hematopoietic stem cells will survive with acquired proleukemogenic mutations is increased.

Alternatively, these data are also consistent with a model in which the Pro form of p53 is protective against t-AML in individuals with lower levels of the MDM2 protein (the TT genotype). Indeed, it has recently been shown that in response to some forms of DNA damage such as hypoxia, the Pro form of p53 has an enhanced apoptotic potential compared with the Arg form.[45] Furthermore, the *TP53* codon 72 Arg allele is preferentially retained in a variety of human cancers, including squamous cell carcinoma,[46,47] hepatocellular carcinoma,[48] and breast cancer,[49] suggesting that in some situations, it is permissive for cancer, whereas the Pro allele is protective against cancer.

We hypothesized an effect of *MDM2* genotype on latency between treatment and onset of t-AML, because MDM2 regulates p53 levels such that GG homozygotes have lower basal levels of p53 than TT homozygotes. After chemotherapy or radiation treatment, lower levels of p53 could promote survival of mutated preleukemic cell clones. In the UC series, the *MDM2* GG genotype was associated with a shorter latency period to the development of t-AML than the TT genotype, with an almost 47-month difference in median times to cancer development (Table 5). In the combined series, there was a trend toward accelerated t-AML development in *MDM2* GG women and not in *MDM2* GG men (Table 6), and persons with the *MDM2* TT genotype who received their prior chemotherapy before age 50 years had longer latency intervals (Table 7), as has been reported for other cancer types.

We also hypothesized that *MDM2* or *TP53* genotypes might modify the risk of t-AML as a function of treatment modality. Whereas *MDM2* SNP309 genotype was not associated with disease when stratified by treatment modality, we found that *TP53* Pro/Pro homozygous individuals were at increased risk for t-AML if they received radiation treatment as the sole treatment modality. Ionizing radiation induces DNA double-strand breaks and activates the p53-mediated apoptosis pathway through the ATM kinase, whereas alkylating chemotherapy leads to p53 activation through the ATR kinase.[50] Hence, we can theorize that the association of the *TP53* codon 72 Pro allele with t-AML in response to radiation may be due to less efficient radiation-induced apoptosis in Pro/Pro homozygotes and, consequently, increased survival of clones with acquired proleukemogenic mutations.

Table 7. Distributions of latencies of patients with t-AML in the combined UC and UK series stratified by *MDM2* genotype and age at primary diagnosis

Genotype	No. of patients	Latency, mo		*P*
		Median	IQR	
Younger than 50 y				.065
TT	26	118	62-197	
TG	33	84	34-162	
GG	10	66	27-122	
Aged 50 y and older				.89
TT	29	47	29-82	
TG	28	71	24-92	
GG	10	40	30-63	

IQR indicates interquartile range.

The observation that the Pro allele is associated with t-AML induced by radiation but is not associated with t-AML induced by prior chemotherapy underscores the importance of context in assessing the contribution of specific genetic variants to cancer risk. Different cytotoxic therapies introduce different DNA lesions, which induce different cellular response pathways. In different cell types and under certain environmental conditions, such as hypoxia, even these responses may differ. Thus, it is difficult to predict associations between variants and cancer risk. Furthermore, sporadically occurring cancer arises as a consequence of chronic low-dose exposure to oncogenic stresses, whereas t-AML arises as a consequence of acute relatively high-dose exposures. Hence, while there is certainly some overlap in susceptibilities between sporadic disease and t-AML, caution must be exercised when extrapolating from one condition to the other because of differences in the length, timing, and intensity of these exposures.

As the number of long-term cancer survivors increases, so too does the need for genetic markers that can be used to identify those at greatest risk of t-AML. Here, we demonstrate an interaction between the *TP53* codon 72 and the *MDM2* SNP309 polymorphism that is associated with an increased risk of t-AML. This finding underscores the importance of the p53 pathway and, in particular, the potential contribution of p53-mediated apoptosis, to the defense against t-AML. These observations illustrate 3 important points. (1) Because t-AML is a rare, albeit usually lethal, consequence of antecedent therapy with DNA-damaging agents, most association studies of t-AML have been undertaken in small cohorts of patients treated at a single institution. To detect subtle or synergistic effects of genetic variation in t-AML, it is essential to test and validate findings in large combined datasets. (2) More patients in the UC cohort were exposed to prior chemotherapy than in the UK cohort, and more patients in the UK cohort were treated with radiation alone than in the UC cohort. Differences in treatment for primary malignancies may provide an explanation for why an association identified in one cohort is less pronounced in another. (3) Finally, the coinheritance of multiple low-penetrance risk alleles can cooperate to increase disease risk even when no single variant on its own exhibits a statistical association. Thus, hypothesis-driven biological plausibility is an important component of candidate gene studies to detect gene-by-gene interactions, and remains an important consideration even in an era of

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genome-wide "hypothesis-free" association testing. With the effect sizes observed in the present study, it is probably premature to use *MDM2* and *TP52* genotype information to influence treatment decisions; however, with further genetic data, it may be possible to identify individuals with much higher risks for whom treatment or surveillance could be altered.

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Authorship

Contribution: N.A.E. analyzed and interpreted the data and wrote the paper; D.H. performed statistical analysis; O.Y. designed and performed research; L.J.W. performed research; M.B. generated lymphoblastoid cell lines; M.M.L. analyzed data, contributed vital new reagents, and drafted the manuscript; R.A.L. analyzed data and contributed vital new reagents; J.M.A. performed research and analyzed data; and K.O. designed the research, analyzed and interpreted the data, and wrote the paper. All authors reviewed and approved the final manuscript.

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The new affiliation for J.M.A. is Northern Institute for Cancer Research, Newcastle University, Framlington Place, Newcastle upon Tyne, United Kingdom.

Correspondence: Kenan Onel, Section of Hematology/Oncology, Department of Pediatrics, University of Chicago, 5841 South Maryland Avenue, C-425, MC 4060, Chicago, IL 60637; e-mail: konel@uchicago.edu.

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