RESEARCH ARTICLE

Prognostic Significance of *TP53* Mutations and Single Nucleotide Polymorphisms in Acute Myeloid Leukemia: A case Series and Literature Review

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Abstract

Background: The response to treatment and overall survival (OS) of patients with acute myeloid leukemia (AML) is variable, with a median ranging from 6 months to 11.5 years. *TP53* is associated with old age, chemotherapy resistance, and worse OS. Using genetic sequencing, we set out to look at our own experience with AML, and hypothesized that both *TP53* mutations and SNPs at codon 72 would mimic the literature by occurring in a minority of patients, and conferring a worse OS. Materials and Methods: We performed a pilot study of randomly selected, newly diagnosed AML patients at Mount Sinai Medical Center, diagnosed from 2005-2008 (n=10). *TP53* PCR sequencing was performed using DNA from bone marrow smears. Analysis was accomplished using Mutation Surveyor software with confirmation of the variants using the COSMIC and dbSNP databases. Results: Fewer than half of the patients harbored *TP53* mutations (40%). There was no significant difference in OS based on gender, AML history, risk-stratified karyotype, or *TP53* mutation. There were possible trends toward improved survival among patients less than 60 (11 *vs* 4 months, p=0.09), Hispanics (8 *vs* 1 months, p=0.11), and those not harboring SNP P72R (8 *vs* 2 months, p=0.10). There was a significant improvement in survival among patients with better performance status (28 *vs* 4 months, p=0.01) and those who did not have a complex karyotype (8 *vs* 1 months, p=0.03). The most commonly observed *TP53* mutation was a missense N310K (40%) and the most commonly observed SNP was P72R (100.0%). Conclusions: Our study confirms previous reports that poor PS and the presence of a complex karyotype are associated with a decreased OS. In our cohort, *TP53* mutations were relatively common, occurring more frequently in male patients with an adverse karyotype. Although there was no significant difference in survival between *TP53* mutated and un-mutated patients, there was a possible trend toward worse OS among patients with SNP P72R. Larger studies are needed to validate these findings.

Keywords: Acute myeloid leukemia - *TP53* - SNP P72R - survival

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Introduction

With an incidence of 3 to 5 per 100,000, acute myeloid leukemia (AML) is the most commonly occurring acute leukemia in adults in the United States (Yamamoto and Goodman, 2008; Sant et al., 2010; Smith et al., 2011; Dores et al., 2012; Siegal et al., 2013). The response to treatment and overall survival (OS) of patients is variable, with a median OS ranging from a few months to over 10 years (Mrózek et al., 2012). Age at diagnosis and patient performance status (PS) has long been established in prognostication. Patients older than age 60 and those with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of greater than 2 have been shown to have inferior outcomes compared with their younger, healthier counterparts (Estey, 2001; Stone, 2002; Appelbaum et al., 2006). Over the past decade, karyotype expression has been established as a predictor of outcome and patients are generally classified under four risk-stratified groups: favorable, normal, intermediate, or adverse karyotype (Grimwade et al., 1998). Due to the fact that over 60% of patients have normal karyotypes, for which there is much inter-group variability, genetic sequencing has begun to take on an important role in prognostication (Summers et al., 2007; Paschka et al. 2010; Green et al., 2010; Zeichner, 2012).

Tumor protein 53 (*TP53*), a tumor suppressor gene located on the short arm of chromosome 17, mediates DNA damage induced cell cycle arrest and apoptosis (Vogelstein et al., 2000). *TP53* mutations are one of the most frequent genetic abnormalities in human cancer and are one of the more promising prognostic markers for AML. Studies

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have shown that *TP53* mutations are present in 5-25% of all AML patients, in 70% of those with complex karyotypes (Rücker et al., 2012), and are associated with old age, chemotherapy resistance, AML secondary to myelodysplastic syndrome/myeloproliferative neoplasms (MDS/MPN), adverse complex karyotypes (CK), and worse OS (Wattel et al., 1994; Nahi et al., 1998; Padua et al., 1998; Nakano et al., 2000; Christiansen et al., 2001; Stirewalt et al., 2001; Melo et al., 2002; Breems et al., 2008; Seifert et al., 2009; Cavalcanti et al., 2010; Harutyunyan et al., 2011; Jädersten et al., 2011; Tavor et al., 2011; Kanagal-Shamanna et al., 2012; Milosevic et al., 2012; Valcárcel et al., 2013). Single nucleotide polymorphisms (SNPs), changes in DNA seen in an appreciable amount of the population, have been examined in AML. The SNP at codon 72, one of the most common SNPs in human cancer, encodes arginine (Arg) or proline (Pro) in the hydrophobic midsection of the *TP53*. The SNP has been shown to affect patient outcomes by altering the efficiency by which p53 induces apoptosis and suppresses malignant transformation (Thomas et al., 1999; Dumont et al., 2003; Pim et al., 2004; Ellis et al., 2008; Dunna et al., 2012). Using genetic sequencing, we set out to look at our own experience with AML, and hypothesized that both *TP53* mutations and the SNP at codon 72 would mimic the literature by occurring in a minority of patients, and conferring a worse OS.

Materials and Methods

Patients

After obtaining approval by the institutional review board, we performed a retrospective pilot study of randomly selected, newly diagnosed AML patients at Mount Sinai Medical Center (MSMC), diagnosed from 2005-2008 (n=10; Table 1). All patients with AML were included in this study. There were no exclusions. All patient information was obtained from the paper and electronic medical records. Patient ages ranged from 52-89 (median 78 years).

Diagnosis

Immunohistochemical (IHC) analysis of bone marrows and peripheral blood smears was assessed via DO-1 antibody on paraffin embedded tissue. Patients were diagnosed with AML if they had greater than 20% blasts in their bone marrow aspirate (from a 500 cell differential count) and had leukemic cells of myeloid origin as demonstrated by either the presence of Auer rods, cytochemical positivity for myeloperoxidase, or presence of sufficient myeloid markers recognized by immunophenotyping. The diagnosis of AML was based on both the previously accepted French, American, and British (FAB) system (Bennett et al., 1985) and the newer World Health Organization (WHO) classification system (Vardiman et al., 2009). Conventional cytogenetic analysis was performed on short-term cultured bone marrow and peripheral blood cells with the use of the Giemsa (G-banding) technique. Karyotypes were described according to the recommendations of the International System for Human Cytogenetic Nomenclature (ICSN). Patients with three or more numerical and/or structural chromosomal abnormalities were defined as complex karyotype (CK). Risk stratified karyotypes were defined according to the classifications created by the Medical Research Council (MRC), the Southwest Oncology Group/Eastern Cooperative Oncology Group (SWOG/ECOG), and the Cancer and Leukemia Group B (CALGB; Grimwade et al., 1998; Wheatley et al., 1999; Grimwade et al., 2010).

Treatment

When feasible, patients younger than age 60 received treatment with a combination of an anthracycline, such as daunorubicin, for three days and "standard" dose cytarabine for seven days. Initial response to treatment was evaluated 7 to 10 days after completion of induction chemotherapy with a unilateral bone marrow aspirate and biopsy. For patients older than age 60, treatment decisions were individualized and took into account the age, PS, and cytogenetics. For those with severe comorbidities, poor physical function, or unfavorable risk disease, we used supportive care alone or less intensive chemotherapy rather than induction chemotherapy. If residual leukemic blasts (greater than 5 to 10 percent of the marrow volume) were found on the bone marrow aspirate, a second cycle of induction was given. Patients receiving induction therapy had daily laboratory testing, which included a complete blood count and chemistries. Patients were transfused packed red blood cells for symptomatic anemia or a hemoglobin ≤7 to 8 g/dL. All patients received prophylactic antiviral, antibacterial, and antifungal medications. For patients who achieved a complete remission, the decision was made, based on patient and tumor specific factors, whether the patient should receive consolidation chemotherapy, autologous hematopoietic cell transplantation (HCT), or allogeneic HCT.

PCR amplification and sequencing/SNP genotyping

All slides were reviewed by a pathologist to determine the areas of interest prior to DNA extraction (exons 1-11). DNA extraction was performed on formalin fixed paraffin embedded tissue (FFPE) using QIAamp DSP DNA FFPE Tissue Kit 50 (version 1. Cat. # 60404 QIAGEN GmbH, QIAGEN Strasse 1, D-40724 Hilden, Germany). Primers for each exon were designed by Integrated DNA Technologies, Inc (Coralville, Iowa 52241 USA). Polymerase chain reactions (PCRs) were performed on an automated heat-block (DNA Thermal-Cycler, Bio-Rad) using 100ng. of genomic DNA, 10pmol. of each primer, and Taq Gold polymerase with Buffer II (Applied Biosystems, N808-0241) in a final volume of 25ul. Thirty PCR cycles were run with each fragment primer set. Thirty-five cycles of each PCR product (5ul) with sequence primer sets were subsequently run. Final PCR products were gel purified with QIAEX II Gel Extraction Kit (QIAGEN, Cat. #: 20021. QIAGEN Sciences, Maryland 20874, USA) and sequenced by ABI (Applied Biosystems) sequencer from two directions. The sequencing was done on 3730XL with BigDye Taq FS Terminator V 3.1 with analysis ABI Sequence Scanner

version 1.0. Data was extracted by Chromas Lite 2.1 software.

Raw data was imported into Mutation Surveyor DNA Variant and Sequence Analysis Software (Softgenetics). The entire sequence and variant calls were reviewed manually. Variants were confirmed as polymorphisms using the Single Nucleotide Polymorphism database, dbSNP, or as mutations using the COSMIC database (Catalogue of Somatic

Statistical analysis

Descriptive frequencies and median survivals were calculated for demographic information (age, sex, ethnicity), laboratory values (white blood cell count, hemoglobin, hematocrit, platelet count, blast percentage), prognostic factors (ECOG PS, AML history, karyotype, gene expression), and treatment variables (chemotherapy, HCT, treatment response). The SPSS statistical packages and Microsoft Excel were used for this analysis. Overall survival rates were estimated using the Kaplan Meier method (Kaplan and Meier, 1958). OS was calculated from the date of diagnosis to the date of death. Categorical data were cross-tabulated and their association was assessed using the Chi-square test of independence and an independent samples t-test. All tests were two-sided, and the Type I error was 0.05.

Results

The median age at diagnosis of the patients in our study was 78 years old. The majority of patients in our pilot study were greater than 60 (80%), male (60%), Hispanic (60%), and had a poor performance status (ECOG 2-3: 60%; Table 1) Most patients had de-novo AML (50%) with an intermediate (50%) non-complex (70%) karyotype with a *TP53* P72R SNP (50%). Four patients had AML secondary to MDS/MPN (40%). One patient had treatment associated AML (t-AML) secondary to cyclophosphamide chemotherapy for non-Hodgkin's lymphoma (NHL) treatment nine years prior. Four patients had an adverse karyotype and one patient had no karyotype information available. Less than half of the patients harbored *TP53* mutations (40%), all of which were missense point mutations. Only 4 patients received chemotherapy, which consisted of the following agents: daunorubicin plus cytarabine, etoposide, and azacitidine. Two patients obtained a CR to chemotherapy and 1 received an allogeneic HCT, but both patients relapsed and died secondary to their disease. The median laboratory values at diagnosis were as follows: hemoglobin 9.1g/dl, hematocrit 25.7%, platelet count 74$\times 10^3$/ul, white blood cell count 6.5$\times 10^3$/ul, bone marrow blasts 49%.

At the date of analysis, all patients were deceased. The median OS was 6 months. There was no significant difference in OS based on gender, AML history, risk-stratified karyotype, or *TP53* mutation. There were possible trends toward improved survival among patients less than 60 (11 *vs* 4 months, p=0.09), Hispanics (8 *vs* 1 months, p=0.11), and those not harboring SNP P72R (8 *vs* 2 months, p=0.10). There was a significant improvement

Table 1. Patient Characteristics of n=10 Newly Diagnosed AML Patients Diagnosed at MSMC

Characteristic		No. (%)	Median Survival (CI)	p value
Age	<60	2 (20.0)	11	0.09
	>60	8 (80.0)	4 (0, 10.2)	
Sex	Female	4 (40.0)	4 (0, 11.2)	0.69
	Male	6 (60.0)	4 (9, 10.2)	
Ethnicity	Hispanic	6 (60.0)	8 (0, 18.8)	0.11
	Non-Hispanic	4 (40.0)	1	
ECOG PS	0-1	3 (30.0)	28 (0.8, 55.2)	0.01
	2-3	6 (60.0)	4 (0, 9.8)	
	Unknown	1 (10.0)		
AML History				
De-novo		5 (50.0)	8 (0, 16.6)	0.67
Secondary to MDS/MPN		4 (40.0)	1	
Treatment-related		1 (10.0)	8	
Karyotype	Favorable	0 (0)	8 (0.49, 15.5)	0.37
	Intermediate	5 (50.0)	1	
	Adverse	4 (40.0)		
	Unknown	1 (10.0)		
Complex Karyotype				
	Yes	2 (20.0)	1	0.03
	No	7 (70.)	8 (0.3, 15.7)	
	Unknown	1 (10.0)		
TP53 mutation	Yes	4 (40.0)	4 (0, 29.5)	0.17
(All)	No	6 (60.0)	1	
SNP	Yes	5 (50.0)	2 (2, 4.1)	0.10
	No	5 (50.0)	8 (0.5, 15.5)	

Table 2. Patient Characteristics of *TP53* Mutated Versus *TP53* Wild-type Patients. A Chi-squared and Independent Samples T-test were Used for Comparison

Characteristics (number, %)	mutated (n=4)		un-mutated (n=6)		p value
Age					
<60	1	(25.0)	1	(16.7)	0.75
>60	3	(75.0)	5	(83.3)	
Sex					
Male	3	(75.0)	3	(50.0)	0.43
Female	1	(25.0)	3	(50.0)	
Ethnicity					
Hispanic	3	(75.0)	3	(50.0)	0.43
Non-Hispanic	1	(25.0)	3	(50.0)	
ECOG PS					
0-1	2	(50.0)	1	(16.7)	0.26
3-Feb	2	(50.0)	4	(66.7)	0.6
Unknown	0		1	(16.7)	
AML History					
De-novo	2	(50.0)	2	(33.3)	
Secondary to MDS/MPN	2	(50.0)	4	(66.7)	0.6
Treatment-related	0		0		
Karyotype					
Intermediate	1	(25.0)	4	(66.7)	0.2
Adverse	2	(50.0)	2	(33.3)	0.6
Unknown	1	(25.0)	0		
Complex Karyotype	0		2	(40.0)	0.2
SNP P72R	2	(50.0)	3	(50.0)	0.35
Treatment					
Chemotherapy	1	(25.0)	3	(50.0)	0.43
Allo-SCT	1	(25.0)	0	0.2	
Unknown	0		1	(16.7)	0.39
Treatment Response					
CR	1	(100%)	1	(33%)	0.75
No CR	0		2		
Characteristics (median, SD)					
White blood cell count ($\times 10^3$/ul)	4.3	(4.5)	7.14	(11.0)	0.43
Hemoglobin (g/dl)	8.0	(3.5)	9.5	(0.9)	0.51
Hematocrit (%)	23.3	(9.3)	27.9	(2.9)	0.43
Platelet Count ($\times 10^3$/uL)	115.5	(54.9)	62	(48.8)	0.43
Blasts (%)	40	(18.7)	51	(28.2)	0.41

in survival among patients with better performance status (28 *vs* 4 months, p=0.01) and those who did not have a complex karyotype (8 *vs* 1 months, p=0.03).

In the *TP53*-mutated patients, there were more patients younger than age 60 (25.0 *vs* 16.7%), who were male (75.0 *vs* 50.0%), had a good performance status (ECOG 0-1: 50.0 *vs* 16.7%), had de-novo AML (50.0 *vs* 66.7%,) and had an adverse karyotype (50.0 *vs* 33%; Table 2). The most common adverse karyotype in this group was the deletion of chromosome 5. The *TP53* group had fewer patients with complex cytogenetics (0 *vs* 40%) and fewer patients who received chemotherapy (25 *vs* 50%). Only 1 patient in each group had a complete response to treatment. In the *TP53* mutated group, the platelet count was higher (115 *vs* 62$\times 10^3$/ul), but the blast percentage (40 *vs* 51%), hemoglobin (8.0 *vs* 9.5g/dl) and white blood cell count was lower (4.3 *vs* 7.1$\times 10^3$/ul).

Patients with a P72R SNP were more often male (80 *vs* 40%), had a worse performance status (ECOG2-3: 80 *vs* 40%), had AML secondary to MDS/MPN (60 *vs* 20%), and had a complex karyotype (40 *vs* 0%; Table 3). The age-range, number of *TP53* mutations, and laboratory values at diagnosis were similar between the two groups. Two patients with and without a P72R SNP received chemotherapy, but only 1 patient in each group had a complete response to treatment.

The most commonly observed *TP53* mutation was N310K (40%) and the most commonly observed SNP was P72R (100%; Table 4). Two patients had both the *TP53* mutation and a SNP. The most common occurring karyotype and/or genetic abnormality were the deletion of chromosome 5 (28.6%) and a normal karyotype (28.6%). Notably, patient number 5, with a V272G mutation at exon 5 and a normal karyotype, had an overall survival of 48 months. Meanwhile, patients 1 and 3 had a SNP P72R on exon 4, had complex karyotypes, and had an overall survival of only 1 month. Only one patient had two *TP53* mutations, and this patient had an OS of 2 months.

Table 3. Patient Characteristics of SNP P72R Versus those without SNP P72R. A Chi-Squared and Independent Samples T-test were used for Comparison

Characteristics (number, %)		P72R (n=5)	No P72R (n=5)	p-value
Age	<60	1 (20.0)	1 (20.0)	1
	>60	4 (80.0)	4 (80.0)	
Sex	Male	4 (80.0)	2 (40.0)	0.20
	Female	1 (20.0)	3 (60.0)	
Ethnicity	Hispanic	2 (40.0)	1 (20.0)	0.49
	Non-Hispanic	3 (60.0)	4 (80.0)	
ECOG PS	0-1	1 (20.0)	2 (40.0)	0.49
	3-Feb	4 (80.0)	2 (40.0)	0.20
	Unknown	0	1 (20.0)	
AML History	De-novo	2 (40.0)	3 (60.0)	0.53
	Secondary to MDS/MPN	3 (60.0)	1 (20.0)	0.2
	Treatment-related	0	1 (20.0)	0.29
Karyotype	Intermediate	1 (20.0)	4 (80.0)	0.06
	Adverse	3 (60.0)	1 (20.0)	0.20
	Unknown	1 (20.0)	0	0.29
Complex Karyotype		2 (40.0)	0	0.11
TP 53 Mutation		2 (40.0)	2 (40.0)	1
Treatment	Chemotherapy	2 (40.0)	2 (40.0)	1
	Allo-SCT	0	1 (20.0)	0.29
	Unknown	0	1 (20.0)	0.29
Treatment Response	CR	1 (50.0)	1(50.0)	1
	No CR	1 (50.0)	1(50.0)	1
Characteristics (median, SD)				
White blood cell count ($\times 10^3$/ul)		7.68 (4.2)	6.4 (12.3)	0.5
Hemoglobin (g/dl)		8.9 (2.4)	9.2 (1.9)	0.25
Hematocrit (%)		25.4 (6.7)	25.8 (5.0)	0.3
Platelet Count ($\times 10^3$/uL)		73 (63.8)	75 (40.1)	0.97
Blasts (%)		61 (28.4)	48 (17.1)	0.19

Discussion

In our pilot study of randomly selected, newly diagnosed AML patients, the OS of all patients was quite low (6 months). Upon further analysis, there are several reasons for this unusual finding. A large percentage of our patient population were greater than 60 and had a poor PS at diagnosis; potentially two reasons why patients were unable to receive standard induction chemotherapy and potential curative allogeneic transplantation. Most patients were only able to receive palliative chemotherapy or supportive measures. In fact, some were so ill at initial diagnosis that they expired the hospital, often succumbing to overwhelming septic shock. Among our patient population, there were a relatively large number of patients who had AML secondary to MDS/MPN (40%) and who had complex cytogenetics (20%), compared with the 8-10% and 10-15%, respectively that would be expected (Bennett et al., 1985; Vogelstein et al., 2000). These patients are known to have a worse survival compared to those with de-novo AML or those with favorable or normal cytogenetics (Bennett et al., 1985; Stirewalt et al., 2001).

In our cohort, we had a unique population of 60% Hispanic patients, for which there was found to be a trend toward improved survival. This finding is in contrast with previous reports of Hispanic patients doing worse with

Table 4. *TP53* Mutations, SNP's, Associated Karyotypes and Gene Abnormalities, and Corresponding OS

Patient	Exon	Mutation	SNP	Karyotype and Gene Abnormalities	AML History	OS
1	4	-	P72R	Trisomy 8, deletion chromosome 16, trisomy 22, trisomy 9, CBFB gene rearrangement	Secondary AML	1
2	4	P89S	P72R	Unknown	De-Novo	4
3	4	-	P72R	Deletion chromosome 20, trisomy 6, trisomy 22, isochromosome 21q, trisomy 11 MLL gene rearrangement	Secondary AML	1
4	4	-	P72R	Normal karyotype	De-Novo	11
5	8	V272G	-	Normal karyotype	De-Novo	48
6	4 7 9	- C238G N310K	P72R - -	Inversion chromosome 3, deletion chromosome 5	Secondary AML	2
7	9	N310K	-	Translocation (1,2), deletion chromosome 5	Secondary AML	28

*OS: Overall Survival; SNP: single nucleotide polymorphism; P72R: proline to arginine at amino acid position 72; P89S: proline to serine at amino acid position 89; V272G: valine to glycine at amino acid position 272; C238G: Cysteine to glycine at amino acid position 238; N310K: Asparagine to lysine at amino acid position 310

AML (Patel et al., 2012; 2013). Also, not surprisingly, there was no clear association between laboratory (hemoglobin, platelet count) and pathologic values (blast percentage) with clinical outcome.

In our cohort, 40% of patients harbored a *TP53* mutation. This figure was greater than the 5-25% we'd expect to see among all newly diagnosed AML patients, but is far lower than that seen among those with complex karyotypes (Rücker et al., 2012). This is not surprising given the fact our patient population, as previously mentioned, was comprised of elderly, fragile patients with a large proportion having adverse, complex karyotypes. As has previously been well documented (Sigal et al., 2000; Krypuy et al., 2007; Stojnev et al., 2010), all of the *TP53* mutations described in this study resided between exon 5 and 9, but interestingly, none of the mutations found were the same as those seen in the seminal article by Rucker et al., 2012. This finding suggests that there may be hundreds of *TP53* mutations, all of which may have a unique significance. Surprisingly, there was no significant difference in response to chemotherapy or OS based on *TP53* mutation status. Although our study was small, this result is in contrast to many other previous studies showing chemotherapy resistance and worse outcomes for those patients with *TP53* mutations (Wattel et al., 1994; Nahi et al., 1998; Padua et al., 1998; Nakano et al., 2000; Christiansen et al., 2001; Stirewalt et al., 2001; Melo et al., 2002; Breems et al., 2008; Seifert et al., 2009; Cavalcanti et al., 2010; Harutyunyan et al., 2011; Jädersten et al., 2011; Tavor et al., 2011; Kanagal-Shamanna et al., 2012; Milosevic et al., 2012; Valcárcel et al., 2013). This finding suggests that *TP53* mutations may not be as strong a predictor of outcome as other previously established patient characteristics and tumor cytogenetics. To our knowledge, there have been no studies showing differences in survival based on particular *TP53* mutations. Studies have suggested that p53-miRNA-34a deregulations, as opposed to solely *TP53* mutations, may be in fact responsible for worse outcomes in AML patients (Rücker et al., 2013). Other studies have demonstrated that particular p53 protein signatures (full length *vs* beta and gamma isoforms) account for survival differences seen in AML patients (Ånensen et al., 2012). Currently, there are preliminary clinical studies testing novel *TP53* targeted agents (Lehmann et al., 2012).

Of the 4 patients in our study with *TP53* mutations, half of them had secondary AML and had the corresponding deletion of chromosome 5 (adverse karyotype). This observation is consistent with previous studies showing a strong relationship between the two karyotype and genetic signatures (Ånensen et al., 2012). Although the one patient in our study with t-AML did not have a *TP53* mutation, other researchers have shown that *TP53* mutations are more often seen in treatment related AML, because the p53 pathway is protective against leukemic transformation (Smith et al., 2003).

Half of the patients in our study population had a SNP, the commonly seen P72R. These patients were more often male, had worse prognostic features (PS, adverse, complex karyotypes), and worse outcomes compared to those without this particular SNP. Other studies have shown similar findings. Ellis et al. found that those individuals carrying both the MDM2 (regulator of *TP53*) G allele and a *TP53* Pro allele were at increased risk for treatment associated AML (Cavalcanti et al., 2010). Meanwhile, other researchers found that the Arg72 form of p53 was more effective at inducing apoptosis, whereas the Pro72 form was more effective at inducing cell cycle arrest (Thomas et al., 1999; Dumont et al., 2003; Dunna et al., 2012). Some even noted that patients with the Arg/Arg genotype were at increased risk of developing AML. This particular SNP is not unique to AML and codon 72 polymorphism has been implicated in susceptibilities to several different cancers (Matakidou et al., 2003; Koushik et al., 2006; Pietsch et al., 2006).

PCR with mutation identification software proved to be a valuable tool in identifying mutations and SNPs. The biggest limitation of our study was the small sample size. Unfortunately, all of our patients were diagnosed with AML before Flt3-ITD (adverse prognosis) and NPM1 (favorable prognosis) mutations became tested in widespread clinical practice. Therefore, it is unclear if our patients had these mutations and whether or not that had any impact on *TP53* mutation status. Studies have shown that Flt3-ITD mutations are common in elderly patients with AML (Paschka et al. 2010) and confer sensitivity to cytarabine, but resistance to doxorubicin in a manner that depends on p53 (Pardee et al., 2011). Flt3-ITD and NPM1 mutations have showed an inverse correlation to p53 deletion in patients with complex karyotype (Paschka et al. 2010). MDM2 SNP309, another commonly described SNP found in human cancer, was not analyzed as it was outside the reading frame of analysis (exon 12). Interestingly, this SNP has been found to not only interact with P72R, but also to increase the risk of the development of AML (Ellis et al., 2008).

In conclusion, our study confirms previous reports that poor PS and the presence of a complex karyotype are associated with a decreased OS. In our cohort, *TP53* mutations were relatively common, occurring more frequently in male patients with an adverse karyotype. Although there was no significant difference in survival between *TP53* mutated and un-mutated patients, there was a possible trend toward worse OS among patients with SNP P72R. These results suggest that different *TP53* mutations and SNPs should not be treated equally, and that some may confer a worse prognosis than others. Larger studies are needed to validate these findings with the goal to incorporate these new prognostic markers into risk stratification classifications.

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