RESEARCH LETTER

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TO THE EDITOR:

Specific subtype distribution with impact on prognosis of *TP53* single-hit and double-hit events in AML and MDS

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TP53 is the most frequently mutated gene in cancer also playing important roles in pathogenesis of hematologic malignancies.[1,2] In patients with de novo acute myeloid leukemia (AML) and with myelodysplastic syndrome (MDS), *TP53* alterations (*TP53*alt) occur in ~5% to 15% of the patients; in patients with therapy-related AML (t-AML) and MDS and in those with relapses, they are detected at higher frequencies (~25% to 40%).[3-8] *TP53* mutations (*TP53*mut) are generally associated with advanced stages of the disease,a complex karyotype, resistance to conventional (chemo-)therapies, and dismal prognosis.[5,8-11] Several targeted drugs and novel therapeutic options have been developed in recent years; however, the optimal treatment strategy for patients with AML and MDS harboring *TP53*alt remains a critical area of unmet need. *TP53*alts comprise not only gene mutations but also allelic imbalances, including deletions (dels) in *TP53* and regions with copy-neutral loss of heterozygosity (cnLOH) comprising 17p/*TP53*. Frequently, both alleles are altered either by biallelic mutations or *TP53*muts, with accompanying allelic imbalance of the other allele (dels or cnLOH).[2,12-15] Although monoallelic mutations in *TP53* (without accompanying dels or cnLOH; single hit [sh]) already show a negative impact on survival, biallelic alterations in *TP53* (double hit [dh]) lead to a dismal outcome both in patients with AML and MDS.[14,15]

The focus of this study is to analyze *TP53*sh (single *TP53*muts, dels in 17p comprising *TP53,* or cnLOH in 17p including *TP53*) and dh events (≥2 *TP53*mut, *TP53*mut + del, and *TP53*mut + cnLOH) in more detail, with a focus on the distribution, type of aberration, and impact on survival in AML and MDS subgroups (classification in accordance with the World Health Organization 2017).[16] A total of 1519 samples were used for analysis, comprising 772 newly diagnosed AML and 747 MDS cases (Table 1). Whole genome sequencing was performed for all 1519 patients at the time of diagnosis (median coverage, 100×). For this, 151bp paired-end reads were generated on NovaSeq 6000 and HiSeq X machines (Illumina, San Diego, CA; for further information, see supplemental Data). Cases were categorized into (1) one *TP53*mut without accompanying del or cnLOH (mut-only), (2) *TP53* del-only, (3) cnLOH-only (1-3; sh events), (4) *TP53*mut and accompanying del in *TP53* (mut + del; might include 1 or more *TP53*muts), (5) *TP53*mut and accompanying cnLOH (mut + cnLOH, might include 1 or more *TP53*muts), and (6) ≥2 *TP53*muts (≥2 mut-only; without accompanying del or cnLOH; 4-6; dh events). For AML, in 84 of 772 cases at least 1 *TP53*alt was detected (11%); for MDS, 96 of 747 cases showed *TP53*alt (13%). Regarding AML, the highest frequency of *TP53*alt were detected in the group having AML with myelodysplasia-related changes (AML-MRC; 65 of 152 cases, 42%; supplemental Figure 1A; Table 1). In addition, *TP53*alt were recurrently found in AML with *KMT2A::MLLT3* (3 of 26, 12%), AML with *GATA2::MECOM* (3 of 32, 9%), AML with *PML::RARA* (4 of 50, 8%), AML with maturation (2 of 52, 4%) and AML with mutated *NPM1* (2 of 160, 1%). In the remaining AML subgroups, *TP53*alt were only found in 1 patient each or were even completely absent (Table 1). These results were validated using classical diagnostic methods performed on 11 796 unselected AML cases, in which very low *TP53*alt frequencies were also detected for the respective subentities (supplemental Table 1). Regarding MDS, *TP53*alt were detected in all analyzed subentities, with the highest frequencies detected in therapy-related MDS (6 of 22, 27%), followed by MDS 5q− (21 of 104, 20%), MDS with

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Table 1. *TP53* alteration frequencies in subgroups of AML and MDS

	Specific subgroup according to WHO 2017	Number of cases	Cases without *TP53* aberration [n]	Cases with *TP53* aberration [n]	Frequency of *TP53* aberration [%]
AML	AML-MRC*	152	87	65	43
	AML with t(9;11)(p21;q23); *KMT2A::MLLT3*	26	23	3	12
	AML with inv(3)(q21q26); *GATA2::MECOM*	32	29	3	9
	Acute promyelocytic leukemia with *PML::RARA*	50	46	4	8
	Acute monoblastic and monocytic leukemia	14	13	1	7
	Therapy-related AML	19	18	1	5
	AML with maturation	52	50	2	4
	AML without maturation	30	29	1	3
	Acute myelomonocytic leukemia	33	32	1	3
	AML with mutated *RUNX1*	43	42	1	2
	AML with mutated *NPM1*	160	158	2	1
	AML with biallelic mutation of *CEBPA*	47	47	0	0
	AML with inv(16)(p13q22); *CBFB::MYH11*	47	47	0	0
	AML with t(6;9)(p23;q34); *DEK::NUP214*	10	10	0	0
	AML with minimal differentiation	14	14	0	0
	AML with t(8;21)(q22;q22); *RUNX1::RUNX1T1*	43	43	0	0
	Total in AML	**772**	**688**	**84**	**11**
MDS	Therapy-related MDS	22	16	6	27
	MDS with isolated del(5q) (MDS 5q−)*	104	83	21	20
	MDS-EB-2*	151	126	25	17
	MDS-EB-1*	149	128	21	14
	MDS-RS-SLD	42	38	4	10
	MDS-RS-MLD*	148	136	12	8
	MDS-SLD	18	17	1	6
	MDS-MLD	113	107	6	5
	Total in MDS	**747**	**651**	**96**	**13**
	In total cohort	**1519**	**1339**	**180**	**12**

AML and MDS subgroups are sorted according to frequency of *TP53* alteration, respectively.

MDS-MLD, MDS with multilineage dysplasia; MDS-RS-MLD, MDS with multilineage dysplasia with ring sideroblasts; MDS-RS-SLD, MDS with single lineage dysplasia with ring sideroblasts; MDS-SLD, MDS with single lineage dysplasia.

*The subgroups in which more than 10 cases had *TP53* alterations are highlighted in gray. Those were thus selected for further detailed analysis of the alteration type and OS.

excess blasts 1 (MDS-EB1) (25 of 151, 17%), and MDS-EB2 (21 of 149, 14%) (supplemental Figure 1B; Table 1). In the total AML cohort, 34 of 84 patients (40%) showed sh (mut-only, 24%; del-only 14%; and cnLOH-only, 2%), whereas *TP53*dh was found in 50 of 84 patients (60%; mut + del, 38%; mut + cnLOH, 6%; and ≥2 mut, 16%). In MDS, sh was found in 56 of 96 patients (58%) and dh in 40 of 96 patients (42%) (mut-only, 50%; del-only, 7%; cnLOH-only 1%; and mut + del, 20%; mut + cnLOH, 9%; and ≥2 mut, 13%). For analysis of *TP53*alt types in the subentities, only subgroups in which *TP53*alt was found in >10 cases were selected (AML-MRC, MDS with multilineage dysplasia with ring sideroblasts, MDS-EB1, MDS-EB2, and MDS 5q; supplemental Table 2). Although patients with MDS 5q− predominantly showed a *TP53*sh caused by a *TP53*mut (81%), those with AML-MRC and MDS-EB2 predominantly showed dh (68% for each type; supplemental Figure 2). In addition, dels comprising *TP53* were frequently found in MDS with multilineage dysplasia with ring sideroblasts (17%) and MDS-EB1 (14%), whereas mut + cnLOH, causing a dh, was detected in 14% of MDS-EB1 cases. Regarding cnLOH, 5 of 50 cases (19%) of all dh cases in AML and 9 of 40 cases (23%) in MDS were caused by mut + cnLOH; hence, this alteration significantly contributes to the formation of *TP53*dh. Overall survival (OS) was significantly less in patients with a *TP53*sh than in patients without *TP53*alt, both for patients with AML and MDS (sh vs no hit; AML: 8 months vs 21 months, $P = .004$; MDS: 46 vs 70 months, $P = .001$). However, in both entities, the presence of a dh worsened the prognosis drastically (dh vs no hit; AML: 1 vs 21 months, $P < .001$; MDS: 11 vs 70 months, $P < .001$) (Figure 1A,B). Moreover, when the cohorts were split per the sh and dh events, a significant negative impact on OS compared with cases without *TP53*alt was observed for all dh events and mut-only sh events, in both patients with AML and MDS. For cases with del-only, a trend toward a lower OS was detected, which was, however, not statistically significant; whereas for cnLOH-only, the number of cases was not sufficient for meaningful analysis (Figure 1C,D). Moreover, as cnLOH results merely in a duplication of the *TP53* wild-type allele, no clinical impact is assumed. Influence of *TP53*alt on OS was also investigated in the selected subgroups (>10 cases

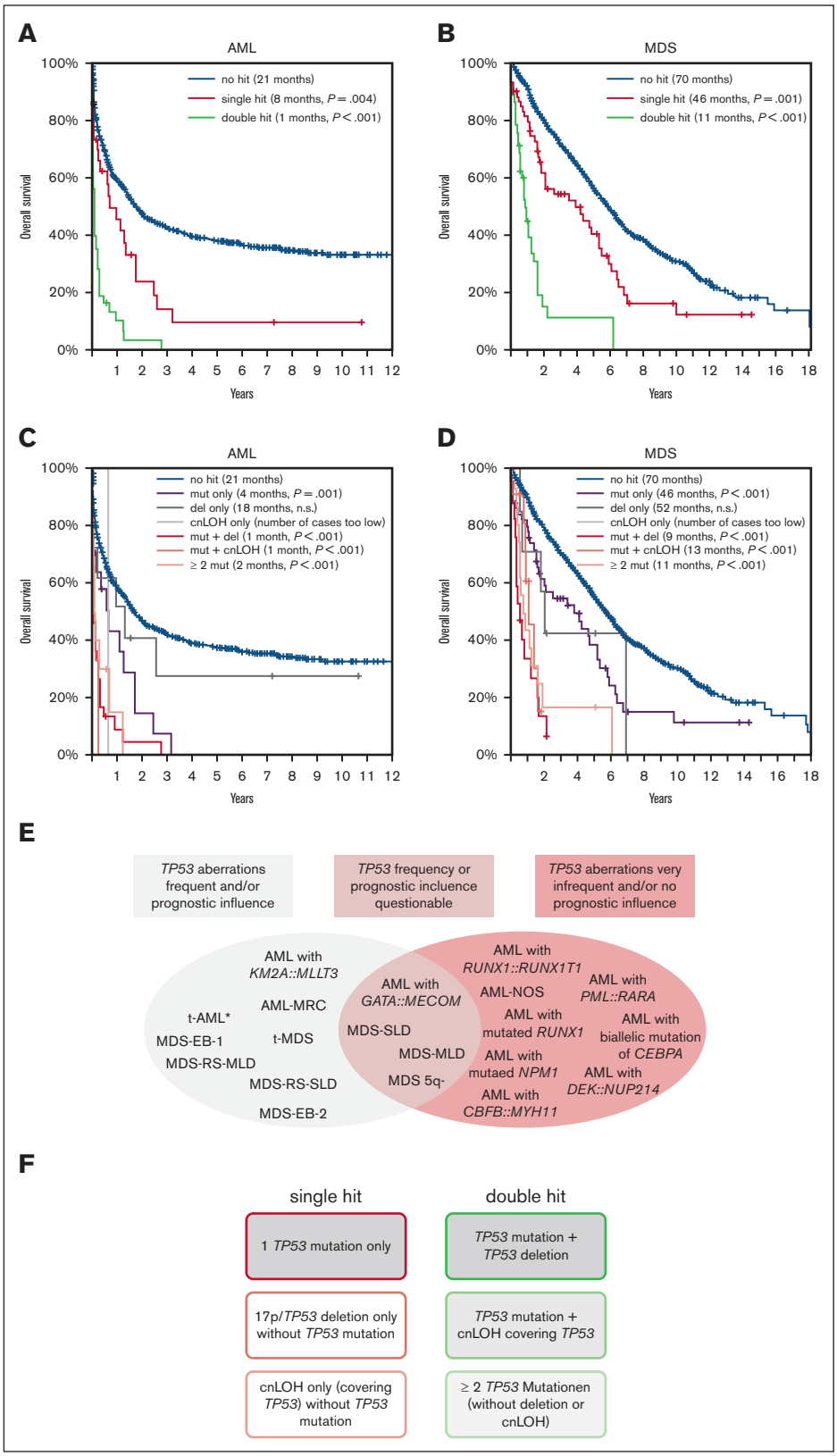

Figure 1. OS of patients with *TP53* alteration in the total AML and MDS cohorts, and suggestion of a diagnostic algorithm. (A-B) In patients with (A) AML and (B) MDS, the OS was analyzed for patients with *TP53* sh (red line), *TP53* dh (green line), and without *TP53*alts (blue line; no hit). In (C) and (D), OS was analyzed in more detail for patients with *TP53* mut-only (purple line), with *TP53* del-only (dark gray line), with cnLOH-only (light gray line), with mut + del (red line), with mut + cnLOH (light red line), with ≥2 mut-only (orange line), and in patients with no hit (blue line). The *P* values denote the significance of the respective alteration in comparison to no hit. Clinical

Figure 1 (continued) data were available for 717 patients with AML and 737 patients with MDS. (E,F) Recommendation of *TP53* analysis for AML and MDS subgroups and suggestion of a diagnostic algorithm. (E) The analyzed entities were categorized into (1) entities in which *TP53* aberrations were frequently detected and/or showed a prognostic influence (marked in gray; left), (2) cases for which the *TP53* alteration frequency was very low or not detectable and/or did not show prognostic relevance (marked in red; right), and (3) entities for which the *TP53* alteration frequency or prognostic influence is questionable (middle). AML without defining genetic abnormalities were summarized as AML-NOS (not otherwise specified). *, *TP53* alterations in t-AML cases were rare in our analysis because of the low number of t-AML cases included in our cohort; however, t-AMLs are known to frequently harbor *TP53* alterations, hence they were included into category 1. (F) Proposed categories for *TP53* sh (left) and *TP53* dh events (right). Marked with gray background are events that have impact on the prognosis in patients with AML and MDS.

with *TP53*alt), the remaining AML cases were summarized and grouped as AML–non-MRC. Interestingly, a significant negative impact of *TP53*alt on OS was found for all subgroups, except for the MDS 5q− subgroup (supplemental Figure 3).

Generally, our results corroborate previous findings that *TP53*alt play an important role in prognosis and risk stratification of patients with AML and MDS.[3,4,17-22] Recently, for MDS, both the World Health Organization classification 2022 and the International Consensus Classification recognized *TP53* mutated cases as a distinct disease entity, although the inclusion criteria vary.[23,24] Our data show that although presence of a single *TP53*mut already influences OS, and a *TP53*dh worsens OS dramatically in both patients with AML and MDS.[14,15,17] In addition, our results demonstrate that the presence of *TP53*alt and influence on prognosis differs markedly within the analyzed AML and MDS subtypes, some depicting low frequencies and/or only minor or no prognostic relevance (see supplemental Data). Moreover, we postulate that for a correct definition of *TP53*dh in a diagnostic setting and for proper risk stratification, (1) the number of *TP53*muts (none, 1, and ≥2), (2) the presence of a 17p/*TP53*del, and (3) the presence of cnLOH involving *TP53* should be investigated (Figure 1E,F). Because of the dismal outcome of both patients with AML and MDS with biallelic *TP53*alts with an urgent need for novel therapeutic options, it can be suggested that these cases should be considered as separate entities in future classification systems. Moreover, as the frequency of *TP53*mut is known to be enriched among patients with relapsed AML and MDS because of the selective advantage of the *TP53* mutated cells caused by their resistance to chemotherapy,[3-8] it raises concerns regarding the use of this treatment option for patients with monoallelic *TP53*muts.

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