Original Article

Correlation of TP53 and MDM2 Genotypes With Response to Therapy in Sarcoma

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BACKGROUND: Relatively few sarcomas harbor *TP53* (tumor protein p53) mutations, but in many cases, amplification of *MDM2* (murine double minute 2) effectively inactivate p53. The p53 pathway activity can also be affected by normal genetic variation. **METHODS:** The mutation status of *TP53* and expression of *MDM2*, *TP53*, and their genetic variants *SNP309* and R72P (Arg72Pro) were investigated in 125 sarcoma patient samples and 18 sarcoma cell lines. Association of the different genotypes and gene aberrations with chemotherapy response and survival, as well as response to MDM2 antagonists *in vitro* was evaluated. **RESULTS:** Twenty-two percent of the tumors had mutant *TP53* and 20% *MDM2* gene amplification. Patients with wild-type *TP53* ($TP53^{Wt}$) tumors had improved survival ($P < .001$) and $TP53^{Wt}$ was an independent prognostic factor (hazard ratio = 0.41; 95% confidence interval = 0.23-0.74; $P = .03$). Interestingly, there was a trend toward longer time to progression after chemotherapy for tumors with the apoptosis-prone p53 variant R72 ($P = .07$), which was strongest with doxorubicin/ifosfamide-based regimens ($P = .01$). Liposarcomas had low R72 frequency (33% versus 56%), but increased levels of *MDM2* and *MDM4* (51% and 11%, $P < .001$). *MDM2* overexpression on a $TP53^{Wt}$ background predicted better response to MDM2 antagonist Nutlin-3a, irrespective of R72P or *SNP309* status. **CONCLUSIONS:** Improved survival after chemotherapy was found in patients with $TP53^{Wt}$ tumors harboring the R72 variant. *MDM2* overexpression in $TP53^{Wt}$ tumors predicted good response to MDM2 antagonists, irrespective of R72P or *SNP309* status. Thus, detailed *TP53* and *MDM2* genotype analyses prior to systemic therapy are recommended. ***Cancer* 2012;000:000–000.** © *2012 American Cancer Society.*

KEYWORDS: *TP53*, *MDM2*, *MDM4 (MDMX)*, *codon72*, R72P, *MDM2SNP309*, sarcoma.

In addition to direct mutations, p53 can be inactivated by abrogation of other components of the signaling pathway or effector molecules that convey p53 downstream effects.[1] In sarcomas, *TP53* (tumor protein p53) mutations are infrequent,[2] but in the wild-type *TP53* ($TP53^{Wt}$) tumors, p53 may be inactivated by amplification of the mouse double minute 2 (*MDM2*) gene ($MDM2^{Ampl}$), in particular, in well-differentiated liposarcomas (WDLS).[3-5] Complementary mechanisms have been suggested,[6,7] but it is still unclear whether and how the pathway is inactivated in the remaining tumors.

In addition to rare predisposition syndromes, such as Li-Fraumeni,[8] more frequent normal genetic variation of *TP53* and *MDM2* is reported to influence cancer risk, progression and overall survival.[9-12] A polymorphism replacing arginine (R72) with proline (P72) at position 72 in p53 (*codon72* polymorphism, referred to as R72P) has been reported to affect p53 function.[13] It has been shown that R72 increases the affinity of p53 to MDM2, affects its export from the nucleus, and provides increased apoptotic potential.[14-16] The *MDM2* polymorphism, *SNP309*, changes a base from *T* to *G* at nucleotide position 309 in the *MDM2* promoter. This polymorphism is located in a binding site for the Sp1 transcription factor, and a *G* gives increased expression level of *MDM2*, hence reduced apoptotic response.[17] The *SNP309G* may also give higher sensitivity to MDM2 antagonists such as Nutlin-3a.[18] The reported frequencies of these polymorphisms vary (eg, 23%-67% for P72,[19] with 58% in Norwegians,[20] and 8%-58% for *SNP309G*, with 55% in Norwegians[21]). Here, we investigate whether these normal variants and somatic aberrations of *TP53* or *MDM2* in tumor material may affect disease outcome, and also how they may relate to Nutlin-3a responses *in vitro*.

MATERIALS AND METHODS

Tissue Samples

A total of 174 sarcoma patient samples were collected between 1980 and 2008. At time of primary diagnosis, all patients signed informed consent to use surplus diagnostic or surgical material in future investigations. The project was approved

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by the ethical committee of Southern Norway (Projects S-06133 and S-06134). Samples were collected immediately after surgery, cut into small pieces, frozen in liquid nitrogen, and stored at −80°C until use. Only malignant tumors confirmed by an experienced pathologist according to World Health Organization guidelines[22] were used, categorized in Table 1. The clinical information was retrieved from the hospital's quality database (http://medinfo.net/medinsight/), and 125 untreated tumor samples were included in the analysis.

Cell Lines

We used 18 sarcoma cell lines; 9 osteosarcomas (OHS, MHM, KPD, IOR/OS14, IOR/OS15, IOR/MOS, U2OS [HTB96], SaOS-2 [HTB85], and OSA [SJSA-1 or CRL2098]) described in Ottaviano et al,[23] 4 liposarcomas (T449, T778, Fu-ddl-S1, and SW872 [HTB92]), and 1 rhabdomyosarcoma (RMS13) described in Müller et al.[24] In addition, SA-4 (CRL7938) was obtained from ATCC (Manassas, Va), WLS-160 was given by Dr. Boven (Academic Hospital Vrije Universiteit, the Netherlands), and RMS4 and RMS28 by Dr. Look and Dr. Houghton (St Jude's Hospital, Memphis, Tenn). All cell lines were grown as described in Ohnstad et al.[25]

DNA Quantification by Real-Time Polymerase Chain Reaction

Frozen tissue was pulverized in liquid nitrogen and DNA isolated by phenol chloroform. Copy number analysis of *MDM2* and *MDM4* (also known as *MDMX*) was done by quantitative real-time polymerase chain reaction (PCR) using FastStartSYBRGreen (Roche). Oligonucleotide primers used were *MDM2*-Forward (5′-AAGCCAAACTG GAAAACTCAACAC-3′), *MDM2*-Reverse (5′-CAGGA ACATCAAAGCCCTCTTC-3′), *MDM4*-Forward (5′-C AGCAGGAGCAGCATATGGTATA-3′) and *MDM4*-Reverse (5′-GAAGCTCTGACGTCCCAGTAG-3′). *ALB* was used as internal reference, using the primers *ALB*-Forward (5′-TTTATTCACATCATTCTCTC-3′) and *ALB*-Reverse (5′-GAGTGAGATATGAGTTGAG-3′). Universal Human Reference DNA (Promega) was used as normal control. The fold-change was determined by the delta-Ct method. Fold-change < 0.5 was scored as loss, between 0.5 and 2 as normal, between 2 and 5 as gain, and amplification as > 5. Samples with PCR failure for target ($n = 2$) or reference ($n = 7$) were excluded from further analysis, 2 samples were missing.

Messenger RNA Quantification by Real-Time Reverse Transcriptase PCR

For expression analysis, total RNA was extracted using Trizol (Invitrogen) according to the manufacturer's

TABLE 1. Clinical and Treatment Characteristics of Study Population

Characteristics		*n* (N = 125)
Median age at diagnosis (range), y		58 (8-87)
Sex	Male	65
	Female	60
Localization	Axial	61
	Extremities	62
	Unknown	2
Primary tumor size	0-10 cm	38
	>10 cm	77
	Unknown	10
Histology	GIST	5
	LMS	8
	LS	35
	MFH	31
	MPNST	8
	OS	17
	SS	8
	Other	13
Grade	Low	27
	High	92
Depth	Superficial	30
	Deep	91
	Unknown	4
Stage[a]	1	1
	2	50
	3	47
	4	18
	Unknown	9
Resection of primary tumor	R0	31
	R1	54
	R2	31
	Unknown	9
Local relapse	Yes	34
	No	89
	Unknown	2
Median interval to first progression (range), m		12 (0-345)
Median number of metastatic events (range)		1 (0-11)
Radiotherapy primary tumor	Yes	30
	No	94
	Unknown	1
Chemotherapy primary tumor	Yes	17
	No	107
	Unknown	1
Chemotherapy[b]	Yes	43
	No	82

Localization: Axial (deep trunk/abdomen/uterus) or extremity (arm/thigh/leg/thoracic wall). Tumor depth: superficial (ie, subcutaneous) or deep (ie, intramuscular/extramuscular/intra-abdominal/extracompartmental [if OS]). GIST: gastrointestinal stromal tumors. LMS: leiomyosarcoma. LS: liposarcoma (well-differentiated [*n* = 16]/myxoid [*n* = 8]/pleomorphic (*n* = *2*)/dedifferentiated (*n* = *0*)/unknown/other (*n* = *9*)), MFH: malignant fibrous histiocytoma (includes myxofibrosarcoma). MPNST: malignant peripheral nerve sheath tumor. OS: osteosarcoma (includes one paraosteal). SS: synovial sarcoma. Others: epitheloid sarcoma/rhabdomyosarcoma/PNET/fibrous sarcoma/angiosarcoma/chondrosarcoma/unclassified.

[a] Stage system.[45,46] Stage3: STS only. Stage3 OS (metastasis) grouped together with Stage 4 STS (distant spread). R0: wide. R1: marginal. R2: intralesional/gross tumor left.

[b] Chemotherapy received at least once during the entire treatment period (doxorubicin/ifosfamide/etoposide/cisplatin/methotrexate/vincristine/dacarbazine/cyclofosfamide/trofosfamide single drugs or combinations according to Scandinavian Sarcoma Group protocols).

instructions, and quantitative real-time reverse transcriptase PCR (RT-PCR) was performed using a High Capacity complementary DNA Archive Kit, TaqMan Gene Expression Assays and the ABI 7500 Real-Time PCR system (Applied Biosystems, Carlsbad, Calif). Assays Hs00234753_m1*, Hs00159092_m1*, and Hs01034249_m1* were used for *MDM2*, *MDM4*, and *TP53*, respectively. *B2M* (Hs99999907_m1) and *TBP* (Hs00427621_m1*) were used as references. Three normal controls were used (human uterus [AM7892], human adipose tissue [AM7956]; Ambion, Austin, Tex; and human bone total RNA). The expression levels were determined by the delta-Ct method. Gene expression was scored as low when fold-change relative to control average was < 0.5, as normal for 0.5 to 2, and as high for > 2.

DNA Sequence Analysis

TP53

TP53 somatic mutations and polymorphic variants (frequency of 1% or higher) in tumors and cell lines were scored by the AmpliChip p53 test (Roche/Affymetrix, in development), a DNA microarray-based sequence-variant scoring method.[26] The PCR failed completely in 4 samples and partly in 7 samples (exon 3 in 6 samples, exon 7 in 1 sample). In cases of partial failure, sequence variations detected were registered, but obviously this could lead to underestimation of mutants and variants.

MDM2SNP309

The region surrounding *SNP309* was amplified using flanking intronic primers, and the restriction enzyme MspA1I was used to cut the PCR products. The fragments were separated by capillary electrophoresis on a Megabase-1000 (GE Healthcare) and the variants indicated as *TT*, *GG*, and *TG*, respectively.[27] Samples without detectable fragments ($n = 7$) were excluded.

Statistical Analysis

Association between genotypes and clinicopathologic factors was assessed using Pearson chi-squared test/Fisher exact test and independent 2-tailed *t* test. Time to recognized metastasis or sarcoma-related death was used as time to progression (TTP). Disease-specific survival (DSS) was time from day of diagnosis until death of disease or last follow-up. Survival probabilities were estimated using the Kaplan-Meier method and assessed by log-rank test. Cox regression analysis was used to adjust for potential confounding clinical variables (Table 1) and Bonferroni correction for multiple testing. Statistical analyses were performed using SPSS (version 16.0, SPSS Inc., Chicago, Ill), and *P* values $< .05$ were considered significant.

TABLE 2. Genotype Frequencies

Characteristics		No. ($n = 125$)	%
TP53	R72[a]	45	36
	P72[a]	49	39
	mutation	27	22
	unknown	4	3
MDM2SNP309[b]	*TT*	55	44
	TG or *GG*	40	32
	GG	19	15
	unknown	11	9
MDM2 copy number	deletion	1	1
	normal	23	18
	gain	65	52
	amplification	25	20
	unknown	11	9
MDM4 copy number	deletion	2	2
	normal	99	79
	gain	9	7
	amplification	4	3
	unknown	11	9

[a] rs 1042522, [b] rs 2279744.

RESULTS

Patient Characteristics

Clinical characteristics of primary tumors and treatment-specific data are given in Table 1 ($n = 125$). A total of 114 samples were informative for *SNP309* and *MDM2*/*MDM4* copy number, 74 for expression (including the major subtypes: liposarcoma [LS], osteosarcoma [OS], leiomyosarcoma [LMS], malignant peripheral nerve sheath tumor [MPNST], and malignant fibrous histiocytoma [MFH]) and 121 for *TP53* genotype. Complete results were available for 62 samples. The genotype frequencies are listed in Table 2.

P72 was most frequent in LS compared to all other subgroups ($P = .05$; Fig. 1A), particularly if WDLS or other/unknown LS ($n = 10$ and 8). Although R72 enrichment was noted in gastrointestinal stromal tumors (GIST), MPNST, and synovial sarcoma (SS), it was not significant. $TP53^{Mut}$ was detected in 27 samples (21.6%), of which 13 had the P72 variant. Fourteen cases had mutation in more than one position. The majority of mutations (23 of 29) occurred in exons 4 through 8, primarily exon 5 and 8, without codon preference. Seven mutations occurred outside the DNA-binding domain. Twelve of the *TP53* mutations were not previously reported in sarcoma.[19,27] $TP53^{Mut}$ dominated in LMS (63%, $P = .01$, Fig. 1A), whereas the frequency of $TP53^{Mut}$ was low in LS (6%, $P = .01$). No $TP53^{Mut}$ was detected in GIST, MPNST or SS, but this was not significant ($P > .2$).

No significant differences in *SNP309* distribution among histological subgroups were found (Fig. 1B). However, there was a tendency toward lower *SNP309G*

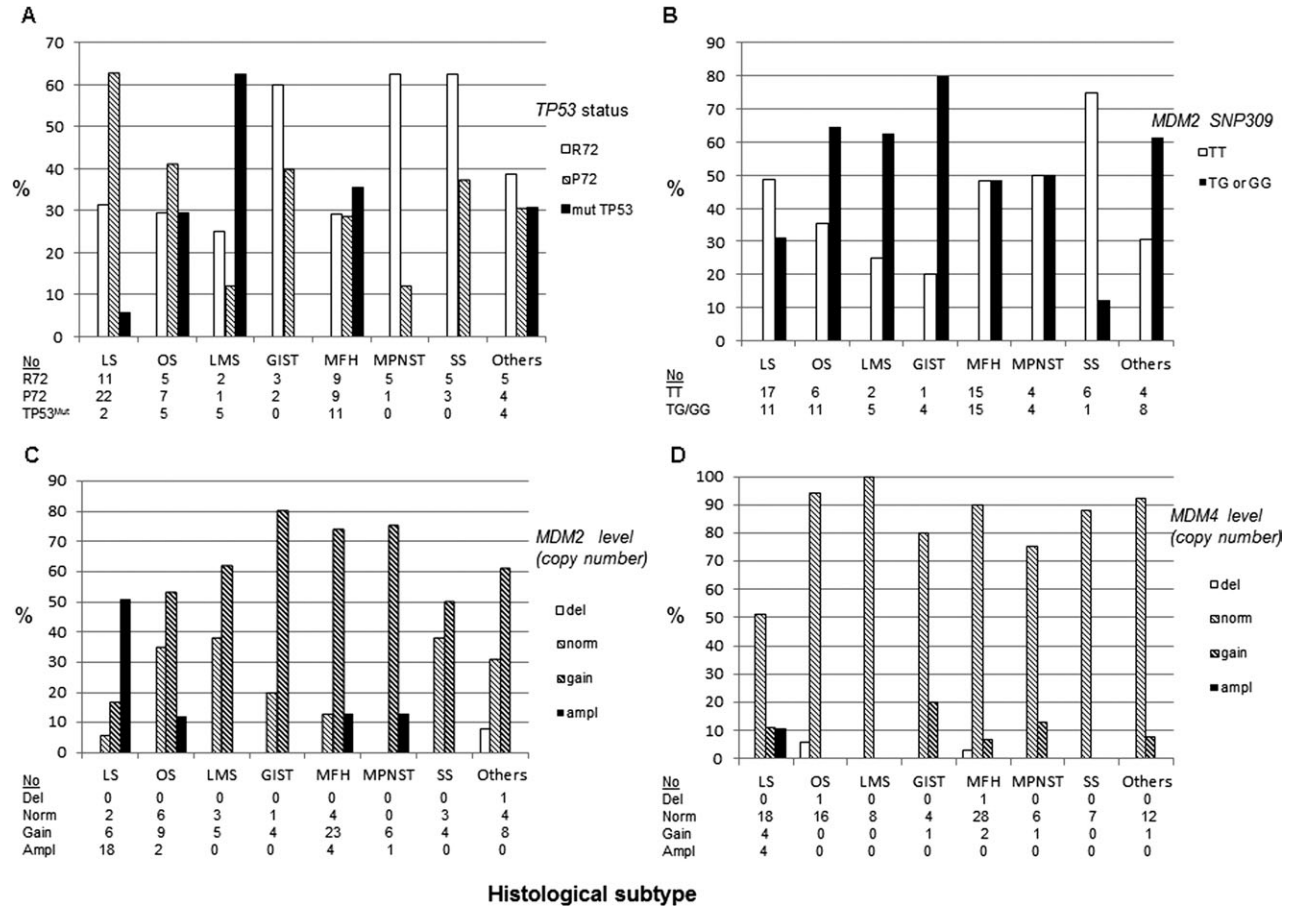

Figure 1. Genotype distributions are shown in different histological subtypes. Bars represent percentages of (A) $TP53^{Wt}$ (R72, P72) and $TP53^{Mut}$, (B) *SNP309* (*TT*, *TG* or *GG*), (C) copy number level of *MDM2*, and (D) copy number level of *MDM4*. The chi-squared test was used to compare each histological subtype to the rest (reference category).

frequency in SS ($P = .06$), and among LS, only WDLS displayed the *GG* genotype. $MDM2^{Ampl}$ was observed in 20% of samples and $MDM4^{Ampl}$ in 3%. As expected, the frequency of $MDM2^{Ampl}$ was high in LS (51%, 81% in WDLS, and 44% in other/unknown LSs) compared with the other subtypes ($P < .001$; Fig. 1C), and $MDM4^{Ampl}$ was only observed in myxoid or other LSs (11%; Fig. 1D).

Correlations Between Genotypes

The samples from major subtypes having a maximum of 2 missing data points were included in the analysis of patterns of genotypes/phenotypes. The results are visualized in a heat map in Figure 2. There was a strong correlation between *MDM2* copy number and expression ($P < .001$). In general, *TP53* mutation and *MDM2* amplification was mutually exclusive with 2 exceptions: LS03 and MFH53. However, none of these expressed high level of *MDM2*. LS stands out with 93% $TP53^{Wt}$ samples, of which 80% had *MDM2* overexpression. Notably, only 24% of $TP53^{Wt}$ samples were scored as $TP53^{Norm}$ for expression. *MDM4* was also overexpressed in a substantial fraction of $TP53^{Wt}$ LS samples (52%, $MDM4^{High}$), with a strong correlation with *MDM2* expression ($P < .001$). No skewed distribution of R72P was observed in $MDM2^{Ampl}$ samples. However, almost all *SNP309G* in LS samples coexisted with *MDM2* overexpression, but not conversely.

The OS samples showed the same pattern of *MDM4* overexpression in $TP53^{Wt}$ samples, where *MDM2* was also overexpressed. P72 was observed together with $TP53^{Mut}$ in 67% of LMS. Except for the MFHs, of which about half had $TP53^{Mut}$ and almost all had $MDM2^{Gain}$, no other specific patterns were found. No clear association of *SNP309G* and other genotypes/phenotypes could be seen.

Correlations With Clinical Data

Survival

At the time of final analysis, 71 patients were deceased due to sarcoma, 18 due to other disease, 8 of

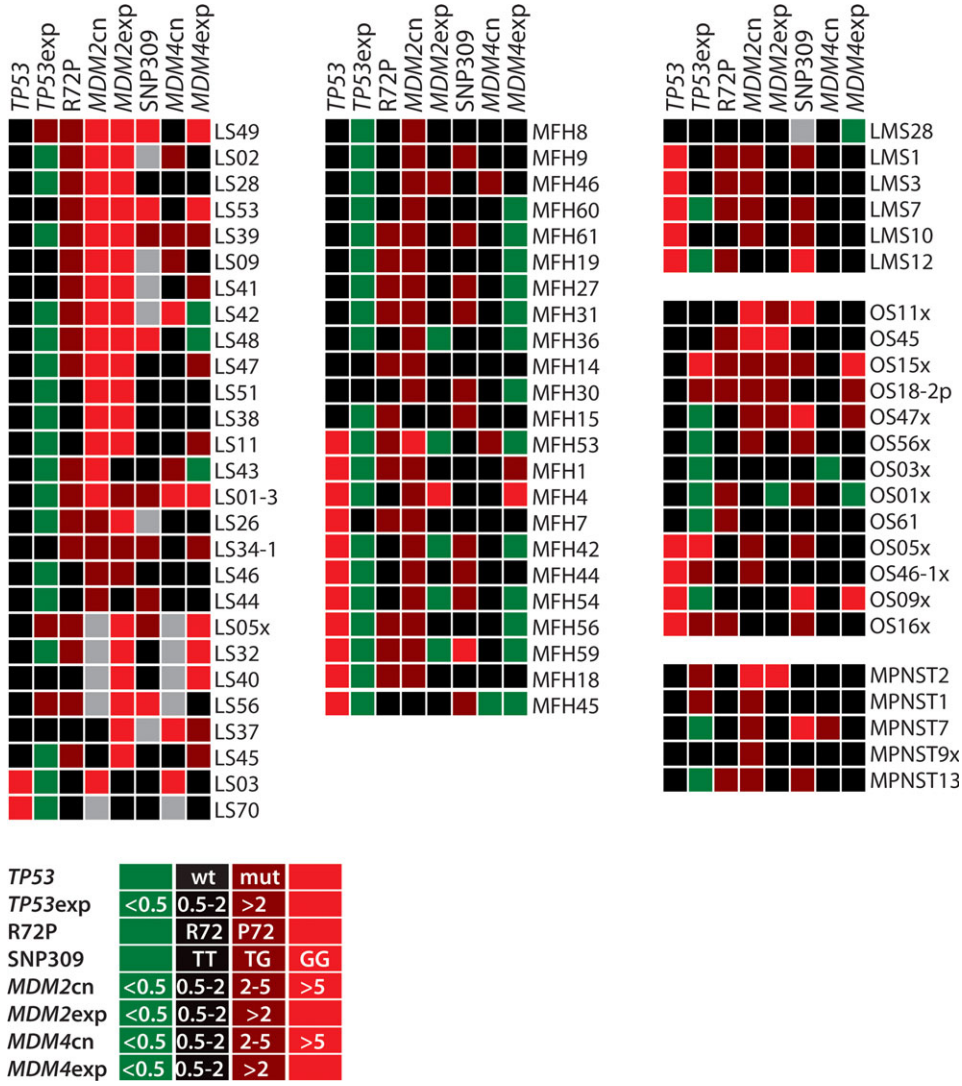

Figure 2. Heat map visualizes the patterns of genotypes/phenotypes across the sarcoma subtypes. Patient tumor sample in rows (LS, liposarcoma; MFH, malignant fibrous histiocytoma; LMS, leiomyosarcoma; OS, osteosarcoma; MPNST, malignant peripheral nerve sheath tumor), characteristics in columns stratified as explained. Expression (exp) is fold-change relative to the average of 3 human reference RNAs. Copy number (cn) is fold-change relative to *ALB* (cn). Gray indicates genotype/phenotype was not available. Abbreviations: mut, mutant; wt, wild type.

unknown cause, and 28 were still alive (1 with persistent sarcoma). The median follow-up was 71 months, DSS was 129 months, and TTP was 36 months.

No statistical differences were observed with respect to R72P polymorphism, but patients with $TP53^{Mut}$ had shorter DSS and TTP than those with $TP53^{Wt}$ ($P < .001$; Fig. 3A). Despite the importance of *TP53* status for survival (hazard ratio[DSS] = 0.41, 95% confidence interval = 0.23-0.74, $P = .03$ and hazard ratio[TTP] = 0.43, 95% confidence interval = 0.25-0.73, $P = .02$), multivariate analysis revealed that axial localization increased the risk of sarcoma-related death 2-fold.

No statistical differences in DSS or TTP were detected for the *SNP309* genotypes (Fig. 3B) or between histological subtypes, except in WDLS, which showed significantly better DSS and TTP than the other LSs. Both *MDM2* copy number and expression levels correlated with prognosis: lower levels indicated shorter DSS and TTP (Fig. 3C,D). However, this was not significant in multivariate analysis, where absence of metastasis at diagnosis correlated with better DSS, and axial localization was associated with more than 3-fold increased risk of sarcoma-related death (hazard ratio = 3.64, 95% confidence interval = 1.76-7.53, $P = .01$). Notably, 77% of

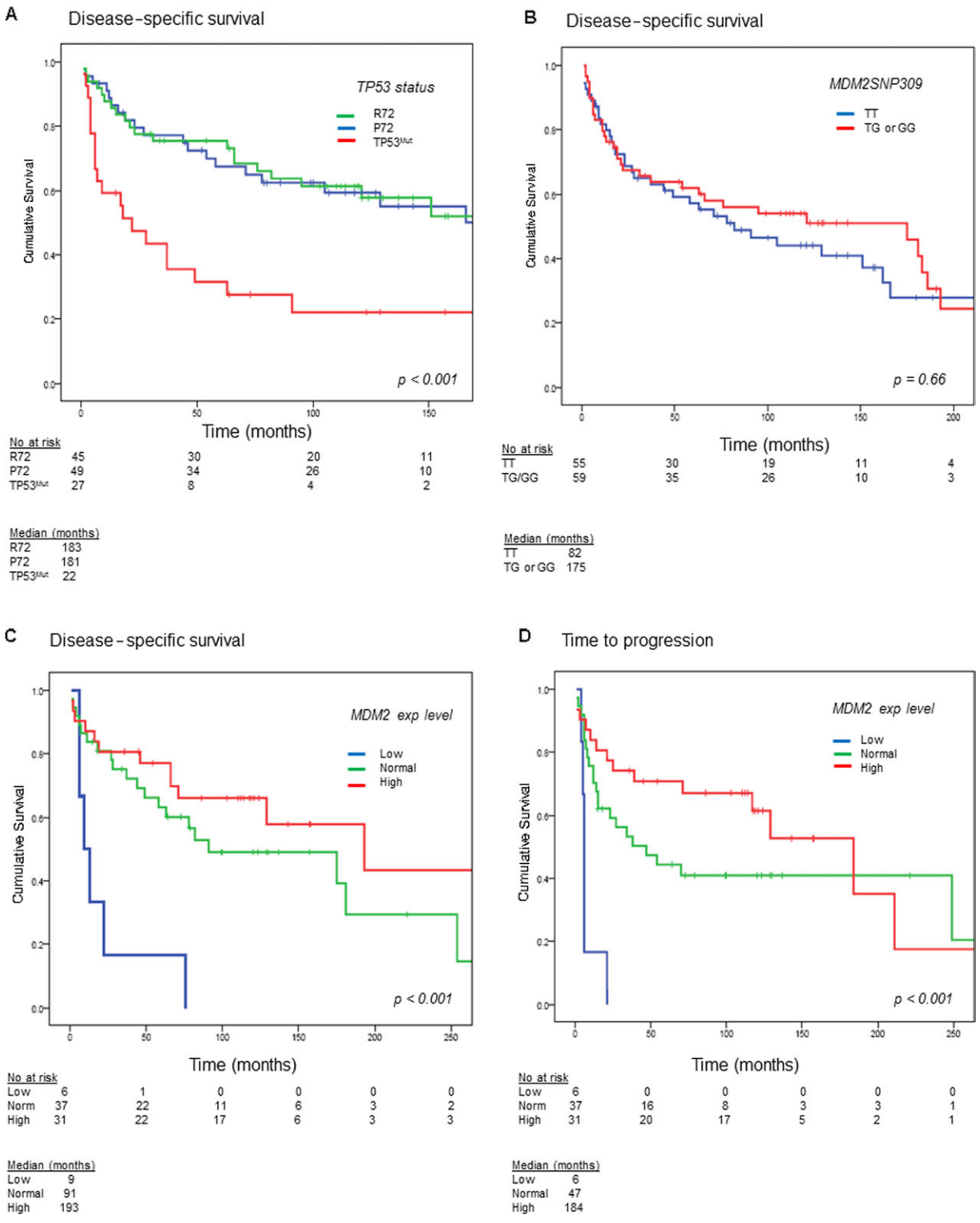

Figure 3. Kaplan-Meyer plots depict (A-C) disease-specific survival and (D) time to progression in study population stratified by (A) *TP53* status; R72, P72 or *TP53*^{Mut}, (B) *MDM2SNP309*; *TT* or *TG/GG*, and (C,D) *MDM2* expression level; low, normal or high. Survival probabilities were assessed by a log-rank test.

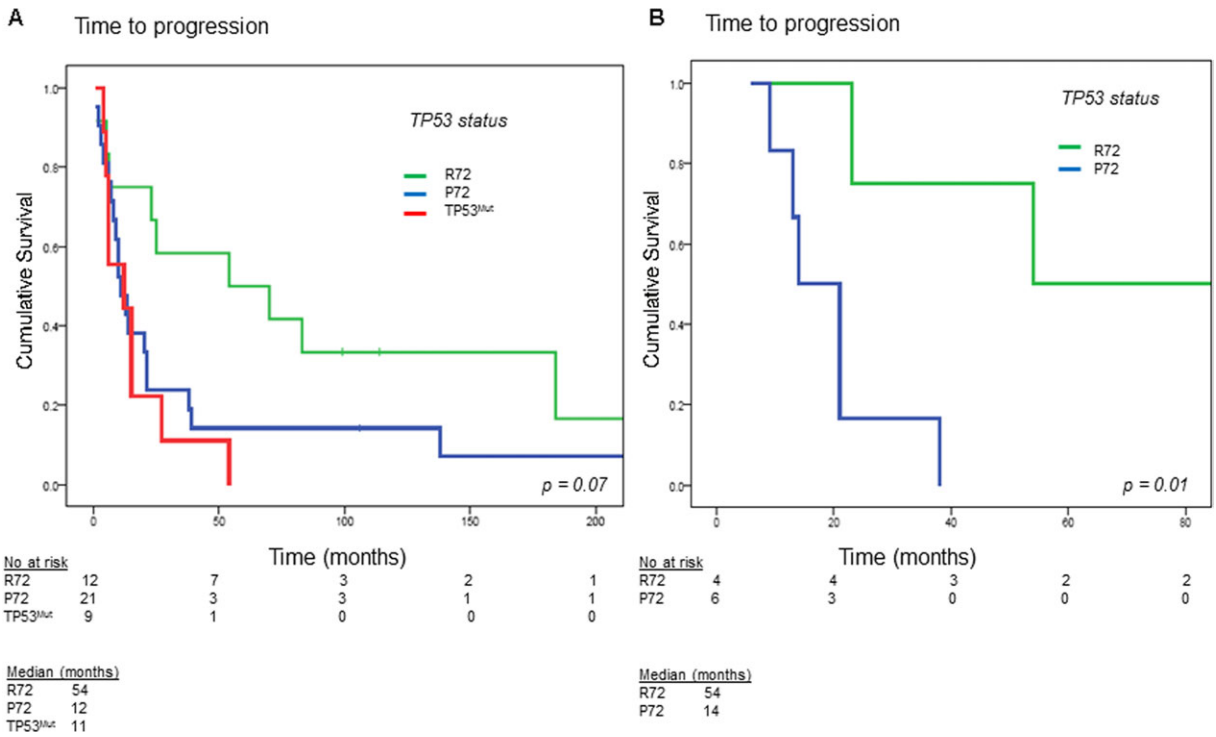

Figure 4. Kaplan-Meyer plots depict time to progression for patients receiving (A) chemotherapy (regimen not specified) or (B) a doxorubicin/ifosfamide combination. Patients were stratified according to *TP53* status; R72, P72, or $TP53^{Mut}$.

$MDM2^{High}$ tumors were LS and presence of WDLS was significantly associated with longer survival in univariate analysis. No significant differences in DSS or TTP were found with relation to *MDM4* copy number or expression level.

Response to Chemotherapy

Complete information about radiotherapy was not available; hence analysis of treatment response was restricted to chemotherapy. A total of 43 patients received chemotherapy at least once: doxorubicin (30%), ifosfamide (26%) or a combination of these (28%) as regimen backbone. Doxorubicin was typically given at a dose of 50 to 75 mg/m^2 and ifosfamide 4.5 to 9 g/m^2 per cycle. Response to treatment was estimated as TTP. Median TTP in this selected cohort was 14 months.

Interestingly, there was a tendency toward longer TTP after chemotherapy with the apoptosis-prone R72 variant (compared with P72, $P = .07$; Fig. 4A). Patients with R72 responded significantly better to the doxorubicin/ifosfamide combination, although the number of patients was limited ($P = .01$; Fig. 4B). No significance was found for ifosfamide alone, doxorubicin alone, or other regimens, and no confounding factors were identified in univariate analysis. Hence, R72P status appears to affect chemotherapy response. Seven treated cases harbored P72 in combination with $TP53^{Mut}$, but their longer TTP (compared to R72/$TP53^{Mut}$, $n = 2$) was not statistically significant. There was no significant relation between *SNP309G* and TTP.

Cell Lines

Response to Nutlin-3a

The cell line characteristics and genotypes are outlined in Table 3. One-third of the cell lines were $TP53^{Mut}$, four with mutations not previously reported in sarcomas. All RMS cell lines were $TP53^{Mut}$, whereas the majority of LS and OS cell lines were $TP53^{Wt}$. None of the $TP53^{Mut}$ lines had *SNP309G*. All $TP53^{Wt}$ LS cell lines expressed high *MDM2* level, whereas $TP53^{Wt}$ OS cell lines were either $MDM2^{Ampl}$/$MDM2^{High}$ or $MDM2^{Gain}$/$MDM2^{Norm}$. In the latter cases, *SNP309G* was always present. Both variants of R72P were present in LS and OS cell lines, R72 only together with *SNP309G* in LS. As all LS were $MDM2^{Ampl}$, no relation between R72 and $MDM2^{Ampl}$ could be observed. Similarly, no association was found in OS cell lines, and no significant relation with *MDM4* was observed.

We reported previously the response of 10 of the cell lines to Nutlin-3a.[24,25,28] All cell lines sensitive to Nutlin-3a harbored $TP53^{Wt}$ together with $MDM2^{Ampl}$ or

TABLE 3. Cell Line Characteristics

Cell Line	Histology	Patient (Sex/Age)	Origin/Site	*TP53*	*SNP 309*	*MDM2* Cn	*MDM2* Exp[d]	*MDM4* Cn	*MDM4* Exp[d]	*TP53* Exp[d]	Nutlin-3a Response IC50 (μM)[e]
SA-4[a]	LS	M/na	na	wt	TG	4	3	4	1		nd
WLS-160	LS	na	na	wt	GG	4	nd	4	nd		nd
SW872[a]	LS	M/36	na	mut	TT	na	3	na	3		>10
T449[a]	WDLS	F/68	PT/retroperitoneum	wt[c]	TT	4	4	2	0		0.6 ± 0.1
T778[a]	WDLS	F/69	LR/retroperitoneum	wt[c]	TT	4	4	2	1		0.7 ± 0.1
Fu-ddl-s1[a]	DDLS	M/61	LR/retroperitoneum	wt[c]	TT	4	4	2	0		0.6 ± 0.2
OSA[a]	OS	M/19	PT/femur	wt	TT	4	4	2	0	2	0.5 ± 0.1
SaOS-2[a]	OS	F/11	Na	mut	TT	4	1	3	0	0	>10
U2OS[a]	OS	F/15	PT/tibia	wt	TG	3	1	3	1	2	1.0 ± 0.5
KPD[a]	OS	M/8	PT/femur	wt	GG	3	1	2	1	0	>10
MHM[a]	OS	M/42	PT/pelvis	wt[c]	TT	4	4	2	0	1	1.9 ± 0.3
OHS	OS	M/14	PT/femur[b]	mut	TT	3	1	2	1		nd
IOR/OS14	OS	M/13	PT/na	wt	TG	3	1	2	0		nd
IOR/OS15[a]	OS	F/12	PT/na	wt[c]	TT	4	2	4	1		nd
IOR/MOS[a]	OS	F/13	PT/na	mut	TG	3	1	2	0		nd
RMS4	ARMS	M/17	PT/arm, lung	mut	TT	2	nd	2	nd		nd
RMS13	RMS	M/17	PT/bone marrow	mut	TT	3	1	3	1		>10
RMS28	ARMS	na	Met/axillary node	mut	TT	3	nd	2	nd		nd

Abbreviations: ARMS, alveolar rhabdomyosarcoma; Cn, copy number; DDLS, dedifferentiated liposarcoma; Exp, expression; IC50, median inhibitory concentration; LR, local relapse; LS, liposarcoma, unspecified; Met, metastasis; mut, mutant; na, not available; nd, not done; OS, osteosarcoma, unspecified; PT, primary tumor; RMS, rhabdomyosarcoma, unspecified; WDLS, well-differentiated liposarcoma; wt, wild type.

[a] Tested and authenticated by fingerprinting.

[b] Multiple lesions in multiple bones.

[d] Expression numbers are: 1 (<0.5-fold change), 2 (0.5- to 2-fold change), 3 (2- to 5-fold change), 4 (>5-fold change).

[e] Values are means ± standard error of the mean for 3 to 5 experiments.[24,25]

MDM2^{Gain} (Table 3). Notably, the only cell line (scored by sequencing as *TP53*^{Wt}) that did not respond was previously indicated as *TP53*^{Mut}, due to failure to amplify exon 8 with the AmpliChip p53 assays, very low expression (fold-change of 0.01), and insensitivity to Nutlin-3a.[24] No association between *SNP309* or R72P and Nutlin-3a response was found in this panel.

DISCUSSION

Mutations in *TP53* are less frequent and more heterogeneous in sarcomas than in other cancer types,[29] and in addition to the amplification and overexpression of *MDM2,* other mechanisms, including *MDM4* amplification, may be involved.[4,6,7,30] The hypothesis that *MDM2*^{Ampl} and *TP53*^{Mut} both provide inactivation of the p53 response has been challenged by the occasional report that the 2 aberrations may be found in the same tumor.[31] We found such apparent double mutations in 2 samples, LS03 and MFH53 (Fig. 2). However, none of these expressed MDM2, thus confirming the hypothesis that *MDM2*^{Ampl} and *TP53*^{Mut} are mutually exclusive. Most likely, the amplification of the locus containing *MDM2* was selected for by overexpression of other genes, such as *CDK4* or *HMGA2*.[32-34] Furthermore, contrary to common reports that *TP53*^{Mut} leads to accumulation of p53 protein, we found low p53 expression in these samples (Fig. 2).

In line with previous reports,[2,5,29] we found *TP53*^{Mut} in only 22% of tumors and 39% of cell lines, with no codon preference. Fourteen of the mutations have not been previously reported in sarcoma. LS had only 6% *TP53*^{Mut}. Thus, drug strategies that activate wild-type p53 may have potential in sarcomas, in particular liposarcomas.[24,35]

Twenty percent of the patient samples had more than 5-fold amplification of *MDM2*, and significantly more in LS, primarily WDLS, confirming previous reports.[36,37] Furthermore, *MDM4*^{Ampl} was found exclusively in the LS patient samples, strongly associated with *MDM2*^{Ampl}, indicating that these aberrations are not mutually exclusive, and suggesting that therapeutic strategies that target both MDM2 and MDM4 (MDMX) may be particularly promising in LS patients.

Normal variation in the *TP53* and *MDM2* genes has also been reported to affect tumor properties.[11,38] The presence of *SNP309G* in the promoter may increase *MDM2* expression.[39] However, no overall enrichment of *SNP309G* was observed in this tumor material compared with that from healthy Norwegians, arguing against any predisposing effect,[21] and no clear association between

MDM2 expression level and *SNP309* was found in this panel. The latter point is perhaps not surprising, because *MDM2* expression is highly associated with gain or gene amplification, and there was no tendency for specific alleles to be overrepresented in tumors with altered *MDM2* copy number.

As shown here, most published studies conclude that mutant *TP53* is associated with poor outcome.[40] Similar to loss of p53 function, overexpression of *MDM2* could be a poor prognostic indicator, although published conclusions differ (reviewed in Onel and Cordon-Cardo[41]). We found association of low *MDM2* (messenger RNA) expression with shorter DSS and TTP. It should be noted that *MDM2* messenger RNA may not correlate with MDM2 protein,[42] and also there are multiple splice variants complicating the interpretation of either. However, the relation to survival could not be verified in multivariate analysis, probably due to the strong influence of WDLS with high *MDM2*, but it is also noteworthy that low *MDM2* level is associated with $TP53^{Mut}$.

Presence of *TP53* R72 has been associated with better induction of apoptosis.[14] However, excluding LS, P72 was less frequent in this tumor material than in healthy Norwegians (30% versus 58%[20]), contradicting a protective role of R72. Interestingly, we found a tendency toward longer TTP after chemotherapy for patients with the R72 variant, as previously reported for squamous carcinomas.[16] Subsequent subgroup analysis revealed that patients with R72 respond significantly better to doxorubicin/ifosfamide combination regimens than do those patients with the P72 variant. Although the number of patients was limited, the association was significant in multivariate analysis. The reduced chemotherapy response often observed in LS could be associated with lower prevalence of R72.

In addition, $TP53^{Mut}$ combined with R72 has previously been shown to reduce apoptosis after chemotherapy,[43] because specific p53 mutants are capable of inhibiting p73, another possible mediator of cytotoxicity. In this series, the number of samples was too small to conclude, but patients with P72/$TP53^{Mut}$ showed longer TTP than their equivalent (R72/$TP53^{Mut}$).

Finally, an important objective of this study was to investigate if *TP53* or *MDM2* polymorphism could modify sensitivity to MDM2 antagonists. In our hands, all cell lines with $MDM2^{Ampl}$ responded well to MDM2 antagonist Nutlin-3a, as long as *TP53* was wild-type. Interestingly, one cell line (U2OS) only having $MDM2^{Gain}$, but *SNP309TG*, also had satisfactory drug-response. In contrast, the $TP53^{Wt}$ OS cell line KPD was not sensitive to Nutlin-3a, despite being homozygous for the variant allele (*SNP309GG*). This did not appear to be due to inactivation by MDM4, as reported,[44] because the *MDM4* expression level was normal. However, *TP53* expression level was very low. Recently, Knappskog et al reported that other *SNPs* (eg, *SNP285* in breast and ovarian tumors) may interact with *SNP309* and modulate the MDM2 phenotype.[21] Thus, extended genotype examination may be necessary to guide future p53-activation therapy.

CONCLUSIONS

Patients with *TP53* wild-type sarcomas and the R72 variant had improved survival and response to chemotherapy. If *TP53* is mutated, presence of R72 may have the opposite effect. *MDM2* overexpression predicts good response to MDM2 antagonists such as Nutlin-3a, but intact p53 pathway is essential. Detailed analysis of aberrations in the p53 pathways may help to predict tumor sensitivity and resistance to p53 activating therapy by MDM2 antagonists

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