haematologica
Journal of the Ferrata Storti Foundation

Clinical interrogation of *TP53* aberrations and its impact on survival in patients with myeloid neoplasms

by Jayastu Senapati, Sanam Loghavi, Guillermo Garcia-Manero, Guilin Tang, Tapan Kadia, Nicholas Short, Hussein A. Abbas, Naszrin Arani, Courtney D. DiNardo, Gautam Borthakur, Naveen Pemmaraju, Betul Oran, Elizabeth Shpall, Uday Popat, Richard Champlin, Sherry Pierce, Sankalp Arora, Ghayas Issa, Musa Yilmaz, Keyur Patel, Koichi Takahashi, Guillermo Montalban-Bravo, Danielle Hammond, Fadi G. Haddad, Farhad Ravandi, Hagop M. Kantarjian, and Naval G. Daver

Received: August 14, 2024.
Accepted: December 3, 2024.

Citation: Jayastu Senapati, Sanam Loghavi, Guillermo Garcia-Manero, Guilin Tang, Tapan Kadia, Nicholas Short, Hussein A. Abbas, Naszrin Arani, Courtney D. DiNardo, Gautam Borthakur, Naveen Pemmaraju, Betul Oran, Elizabeth Shpall, Uday Popat, Richard Champlin, Sherry Pierce, Sankalp Arora, Ghayas Issa, Musa Yilmaz, Keyur Patel, Koichi Takahashi, Guillermo Montalban-Bravo, Danielle Hammond, Fadi G. Haddad, Farhad Ravandi, Hagop M. Kantarjian, and Naval G. Daver. Clinical interrogation of TP53 aberrations and its impact on survival in patients with myeloid neoplasms.
Haematologica. 2024 Dec 12. doi: 10.3324/haematol.2024.286465 [Epub ahead of print]

Clinical interrogation of *TP53* aberrations and its impact on survival in patients with myeloid neoplasms

Jayastu Senapati[1]*, Sanam Loghavi[2]*, Guillermo Garcia-Manero[1], Guillin Tang[2], Tapan Kadia[1], Nicholas J. Short[1], Hussein A. Abbas[1], Naszrin Arani[3], Courtney D. DiNardo[1], Gautam Borthakur[1], Naveen Pemmaraju[1], Betul Oran[4], Elizabeth Shpall[4], Uday Popat[4], Richard Champlin[4], Sherry Pierce[1], Sankalp Arora[3], Ghayas Issa[1], Musa Yilmaz[1], Keyur Patel[1], Koichi Takahashi[1], Guillermo Montalban-Bravo[1], Danielle Hammond[1], Fadi G. Haddad[1], Farhad Ravandi[1], Hagop M. Kantarjian[1], Naval G. Daver[1]

[1]Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.

[2]Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.

[3]Department of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.

[4]Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.

* These authors contributed equally

Running Title: *TP53* aberrations in myeloid neoplasm and outcomes

Keywords: *TP53*, MDS, AML. Venetoclax, allogeneic stem cell transplantation

Corresponding author:

Naval G. Daver
Professor of Medicine,
Department of Leukemia,
The University of Texas MD Anderson Cancer Center,
1515 Holcombe Blvd, Houston 77030, USA
Email: ndaver@mdanderson.org

Funding: The study was supported by University of Texas MD Anderson Cancer Center Grant (CA016672) and University of Texas MD Anderson SPORE (C1100632).

Data availability statement: Data available from corresponding author on reasonable request

Author COI:

GGM has received research funding from Astex Pharmaceuticals, Novartis, AbbVie, BMS, Genentech, Aprea Therapeutics, Curis, and Gilead Sciences; has been a consultant for Astex Pharmaceuticals, Acceleron Pharma, and BMS; and has received honoraria from Astex Pharmaceuticals, Acceleron Pharma, AbbVie, Novartis, Gilead Sciences, Curis, Genentech, and BMS. TMK has been a consultant for AbbVie, Agios, BMS, Genentech, Jazz Pharmaceuticals, Novartis, Servier, and PinotBio; has received research funding from AbbVie, BMS, Genentech, Jazz Pharmaceuticals, Pfizer, Cellenkos, Ascentage Pharma, GenFleet Therapeutics, Astellas Pharma, AstraZeneca, Amgen, Cyclacel Pharmaceuticals, Delta-Fly Pharma, Iterion Therapeutics, GlycoMimetics, and Regeneron Pharmaceuticals; and has received honoraria from Astex Pharmaceuticals. NJS has been a consultant for Takeda Oncology, AstraZeneca, Amgen, Novartis, and Pfizer and received research funding from Takeda Oncology, Astellas, and Stemline Therapeutics as well as honoraria from Amgen. CDD has been a board of directors or advisory committee member for Genmab, GSK, Kura Oncology, and Notable Labs; has received honoraria from Kura, Astellas Pharma, Bluebird Bio, Bristol Myers Squibb, Foghorn Therapeutics, Immune-Onc Therapeutics, Novartis, Takeda Oncology, Gilead Sciences, and Jazz Pharmaceuticals; is a current holder of stock options for Notable Labs; has been a consultant for AbbVie and Servier; and has received research funding from Servier, Bristol Myers Squibb, Foghorn, Immune-Onc Therapeutics, Loxo Oncology, Astex Pharmaceuticals, Cleave, and Forma. GB has received research funding from Astex Pharmaceuticals, Ryvu Therapeutics, and PTC Therapeutics; has been a board of directors or advisory committee member for Pacyclex Pharmaceuticals, Novartis, CytomX, and Bio Ascend; and has been a consultant for Catamaran Bio, AbbVie, PPD Development, Protagonist Therapeutics, and Janssen. NP has received Consultancy/Scientific Advisory Board/Speaking from Pacylex Pharmaceuticals, Astellas, Pharma US, Aplastic Anemia & MDS International Foundation, CareDx, ImmunoGen, Inc, Bristol-Myers Squibb Co., Cimeio Therapeutics AG, EUSA Pharma, Menarini Group, Blueprint Medicines, CTI BioPharma, ClearView Healthcare Partners, Novartis Pharmaceutical, Neopharm, Celgene Corporation, AbbVie Pharmaceuticals, Pharma Essentia, Curio Science, DAVA Oncology, Imedex, Intellisphere, CancerNet, Harborside Press, Karyopharm, Aptitude Health, Medscape, Magdalen Medical Publishing, Morphpsys. GCI has been a consultant for Novartis, Kura Oncology, and NuProbe and received research funding from Celgene, Kura Oncology, Syndax, Merck, Cullinan, and Novartis. MY has received research funding from DaiichiSankyo and Pfizer. HMK has received research funding from AbbVie, Amgen, Ascentage Pharma, BMS, Daiichi Sankyo, ImmunoGen, Jazz Pharmaceuticals, and Novartis as well as honoraria from AbbVie, Amgen, Amphista Therapeutics, Ascentage Pharma, Astellas Pharma, Biologix, Curis, Ipsen, KAHR, Novartis, Pfizer, Precision Biosciences, Shenzhen TargetRx, and Takeda Oncology. ND has received research funding from Astellas Pharma, AbbVie, Genentech, Daiichi Sankyo, Gilead Sciences, ImmunoGen, Pfizer, Bristol Myers Squibb, Trovagene, Servier, Novimmune, Incyte, Hanmi Pharm, Fate Therapeutics, Amgen, Kite Pharma, Novartis, Astex Pharmaceuticals, KAHR, Shattuck, Sobi, GlycoMimetics, and Trillium; has been an advisor for Astellas Pharma, AbbVie, Genentech, Daiichi Sankyo, Novartis, Jazz Pharmaceuticals, Amgen, Servier, Karyopharm Therapeutics, Trovagene, Trillium, Syndax, Gilead Sciences, Pfizer, Bristol Myers Squibb, Kite Pharma, Actinium Pharmaceuticals, Arog Pharmaceuticals, ImmunoGen, Arcellx, and Shattuck; has been a data monitoring committee member for Kartos Therapeutics and Jazz Pharmaceuticals; has been a consultant or board of directors or advisory committee member for Agios, Celgene, Sobi, and STAR Therapeutics; and has received research funding from Karyopham Therapeutics and Newave Pharmaceutical. The other authors have nor relevant disclosures.

Author contribution:

JS, SL and ND designed the manuscript. JS and SP collected the data. JS, SL and ND analyzed the data. SL and GT provided the laboratory data. JS, NA, SA made the figures. JS, GGM, GT, TK, NJS, HA, CDD, GB, NP, BO, ES, UP, RC, GI, MY, KP, KT, GMB, DH, FGH, FR, HMK and ND provided patients. JS wrote the first manuscript draft. SL and ND revised the drafts. All others reviewed the final draft and approved the final draft

Abstract:

In myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) with *TP53* aberrations, dissecting the interaction amongst patient, disease and treatment factors are important for therapeutic decisions and prognostication. This retrospective analysis included patients with newly diagnosed MDS (>5% blasts) and AML with *TP53* mutation(s) treated at MD Anderson Cancer Center. We factored patient age, *TP53* aberration burden, therapy intensity and use of venetoclax in the AML subgroup, and allogeneic hematopoietic stem cell transplantation (HSCT) to interrogate outcomes. *TP53* was annotated as high-risk (*TP53*^{HR}) if >1 mutation, one mutation + allelic deletion or a single mutation with variant allele frequency (VAF) ≥40%; *TP53* low risk (*TP53*^{LR}) included a single *TP53* mutation VAF <40%. 413 patients (291 AML, 122 MDS) at a median age of 69.4 years were included, 350 (85%) with *TP53*^{HR} (253 AML [87%], 97 [79%] MDS). Overall response (OR) rate was 53% in AML and 62% in MDS. OR and composite complete response (CRc) rates was similar in patients with AML irrespective of treatment intensity, but higher when treated with venetoclax. At a median follow-up of 77 months, median OS was superior in patients with MDS than AML (10.8 versus 5.9 months). On multivariate analysis (MVA) MDS had lower hazards of death compared to AML, as was *TP53*^{LR} and HSCT. In the AML cohort, *TP53*^{LR} and HSCT were favorable on MVA, though venetoclax did not improve survival. Both the diagnosis of MDS or AML and burden of *TP53* aberrations dictated outcomes in our analysis and HSCT consistently led to improved survival outcomes.

Introduction:

The approval of several novel agents has improved the outcomes of patients with newly diagnosed acute myeloid leukemia (AML), though the outcomes of most patients with adverse-risk AML remain dismal [1-5]. AML with *TP53* aberrations has particularly dismal outcomes due to resistance to various treatments, including cytotoxic chemotherapy, epigenetic therapies, and apoptosis-inducing therapies such a venetoclax [2,6-8]. Recent attempts to improve the outcomes of patients with *TP53* mutated (*TP53*^{mut}) AML have failed to improve survival, however extensive efforts are ongoing to leverage non-chemotherapy-based approaches to improve outcomes in these patients [9-12]. Mutation-agnostic agents including venetoclax, in combination with low-intensity therapy have failed to improve outcomes of patients with *TP53*^{mut} AML [12-16]. However, whether these outcomes are homogenously dismal regardless of the type of *TP53* aberrations, treatment intensity, or type of therapy needs to be better understood. Despite allogeneic hematopoietic stem cell transplantation (HSCT) being the only potentially curative option for patients with *TP53* mutated AML, post-HSCT survival remains generally poor. In addition, the impact of baseline *TP53* mutational burden and concomitant cytogenetics on HSCT outcomes remains poorly defined.

Previous studies have annotated the allelic status of *TP53* using the mutational burden [variant allele fraction (VAF)] and *TP53* allelic loss (*TP53*^{loss}) through cytogenetic assessment [17-19]. These studies have made important contributions in correlating the burden of *TP53* aberrations with clinical outcomes; however, the interplay of blast burden and treatment regimens in conjunction with the burden of *TP53* aberrations is not well known and needs further characterization. A number of studies have suggested minimizing or even obviating the need for blast cutoffs in distinguishing *TP53* mutated AML from *TP53* mutated myelodysplastic neoplasms (MDS) with increased blasts, mostly due to their similarly poor outcomes irrespective of blast percentage and need for inclusion in clinical trials [20-24].

Routine laboratory methods such as conventional karyotyping, fluorescent *in situ* hybridization (FISH), bulk next-generation sequencing (NGS), and array-specific comparative genomic hybridization (aCGH) can be used to infer the allelic status and the burden of *TP53* aberrations, which along with patient age, fitness, blood and/or bone marrow (BM) blast percentage, can be used for prognostication and to guide treatment decision-making. In view of evolving data dissecting the outcomes of patients with *TP53* mutated MDS and AML based solely on the burden of *TP53* aberrations, we attempted to more comprehensively study the outcomes of patients with newly diagnosed MDS and AML with *TP53* mutation at MD Anderson Cancer Center (MDACC) incorporating not just *TP53* burden, but also age, pathologic diagnosis (MDS vs. AML), treatment intensity, use of venetoclax and HSCT. The primary

objective of the study was to compare the outcomes of patients with MDS and AML with *TP53* mutations and then to focus on factors affecting outcomes of patients in the AML cohort.

Methods:

Patients and treatment

We performed a retrospective analysis of adult patients (≥18 years) with newly diagnosed MDS (and ≥5% blasts) and AML as per World Health Organization 2016 criteria[25] at MDACC harboring a pathogenic *TP53* mutation +/- concurrent deletion. Baseline parameters, including complete blood counts, BM blast percentage, cytogenetics and mutations, treatment intensity, use of frontline venetoclax and HSCT in first remission, were obtained from the electronic medical records. The study was approved by the Institutional Review Board and conducted in accordance with the declaration of Helsinki. Informed consent was obtained from all participants.

Assessment of *TP53* aberrations

TP53 mutation analysis:

Next-generation sequencing (NGS) was performed using our clinically-validated myeloid panels, interrogating the entire exonic or hotspot regions of 28, 53 or 81 genes (depending on the time of presentation) frequently mutated in myeloid malignancies, including *TP53,* with a lower limit of detection of VAF ≥2%, as described previously and detailed in the supplement [26,27].

TP53 allelic loss/deletion:

Allelic loss/deletion at the *TP53* locus was studied using a combination of conventional karyotyping, FISH and aCGH. Allelic loss/deletion was defined as described previously and detailed in the supplement[28,29].

Annotation of TP53 aberrations:

We classified our patient population based on the burden of *TP53* aberrations into *TP53* low-risk ($TP53^{LR}$) and $TP53^{HR}$ with a focus on clinical relevance. Multihit *TP53* aberrations included patients with >1 mutation, one *TP53* mutation + deletion, and a *TP53* mutation + copy neutral loss of heterozygosity (cnLOH) (assessed either through a-CGH or with high VAF (≥40%) as a surrogate)[19]. Thus, our $TP53^{HR}$ group included patients with documented multihit status (**Group 1**: > 1 *TP53* mutation with VAF ≥2%, [Supplemental Figure 1]; **Group 2**: One *TP53* mutation [VAF ≥2%] and concurrent 17p.13 deletion, and

Group 3: *TP53* single hit mutations with VAF ≥40% [19]. The *TP53*^{LR} group included patients with a single mutation with a VAF <40% and no concurrent 17p.13 deletion.

Response and outcomes

Response was annotated per the European LeukemiaNet (ELN) 2017 guidelines for AML[30] and the 2006 International Working Group criteria for MDS [31]. Best response after frontline therapy was recorded, prior to HSCT. Overall response (OR) included a combination of complete remission (CR), complete remission with incomplete counts recovery (CRi) and morphological leukemia free state (MLFS) for AML, and CR and BM CR for MDS. Relapse-free survival (RFS) was calculated from the time of best response to relapse, transformation to AML (for MDS patients) or death. We did not censor for HSCT. Overall survival (OS) was calculated from the time of therapy initiation to death from any cause and censored at last follow-up.

Statistical analysis

Mann-Whitney U test and a 2-sided Fisher T test were used to compare continuous and categorical baseline variables. A *P* value of <0.05 was considered significant. Survival data were calculated using the Kaplan Meier test and compared using the Mantel Cox log-rank test. Follow up was calculated using reverse Kaplan Meier method. Cox proportional hazard was used to study disease and treatment factors associated with OS through univariate (UVA) and multivariate analysis (MVA). For MVA, factors biologically relevant on the UVA model and/or those with P<0.1 were used. We used a classification and regression tree (CRT) model as a predictive decision-making tool for survival at 1-year. Propensity score analysis was done by comparing logit of propensity scores from baseline covariates and using a caliper width of sigma 0.1 with an optimal selection algorithm. Analysis was done using GraphPad Prism version 9.3.1 (GraphPad Software, Boston, MA) and the Lumivero (New York, NY) (2023) XLSTAT statistical solution.

Results:

We identified 413 unique patients with newly diagnosed AML (291 [70.5%]) and MDS (122 [29.5%]) harboring a known pathogenic *TP53* mutation with available cytogenetic (CTG) data between January 2013 to July 2022. Baseline characteristics are summarized in **Table 1.** The median age at diagnosis was 69.4 years (range, 18.2-90.4 years); 321 patients (78%) were ≥ 60 years. In the AML group, the median age was 70 years (range, 20.1-87 years). The median age in the MDS cohort was 69 years (range 18.2-

90.4 years). The median BM aspirate blast percentage in the MDS cohort was 9 (range, 1-18), 57 patients (47%) had ≥10% BM blasts at diagnosis.

***TP53* allelic status:**

358 patients (87%) had a complex karyotype (CK) including 190 patients (46%) with *TP53* deletion. Only 2 patients (1%) with a *TP53* deletion did not have a complex karyotype. Based on our HR vs LR algorithm, 63 patients (15%) had $TP53^{LR}$ [38 patients (13%) with AML and 25 patients (20%) with MDS, p=0.07] **(Supplemental Figure 1 [Figure S1]).** The median VAF in the patients with $TP53^{LR}$ was 21% (range, 2%-39%) and 45/63 patients (71%) had a VAF ≥10%. The median $TP53^{LR}$ VAF was 20% (range, 2%-37%) in the MDS group and 23% (range, 2%-39%) in the AML group, P=0.40. In the $TP53^{LR}$ group 37 patients (59%) had a complex karyotype (20 of 38 [53%] with $TP53^{LR}$ AML and 17 of 25 [68%] with $TP53^{LR}$ MDS).

A total of 350 patients (85%) had a $TP53^{HR}$ aberration, of whom 111 patients (32%) had multiple *TP53* mutations (Group 1). Among these, 65 patients (59%) had a VAF sum of ≥50% (43 with AML and 35 with MDS) and 47 (41%) had a VAF sum <50% (35 with AML and 12 with MDS). There was no difference in OS based on *TP53* VAF sum in the full cohort or the AML and MDS cohorts, when analyzed separately **(Figure S2).** Based on this, all patients with multiple *TP53* mutations were considered to have multihit *TP53* status irrespective of the VAF sums, for subsequent analysis. The $TP53^{HR}$ cohort also included 112 (32%) patients with a single *TP53* mutation and a *TP53* deletion [Group 2; median *TP53* VAF:33% (range, 2%-93%);71/112 (63%) with *TP53* VAF <40%]. and 127patients (36%) with a single *TP53* mutation ≥40% [Group 3; median *TP53* VAF:69% (range, 40%-97%)]. We validated the VAF cutoff for patients with single a TP53 mutation in the AML cohort using decision trees from a CRT model. A TP53 VAF >37.5% (very close to our VAF cutoff of 40% for calling $TP53^{HR}$) had the best discriminatory power to predict OS at 1 year and was associated with OS < 1 year in 78% patients **(Figure S3).** Further details are in the supplement **(Figure S4-S5).**

Missense mutations were the most common, occurring in 361 patients (87%), followed by frameshift in 42 patients (10%), nonsense in 31 patients (7%) and splice site mutations in 10 patients (2%) **(Figure S6).**

Treatment and response outcomes:

Overall, 319 patients (77%) were treated on a clinical trial, 219 (75%) patients with AML and 100 patients (82%) with MDS. In the AML group, 234 patients (80%) were treated with low-intensity therapy, 201 (86%) of whom received hypomethylating agent (HMA)-based therapy. Amongst these 234

patients, 81 (35%) received venetoclax and 213 patients (91%) were ≥60 years old. The other 57 patients with AML were treated with intensive chemotherapy-based approaches; 42 (74%) patients were <60 years and only 9 patients (15%) received concurrent venetoclax **(Table S1).**

Amongst patients with AML, an OR was attained in 155 patients (53%) and a composite complete response (CRc) [CRc=CR+CRi] in 129 (44%) **(Figure S7).** Thirty-one patients (11% of full AML group and 20% of responders) proceeded to HSCT in first remission. The median age of the transplanted patients at AML diagnosis was 61.4 years (range, 20-74 years) and 20 patients (64%) had been treated on a low-intensity regimen prior to HSCT **(Figure S8A).**

The most common treatment in the MDS group was HMA, used in 114 patients (93%) and cumulatively 8 patients (6%) had received venetoclax as part of frontline therapy. An OR was attained in 76 patients (62%), of whom 43 patients (30%) had a CR **(Figure S7).** Overall, 23 responders (20% of full MDS group) proceeded to an HSCT. Twenty-two patients (18%) transformed to an AML, 16 of whom were responders and 6 patients amongst them had transformed after HSCT **(Figure S8B).**

***TP53* aberration status and survival outcomes:**

The median follow-up of the whole cohort was 77.8 months (95% CI 77-90 months); 78 months for AML and not reached for the MDS group. The median RFS and OS in the patients with AML was 4.7 months (95% C.I. 2.5-5.6 months) and 5.9 months respectively (95% C.I. 5.3-7.3 months); for patients with MDS the median RFS and OS were 5.9 months (95% C.I. 3.9-7.7 months) and 10.8 months (95% C.I. 9.1-11.9 months) respectively **(Figure 1).**

In the full cohort, the median OS was significantly longer in patients with $TP53^{LR}$ compared with $TP53^{HR}$ (12.1 months versus 6.8 months, P<0.001) while there was no significant difference between type of $TP53^{HR}$ **(Figure 2A, S9A).** Stratifying by diagnosis, in the MDS cohort, OS was significantly better in $TP53^{LR}$ compared to the $TP53^{HR}$ (14.3 vs. 9.3 months, P= 0.06 **(Figure 2B)** There was no difference amongst the 3 $TP53^{HR}$ groups (11.6 months in Group 1 versus 10.1 months in Group 2 and 8.4 months in Group 3, *p* log-rank for trend 0.67) **(Figure S9B).** Patients with $TP53^{LR}$ AML had a better OS of 10.4 months compared to 5.4 months in patients with $TP53^{HR}$ AML (P= <0.01) **(Figure 2C).** The median OS was slightly better in Group 2 $TP53^{HR}$ at 6.9 months compared with 5.6 months (Group 3) and 4.4 months (Group 1) (P= for trend 0.02). Despite statistical significance the absolute durations of response were short and dismal in

all 3 *TP53*^{HR} sub-groups **(Figure S9C).** Twenty of 38 (53%) patients with AML *TP53*^{LR} had a CK (without *TP53* deletion) and an inferior survival of 8.2 months, compared to the remainder *TP53*^{LR} patients with AML who did not have a CK (12.7 months, P= 0.04). The median *TP53* VAF was not different between the 2 groups (25% versus 22%, P=0.63) **(Figure S10A).** Though limited by very small patient numbers, there was no different in OS in the MDS *TP53*^{LR} group based on CK **(Figure S10B).**

MDS vs AML survival outcomes

In patients with MDS, there was no difference in median OS based on 5-10% and ≥10% BM blasts (10.7 versus 11.6 months, P=0.21). However, OS was superior in patients with MDS, irrespective of blast percentage, compared to AML **(Figure S11).** Amongst patients with *TP53*^{HR}, there was a significant difference in OS between the MDS and AML group (9.3 and 5.4 months respectively, P=0.001), however there was no significant difference in OS between *TP53*^{LR} MDS and AML (14.3 versus 10.5 months respectively, P=0.83) **(Figure S12).** We then selected the patients with *TP53*^{HR} AML (n=23) and MDS (n=16) who underwent HSCT; the median survival was similar at 12.6 months and 15.4 months respectively, P= 0.73 **(Figure S13).** The numbers in the *TP53*^{LR} HSCT arms were too small for a salient comparison.

We performed Cox regression analysis accounting for MDS or AML diagnosis, age (</≥60 years), *TP3* status (*TP53*^{HR} versus *TP53*^{LR}), CTG (CK or not), use of venetoclax and HSCT; on MVA patients with MDS had significantly reduced risk of death (Hazard ratio [hr]=0.76, 95% CI 0.61-0.94) compared to patients with AML, along with *TP53*^{LR} (hr=0.66, 95% CI 0.48-0.89) and HSCT (hr=0.42, 95% CI 0.30-0.57) while other covariates were not significant **(Table 2).** We subsequently performed a propensity matched comparison of patients in the MDS cohort to the AML cohort, including age (continuous), *TP53* status (*TP53*^{HR} versus *TP53*^{LR}), attainment of OR and HSCT as variables; amongst 23 matched pairs there was no difference of median OS between the 2 groups (17.3 versus 13.9 months respective, P= 0.69); incidentally this matching selected 23 patients in each group who had undergone an HSCT. We thus matched again discounting the patients who had undergone HSCT and maintaining the other variables; all 99 non-transplanted patients in the MDS group could be adequately matched to 99/260 non-transplanted patients in the AML group. The median OS was significantly shorter in the AML compared to the MDS group; 5.3 versus 9.3 months respectively, P=0.001 **(Figure 3)**. Finally, we used the full dataset of 413 patients (AML + MDS) and proceeded with a CRT using the diagnosis (MDS vs AML), age ≥/< 60 years and *TP53* aberration status; the primary decision node was *TP53* status, with a 75%

mortality within 1 year in patients with *TP53*^{HR}. The *TP53* status predicted survival at 1 year more strongly than the diagnosis of MDS or AML **(Figure S14).**

HSCT, *TP53* status and outcomes in AML and MDS

A total of 32 patients (11%) (9 of 38 [24%] patients with *TP53*^{LR} and 23 of 253 [9%] patients with *TP53*^{HR}, P =0.02) with AML at a median age of 61.3 years (range, 20-73.6 years) could proceed to HSCT at a median of 3.7 months of therapy (range, 2.1-7.4 months) leading to a median survival of 14 months and 2-year OS of 32%; this was significantly superior to a landmark comparison of patients <70 years who attained a response but did not undergo HSCT (14 versus 9.4 months, *p* log-rank 0.001) **(Figure 4A-B).** When comparing the *TP53*^{HR} AML, again HSCT led to a statistically significant improvement in OS (12.6 versus 9 months, *P*= 0.009). Amongst the patients with AML who underwent an HSCT, 17 (7 *TP53*^{LR} and 16 *TP53*^{HR}) had a best response of MRD negative CRc before HSCT, and the other 15 patients (3 *TP53*^{LR} and 12 *TP53*^{HR}) were positive for MRD by flow cytometry (13 CRc, 1 MLFS and 1 stable disease). The median OS of patients with MRD negative before HSCT was 29.5 months compared to 10.0 months for those who were MRD positive before HSCT (p<0.001). Selecting only patients with TP53^{HR}, the median OS was 26.4 months who were MRD negative compared to 9.5 months who were MRD positive before HSCT (p=0.003) **(Figure 4C-D).**

In the MDS group 23 patients (18.8%) (median age of 63.4 years, range 18.2-76 years) underwent an HSCT [7 of 25 (28%) patients with *TP53*^{LR} and 16 of 97 (16%) patients with *TP53*^{HR}] with a median OS of 17.3 months and 2-year OS of 32%. The median time to HSCT post therapy initiation was 5.4 months (range 2.9-17.7 months). On a landmark analysis comparing transplanted patients to non-transplanted patients who had a response and <70 years of age at diagnosis, HSCT significantly improved OS (17.3 versus 12.4 months, P=0.02) **(Figure 4E-F).** On selecting only *TP53*^{HR} patients in the transplanted and comparator group, again transplanted patients had a superior OS (15.5 versus 11.9 months, P=0.05) on landmark analysis.

Assessment of factors affecting survival in AML

Focusing on the AML cohort we analyzed disease and treatment related factors that affected the rates of HSCT and survival.

Venetoclax, treatment intensity and outcomes in AML:

A CRc and OR was attained in 21/38 (55%) and 27/38 (71%) patients with *TP53*^{LR} compared to 108/253 (43%) (*P*=0.16) and 128/253 (36%) (*P*=0.02) patients with *TP53*^{HR}. On UVA, *TP53*^{LR} and use of venetoclax was associated with higher odds of response in AML while CK was associated with lower odds for OR. On MVA including CTG, *TP53* status, use of venetoclax and treatment intensity as variables, *TP53*^{LR} continued to favor odds of response compared to *TP53*^{HR} (Odds ratio 2.415, 95% CI 0.99-4.95) along with venetoclax (Odds ratio 2.09, 95% CI 1.30-3.78) while other variables were not independently significant **(Table S2).**

Two-hundred thirty-four (80%) patients with AML received a low-intensity therapy of whom 202 (86%) had *TP53*^{HR} **(Table S3).** Amongst the 57 patients treated with intensive therapy, 51 (89%) patients had *TP53*^{HR}. An OR was seen in 130 patients (56%) (CRc:44%) treated with low-intensity therapy and 107/202 (53%) (CRc: 43%) patients with *TP53*^{HR} treated with low-intensity therapy. In the intensively treated arm, an OR was seen in 25/57 (44%) (CRc:44%) and in 21/51 (41%) (CRc:41%) patients with *TP53*^{HR}. There was no difference in RFS and OS based on treatment intensity **(Figure S15).** The rates of HSCT were higher for patients treated with intensive therapy compared to low-intensity therapy (19% versus 9%, *P*=0.03) despite comparable response rates between the two arm, possibly because of the lower median age in the intensively treated patients (56.6 years versus 72.2 years, P<0.0001). In patients <60 years of age (n=63), there was no difference in OS between intensive and low-intensity therapy, irrespective of *TP53* burden **(Figure S16).**

With respect to venetoclax, overall, 90 patients (31%) had received venetoclax of whom 81 patients (90%) were treated with low-intensity therapy **(Table S4).** The rates of CRc and OR was higher in the patients treated with venetoclax containing regimens (54% and 66%) compared to those who did not receive venetoclax (40%, P=0.02 and 48%, P=0.005 respectively). When selecting only patients with *TP53*^{HR}, again, rates of CRc and OR was higher when patients were treated with venetoclax (54% vs. 37%, P=0.02 and 63% vs. 44%, P=0.005 respectively). However, HSCT rates, RFS and OS were similar between patients treated with or without venetoclax in the full AML cohort as well as *TP53*^{HR} AML group **(Figure S17)**. The 60-day mortality was similar in patients who received low-intensity therapy with or without venetoclax (15/81 [18%] versus 22/153 [14%], P=0.45). Characteristics and outcomes of patients treated only with HMA based low-intensity therapy is described in **Table S5**.

Finally, we did a Cox proportional hazard analysis to understand the independent significance of disease and treatment factors affecting survival in patients with AML. On UVA, using age </≥60 years, *de novo* versus secondary or therapy-related AML, *TP53*^{HR} versus *TP53*^{LR}, CTG (CK versus others), use of venetoclax and HSCT, *de novo* AML and HSCT were favorable risk factors while CK was adverse. Including these significant factors on a stepwise multivariate Cox, *de novo* AML (hr=0.73, 95% CI 0.57-0.93), *TP53*^{LR} (hr=0.58, 95% CI 0.38-0.86) and HSCT (hr=0.40, 95% CI 0.26-0.60) continued to remain significant **(Table 3A).** MVA for factors affecting survival only in patients who attained ORR is in **Table 3B.**

Discussion

We present a comprehensive analysis on the impact of *TP53* aberrations on outcomes among a large contemporary cohort of patients with *TP53* mutated AML and MDS. Our report includes well curated data at a single large academic center with >75% patients treated on clinical trials with a median follow-up of >6 years. Importantly, we have tried to analyze the clinical relevance of the *TP53* allele status on survival outcomes, and better define what constitutes truly high-risk *TP53* mutations in MDS and AML from a clinical standpoint. The focus of our analysis was to understand the interplay between MDS and AML (based on historical blast percentage cutoffs) and baseline *TP53* burden on clinical outcomes, and to study the impact of therapeutic interventions including HSCT, intensive vs non-intensive therapy, and venetoclax use on response and survival in relation to the baseline *TP53* burden in patients with AML. Using a machine learning algorithm, we also validated the cutoff of a single *TP53* mutation (without an allelic loss) in AML that is associated with inferior outcomes; our present finding of 37.5% is close to the 40% reported in previous analysis.

TP53^{HR} was associated with inferior ORR in patients with AML but not with MDS, and this remained significant even on MVA. Though median survival was <12 months in both the MDS and AML group, in mutation burden unstratified analysis, median survival for AML (defined as >/=20% blasts) was significantly shorter than MDS (5.9 versus 10.8 months). There was no difference in survival within the MDS group based on the blast percentage either for the entire population (5-10% vs >10% both with median OS approximately 11 months, **Figure S6)** or for the *TP53*^{HR}. Although studies have claimed diminutive (in some cases even irrelevant) effects of blast percentage defining MDS and AML on OS in patients with high burden *TP53* aberrations, in our analysis with a large number of well-annotated and contemporary patients we see a statistically better survival in patients with MDS (5-19%) compared to

patients with AML (>/=20% blasts). Although the outcomes remain dismal for both MDS and AML with *TP53*^{HR} it is important to have the OS expectations clearly defined and differentiated between these 2 populations to enable critical and realistic appraisal of emerging data from phase I/II single arm studies in the right context. For example, an OS of 12 months may be considered encouraging in a study of frontline *TP53*^{HR} AML but is very similar to expected historical outcomes in frontline *TP53*^{HR} MDS. In further interrogation towards this effect, we found on MVA that both the diagnosis (MDS or AML) as well as the *TP53* allele status had an independent impact on OS. However, on the CRT analysis, the *TP53*^{HR} status indeed carried more weight and had the most discriminatory role in predicting poor survival at 1 year. Putting these into perspective, we can draw the conclusion that both the diagnosis of AML and MDS as well as the *TP53* allele status at baseline are important prognostic variables.

Our study shows important evidence in assessing *TP53* aberration burden and that patients with *TP53*^{LR} have better outcomes in both MDS and AML, validated with independent statistical models. The lack of convergence of OS of patients with high-blast MDS and AML was surprising and different from analysis by other groups [17]. Though few patients in both disease groups proceeded to HSCT, we show that transplanted patients with MDS and AML had similar survival to each other, and led to improved OS on a landmark analysis comparing to patients who did not undergo HSCT; the independent benefit from HSCT was also maintained on multivariate Cox regression analysis. This again underlines the need to facilitate HSCT in patients with *TP53* aberrations whenever feasible. Though HSCT in *TP53* mutated myeloid disorders is often debated, it remains the only line of management which has the potential to offer an improved survival over any other form of non-transplant therapy and should be the goal after some form of BM remission is attained in a transplant eligible patient. The presence of *TP53* aberrations should in isolation not preclude patients from an HSCT. Next, we show that treatment intensity does not have a significant bearing on long-term survival outcomes. In patients <60 years of age, intensive and low-intensity therapy fared equivocally both in terms of response rates as well as OS. Though more patients treated with intensive therapy proceeded to an HSCT, this was a function of the lower median age and more patients <60 years of age in the intensive treated arm compared to the low-intensity treatment arm, which would have driven their therapy choice in the first place. In the context of venetoclax, we showed that despite the higher rates of CRc and OR in patients with AML who received venetoclax along with their treatment, these responses were not durable and did not lead to higher rates of HSCT or improved survival.

Our study had few limitations, primarily being the retrospective nature of this analysis. However, majority of our patients were treated on clinical trials and the analyses stem from well curated prospectively collected data. Enrollment of patients on clinical trials could however be associated with potential selection biases secondary to trial enrollment criteria, though clinical trials remain the ideal treatment decision for patents with high-risk AML (including *TP53*^{mut} AML) given dismal outcomes with standard of care therapy. Secondly the *TP53* allele loss call was from a combination of cytogenetic data, FISH and aCGH and might have missed some cn-LOH. Nonetheless the use of routine laboratory tools for the annotation of *TP53* aberration in our patients make the interpretation of this data clinically robust and widely adaptable.

In summary, our study shows that *TP53* aberrations (mutations and/or allelic loss) is an independent factor that affects survival outcomes, and this impact is dependent on the burden of this aberration. Secondly, the diagnosis of MDS or AML, and the burden of *TP53* aberration independently affect survival, and HSCT lead to equivalent improved outcomes in both diagnosis groups.

References:

1. Daver NG, Iqbal S, Huang J, et al. Clinical characteristics and overall survival among acute myeloid leukemia patients with TP53 gene mutation or chromosome 17p deletion. Am J Hematol. 2023;98(8):1176-1184.
2. Daver NG, Maiti A, Kadia TM, et al. TP53-Mutated Myelodysplastic Syndrome and Acute Myeloid Leukemia: Biology, Current Therapy, and Future Directions. Cancer Discov. 2022;12(11):2516-2529.
3. Kantarjian H, Kadia T, DiNardo C, et al. Acute myeloid leukemia: current progress and future directions. Blood Cancer J. 2021;11(2):41.
4. Bazinet A, Kantarjian HM. Moving toward individualized target-based therapies in acute myeloid leukemia. Ann Oncol. 2023;34(2):141-151.
5. Lachowiez CA, Long N, Saultz J, et al. Comparison and validation of the 2022 European LeukemiaNet guidelines in acute myeloid leukemia. Blood Adv. 2023;7(9):1899-1909.
6. Nechiporuk T, Kurtz SE, Nikolova O, et al. The TP53 Apoptotic Network Is a Primary Mediator of Resistance to BCL2 Inhibition in AML Cells. Cancer Discov. 2019;9(7):910-925.
7. Prokocimer M, Molchadsky A, Rotter V. Dysfunctional diversity of p53 proteins in adult acute myeloid leukemia: projections on diagnostic workup and therapy. Blood. 2017;130(6):699-712.
8. Desai PN, Wang B, Reville PK, et al. Single-Cell Characterization of TP53-Mutated AML Patients Treated with Frontline Azacitidine, Venetoclax, and Magrolimab Reveals Mechanisms of Response and Resistance. Blood. 2023;142(Supplement 1):64.
9. Daver N, Senapati J, Maiti A, et al. Phase I/II Study of Azacitidine (AZA) with Venetoclax (VEN) and Magrolimab (Magro) in Patients (pts) with Newly Diagnosed (ND) Older/Unfit or High-Risk Acute Myeloid Leukemia (AML) and Relapsed/Refractory (R/R) AML. Blood. 2022;140(Supplement 1):141-144.
10. Senapati J, Loghavi S, Reville PK, et al. Outcomes of Patients (pts) with Newly Diagnosed Acute Myeloid Leukemia (AML) and TP53 mutation/Loss Treated on the Phase 2 Study of Venetoclax (Ven) Added to Alternating Cladribine (Clad) Plus Low-Dose Cytarabine (LDAC) and Azacitidine (Aza): A Subgroup Analysis. Blood. 2023;142(Supplement 1):4282.
11. Garcia-Manero G, Goldberg AD, Winer ES, et al. Eprenetapopt combined with venetoclax and azacitidine in TP53-mutated acute myeloid leukaemia: a phase 1, dose-finding and expansion study. Lancet Haematol. 2023;10(4):e272-e283.
12. Daver NG, Iqbal S, Renard C, et al. Treatment outcomes for newly diagnosed, treatment-naïve TP53-mutated acute myeloid leukemia: a systematic review and meta-analysis. J Hematol Oncol. 2023;16(1):19.
13. Pollyea DA, Pratz KW, Wei AH, et al. Outcomes in Patients with Poor-Risk Cytogenetics with or without TP53 Mutations Treated with Venetoclax and Azacitidine. Clin Cancer Res. 2022;28(24):5272-5279.
14. Wei AH, Panayiotidis P, Montesinos P, et al. Long-term follow-up of VIALE-C in patients with untreated AML ineligible for intensive chemotherapy. Blood. 2022;140(25):2754-2756.
15. Döhner H, Pratz KW, DiNardo CD, et al. ELN Risk Stratification Is Not Predictive of Outcomes for Treatment-Naïve Patients with Acute Myeloid Leukemia Treated with Venetoclax and Azacitidine. Blood. 2022;140(Supplement 1):1441-1444.
16. Bataller A, Bazinet A, DiNardo CD, et al. Prognostic risk signature in patients with acute myeloid leukemia treated with hypomethylating agents and venetoclax. Blood Adv. 2024;8(4):927-935.
17. Grob T, Al Hinai ASA, Sanders MA, et al. Molecular characterization of mutant TP53 acute myeloid leukemia and high-risk myelodysplastic syndrome. Blood. 2022;139(15):2347-2354.
18. Bahaj W, Kewan T, Gurnari C, et al. Novel scheme for defining the clinical implications of TP53 mutations in myeloid neoplasia. J Hematol Oncol. 2023;16(1):91.

19. Short NJ, Montalban-Bravo G, Hwang H, et al. Prognostic and therapeutic impacts of mutant TP53 variant allelic frequency in newly diagnosed acute myeloid leukemia. Blood Adv. 2020;4(22):5681-5689.
20. DiNardo CD, Garcia-Manero G, Kantarjian HM. Time to blur the blast boundaries. Cancer. 2022;128(8):1568-1570.
21. Estey E, Hasserjian RP, Döhner H. Distinguishing AML from MDS: a fixed blast percentage may no longer be optimal. Blood. 2022;139(3):323-332.
22. DiNardo CD, Garcia-Manero G, Pierce S, et al. Interactions and relevance of blast percentage and treatment strategy among younger and older patients with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). Am J Hematol. 2016;91(2):227-232.
23. Döhner H, Wei AH, Appelbaum FR, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022;140(12):1345-1377.
24. Arber DA, Orazi A, Hasserjian RP, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. Blood. 2022;140(11):1200-1228.
25. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-2405.
26. ISCN 2016: An International System for Human Cytogenomic Nomenclature (2016): S.Karger AG; 2016.
27. Ok CY, Loghavi S, Sui D, et al. Persistent IDH1/2 mutations in remission can predict relapse in patients with acute myeloid leukemia. Haematologica. 2019;104(2):305-311.
28. Montalban-Bravo G, Kanagal-Shamanna R, Benton CB, et al. Genomic context and TP53 allele frequency define clinical outcomes in TP53-mutated myelodysplastic syndromes. Blood Adv. 2020;4(3):482-495.
29. Short NJ, Montalban-Bravo G, Hwang H, et al. Prognostic and therapeutic impacts of mutant TP53 variant allelic frequency in newly diagnosed acute myeloid leukemia. Blood Adv. 2020;4(22):5681-5689.
30. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424-447.
31. Cheson BD, Greenberg PL, Bennett JM, et al. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood. 2006;108(2):419-425.

Tables

Table 1: Baseline characteristics of the patients

Parameters		MDS (n=122)	AML (n=291)	*p*-value
		N (%), median[range]		
Age (years)		69 [18-90]	70 [20-87]	0.48
	Age ≥ 60 years	94 (77)	227 (78)	0.90
Gender	Female	58 (47)	138 (47)	0.99
Race	White	99 (81)	219 (75)	
	Black	9 (7)	27 (9)	
	Hispanic	5 (4)	10 (3)	
	Other	9 (7)	24 (8)	
Baseline CBC	Hb (g/L)	9.2 [6.9-13.8]	9.6 [6.3-13.2]	
	WBC (1x 10^9/L)	3.8 [0.6-13.5]	6.8 [0.1-77.3]	
	Platelet (1x 10^9/L)	37 [2-647]	30 [1-271]	
BM blasts on aspirate (%)		9 [5-18] *	32 [7-97] #	
Cytogenetics	Complex	104 (85)	254 (87)	0.63
***TP53* aberrations**	-17/del17p	42 (34)	148 (51)	0.002
	Single mutation	87 (71)	215 (74)	0.63
	>1 mutation	35 (29)	76 (26)	
Myeloid mutations	*ASXL1*	2 (2)	6 (2)	
	DNMT3A	6 (5)	29 (10)	
	FLT3 TKD	0	6 (2)	
	FLT3 ITD	0	9 (3)	
	IDH1	0	14 (5)	
	IDH2	2 (2)	6 (2)	
	NPM1	0	6 (2)	
	RAS	4 (3)	20 (7)	
	RUNX1	5 (4)	8 (3)	
	SF3B1	2 (2)	5 (2)	
	TET2	4 (3)	26 (9)	
Therapy related myeloid neoplasm		61 (50)	76 (26)	
Secondary AML		-	50 (17)	

*Blast percentages mentioned here are as per estimates on aspirate smear.

#Blast percentages mentioned here are as per estimates on aspirate smear. For patients with < 20% blasts on aspirate smear, bone marrow morphology or immunohistochemistry showed features suggestive of higher blast counts

Abbreviations: MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; CBC, complete blood count; Hb, hemoglobin; WBC, white blood cell count; BM, bone marrow

Table 2: Multivariate Cox proportional hazard model to analyze factors affecting overall survival in the full patient cohort (n=413).

Univariate analysis				Multivariate analysis				Evaluable	Covariate frequency (n/n)	Events (n/n)	Events (%/%)
Co-variates	**HR**	**95% CI**	**P value**	**Co-variates**	**HR**	**95% CI**	**P value**				
MDS	0.70	0.56-0.87	0.001	MDS	0.76	0.61-0.94	0.01	413	122/291	115/280	94/96
Age ≥ 60 years	1.07	0.84-1.36	0.59					413	322/91	307/88	95/97
TP53^{LR}	0.54	0.41-0.71	<0.0001	*TP53*^{LR}	0.66	0.48-0.89	0.008	413	63/350	57/338	90/97
CK	1.63	1.21-2.27	0.002	CK	1.23	0.89-1.74	0.23	410	357/53	347/46	97/87
Venetoclax	1.15	0.90-1.44	0.26					413	98/315	91/304	93/96
HSCT	0.37	0.27-0.51	<0.0001	HSCT	0.42	0.30-0.57	<0.0001	413	55/358	45/350	82/98

Abbreviations: HR, hazard ratio; MDS, myelodysplastic syndrome; CK, complex karyotype; HSCT, allogeneic hematopoietic stem cell transplantation

Table 3: Multivariate Cox proportional hazard model of factors affecting overall survival (OS) in AML

3A. Cox proportional hazard model of factors affecting OS in all patients with AML											
Univariate analysis				**Multivariate analysis**				**Evaluable**	**Covariate frequency (n/n)**	**Events (n/n)**	**Events (%/%)**
Co-variates	**HR**	**95% CI**	**P value**	**Co-variates**	**HR**	**95% CI**	**P value**				
Age ≥ 60 years	1.31	0.85-1.52	0.40					291	228/63	219/61	96/97
Denovo AML	0.74	0.59-0.94	0.01	Denovo AML	0.73	0.57-0.93	0.01	291	158/133	150/130	95/98
TP53^{LR}	0.51	0.35-0.73	0.0004	*TP53*^{LR}	0.58	0.38-0.86	0.009	291	38/253	33/247	87/98
CK	1.73	1.20-2.58	0.005	CK	1.16	0.77-1.80	0.50	290	254/36	249/30	98/83
Venetoclax	1.06	0.82-1.37	0.65					291	90/201	85/195	94/97
HSCT	0.36	0.23-0.53	<0.0001	HSCT	0.40	0.26-0.60	<0.0001	291	32/259	26/254	81/98
3B. Cox proportional hazard model of factors affecting OS in patients with AML who had an Overall Response											
Univariate analysis				**Multivariate analysis**				**Evaluable**	**Covariate frequency (n/n)**	**Events (n/n)**	**Events (%/%)**
Co-variates	**HR**	**95% CI**	**P value**	**Co-variates**	**HR**	**95% CI**	**P value**				
Age ≥ 60 years	1.22	0.81-1.91	0.36					155	126/29	118/27	94/93
Denovo AML	0.57	0.41-0.80	0.001	Denovo AML	0.49	0.34-0.70	<0.0001	155	85/70	78/67	92/96
TP53^{LR}	0.51	0.31-0.79	0.004	*TP53*^{LR}	0.50	0.29-0.83	0.01	155	27/128	22/123	81/96
CK	1.80	1.14-2.99	0.02	CK	0.78	0.45-1.33	0.41	155	129/26	125/20	97/77
Venetoclax	1.19	0.84-1.66	0.32					155	59/96	54/91	91/95
HSCT	0.46	0.29-0.70	0.0006	HSCT	0.48	0.30-0.73	<0.0001	155	31/124	25/120	81/97

Abbreviations: HR, hazard ratio; AML, acute myeloid leukemia; CK, complex karyotype; HSCT, allogeneic hematopoietic stem cell transplantation

Figure Legends

Figure 1: Comparative relapse free survival and overall survival of the full myelodysplastic syndrome and acute myeloid leukemia cohorts

A. Relapse free survival (RFS)
B. Overall survival (OS)

Figure 2: Overall Survival (OS) of patients stratified by the *TP53* aberration burden.

A. OS in the full cohort based on *TP53* aberration
B. OS in the myelodysplastic syndrome (MDS) cohort based on *TP53* aberration
C. OS in the acute myeloid leukemia (AML) cohort based on *TP53* aberration

Figure 3: Propensity matched survival outcomes between patients with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML)

A. Unselected propensity matching

   *this matching incidentally selected all transplanted patients in both groups (matched 23/23 transplanted patients in the MDS group to 23/31 transplanted patients in the AML group)

B. Propensity matching excluding transplanted patients

   #all 99 non-transplanted patients in the MDS cohort could be adequately matched to 99/260 non-transplanted patients in the AML group

Figure 4: Outcomes with allogeneic hematopoietic stem cell transplantation (HSCT)

A-B: Landmark comparison based on transplant in the AML cohort

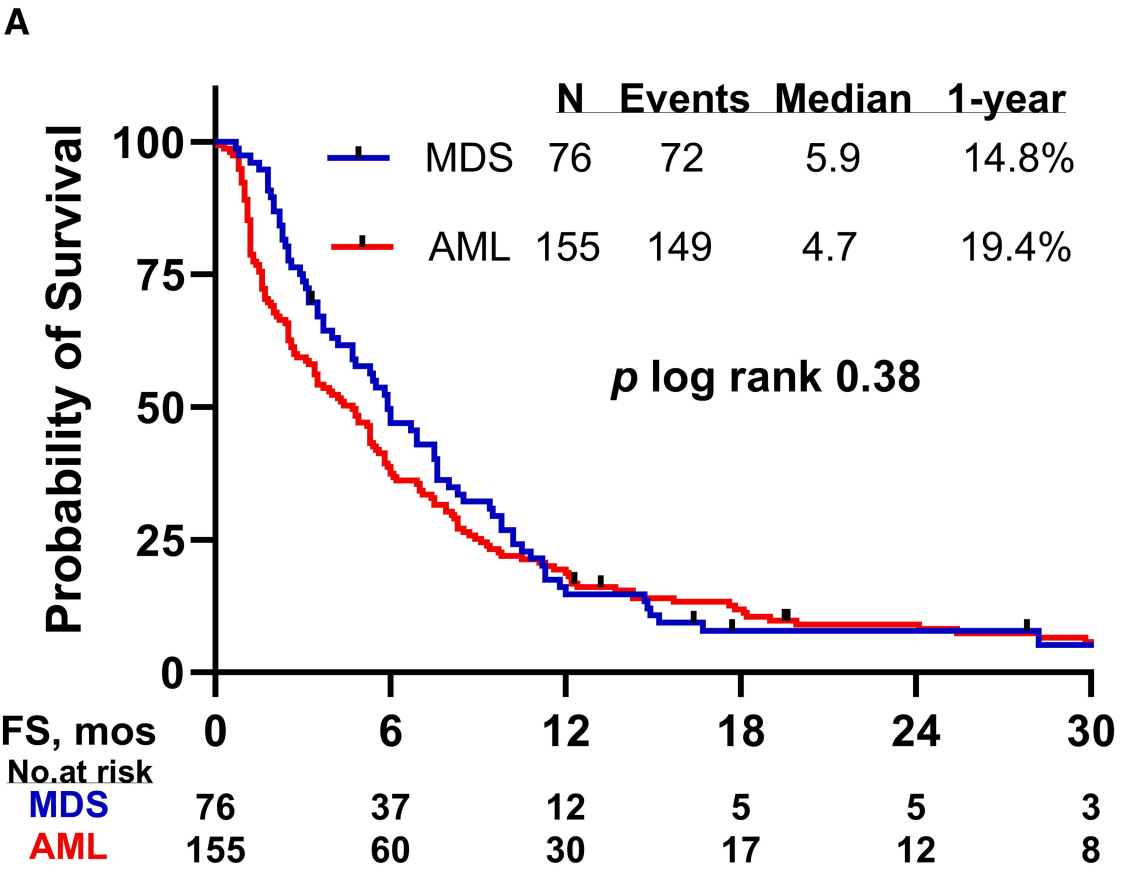
A. Overall survival of transplanted versus non-transplanted patients
B. Overall survival of transplanted versus non-transplanted patients with *TP53*^{HR}

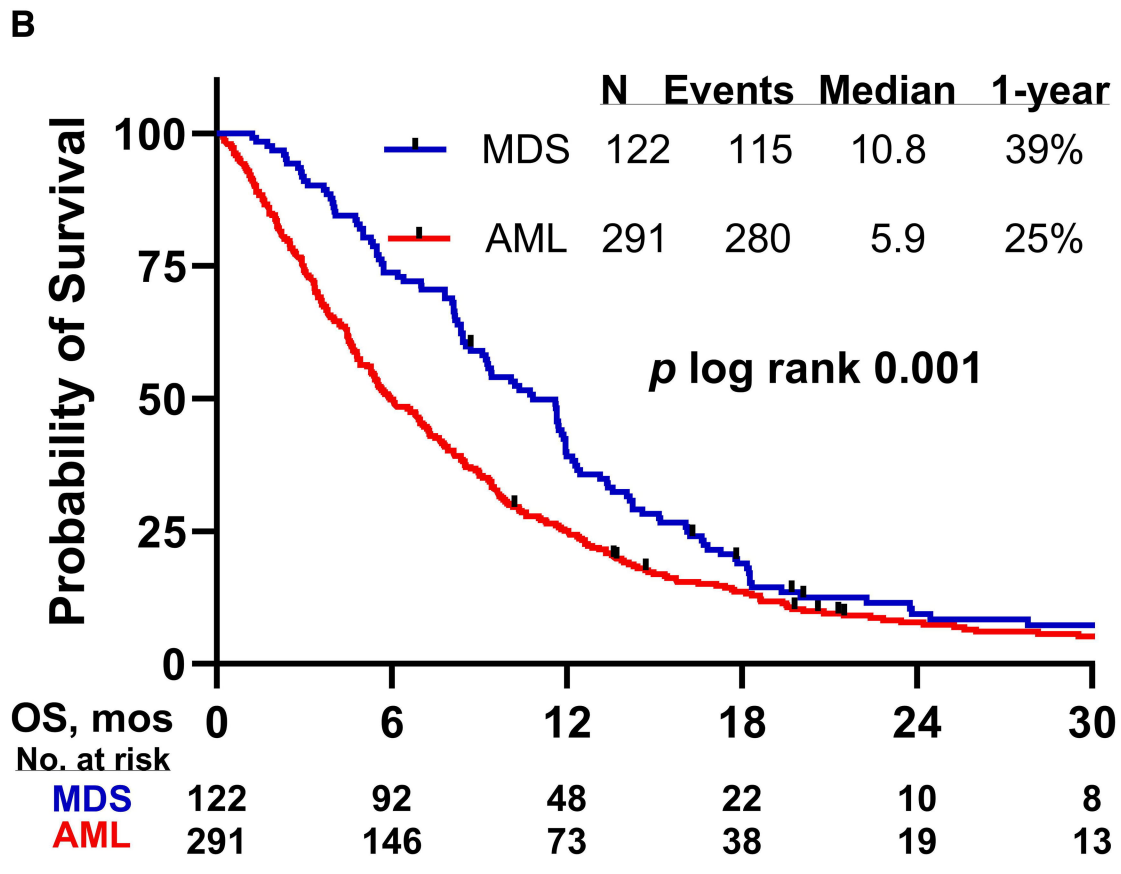
B-C: Outcomes with allogeneic hematopoietic stem cell transplantation (HSCT) in the acute myeloid leukemia cohort stratified by the pre-transplant measurable residual disease

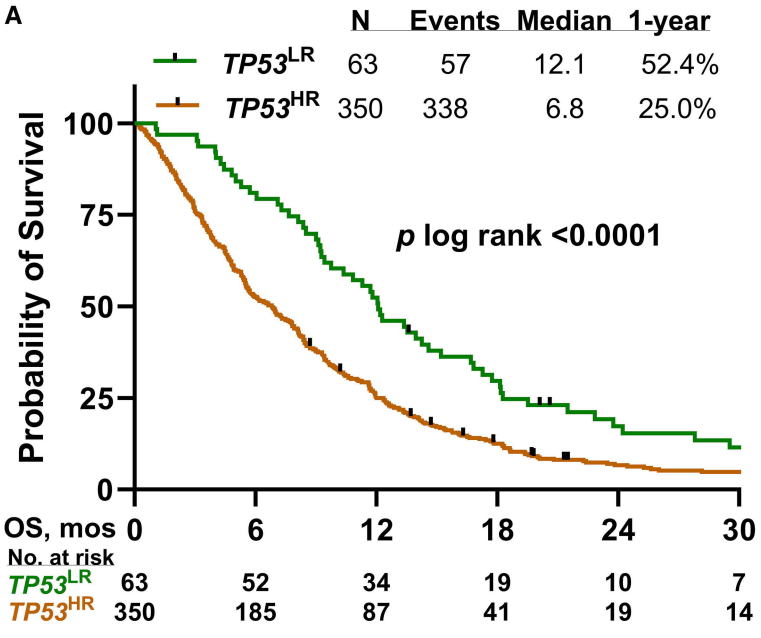
C. Overall survival in the transplanted acute myeloid leukemia cohort
D. Overall survival in the transplanted acute myeloid leukemia cohort with *TP53*^{HR}

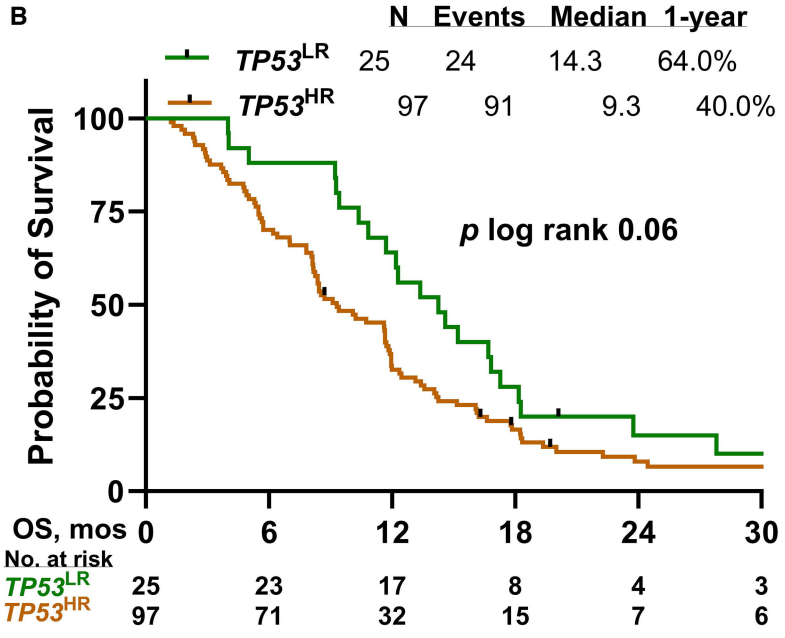
E-F: Landmark comparison based on allogeneic hematopoietic stem cell transplantation in the myelodysplastic syndrome (MDS) cohort

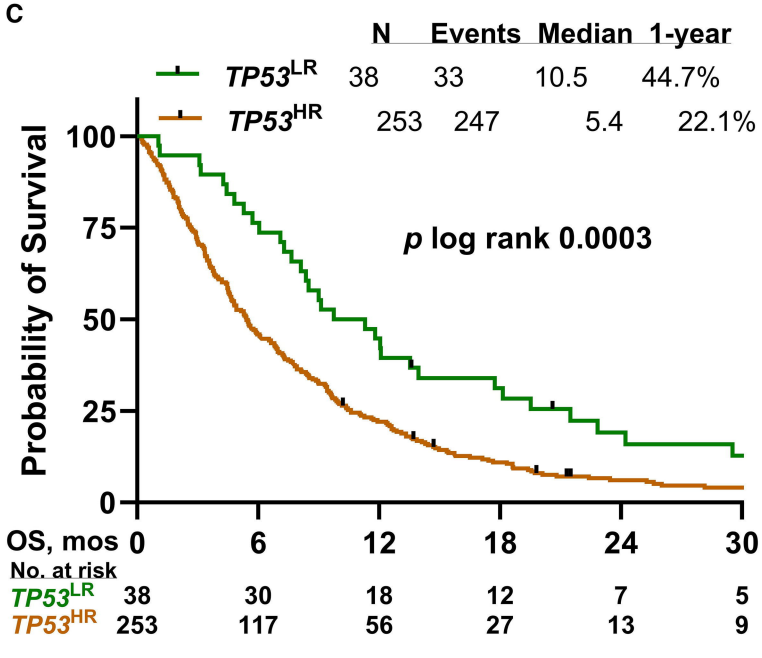
E. Overall survival of transplanted versus non-transplanted patients
F. Overall survival versus transplanted versus non-transplanted patients with *TP53*^{HR}

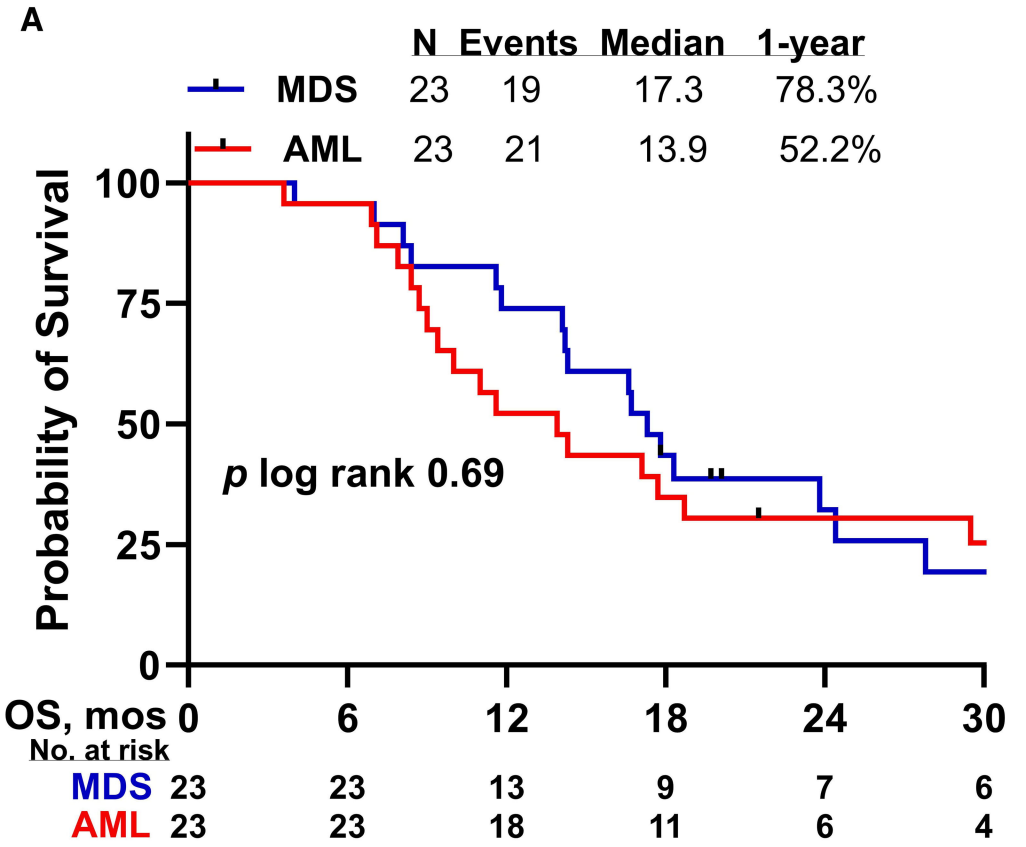

A
N Events Median 1-year
MDS 76 72 5.9 14.8%
AML 155 149 4.7 19.4%
p log rank 0.38
Probability of Survival
100
75
50
25
0
RFS, mos
0
6
12
18
24
30
No.at risk
MDS 76 37 12 5 5 3
AML 155 60 30 17 12 8

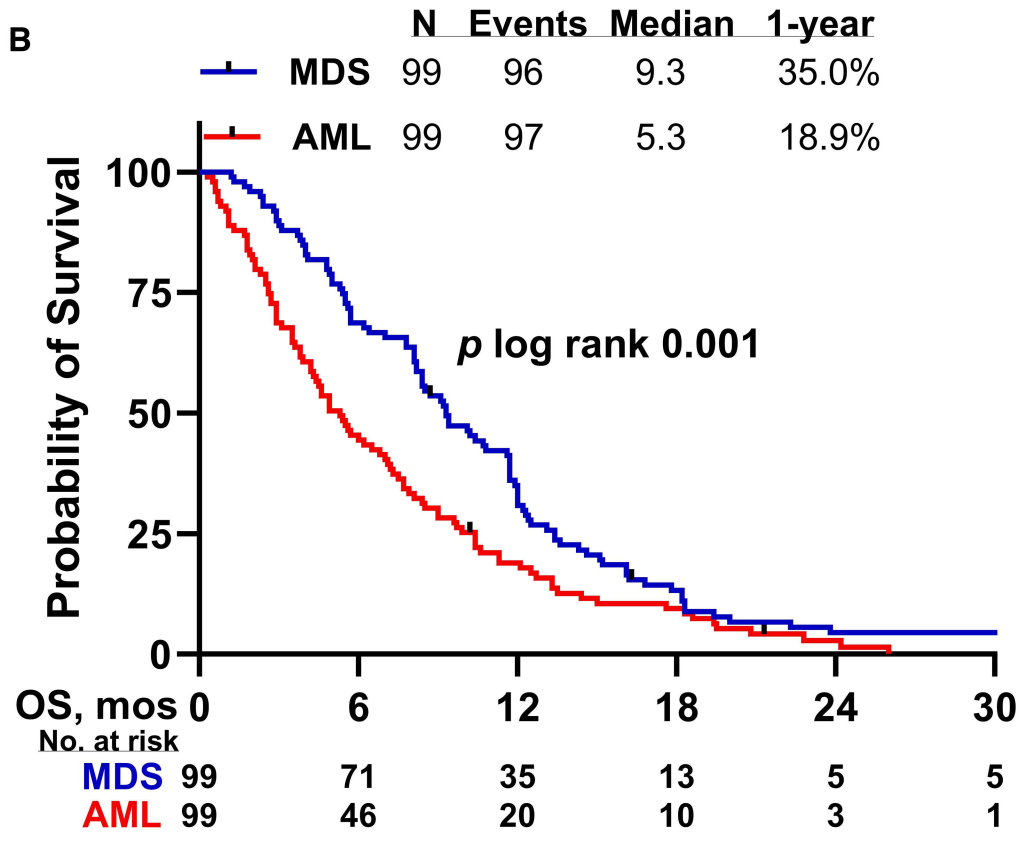

B
N Events Median 1-year
MDS 122 115 10.8 39%
AML 291 280 5.9 25%
p log rank 0.001
Probability of Survival
100
75
50
25
0
OS, mos
0
6
12
18
24
30
No. at risk
MDS 122 92 48 22 10 8
AML 291 146 73 38 19 13

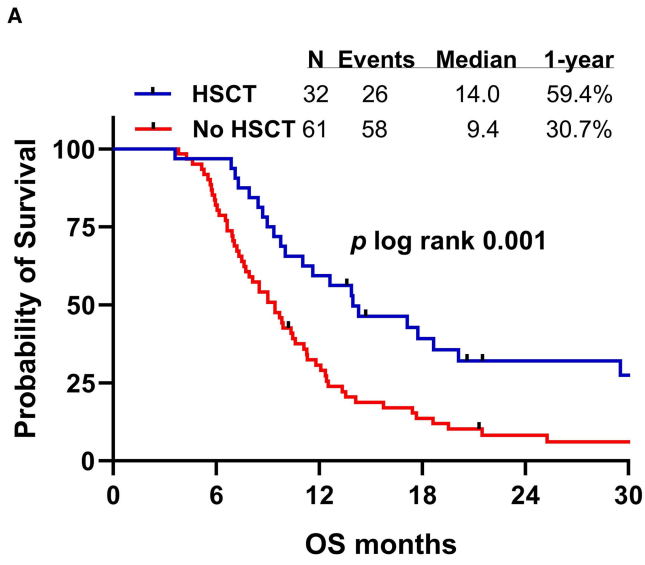

A
N Events Median 1-year
TP53LR 63 57 12.1 52.4%
TP53HR 350 338 6.8 25.0%
p log rank <0.0001
Probability of Survival
100
75
50
25
0
OS, mos 0 6 12 18 24 30
No. at risk
TP53LR 63 52 34 19 10 7
TP53HR 350 185 87 41 19 14

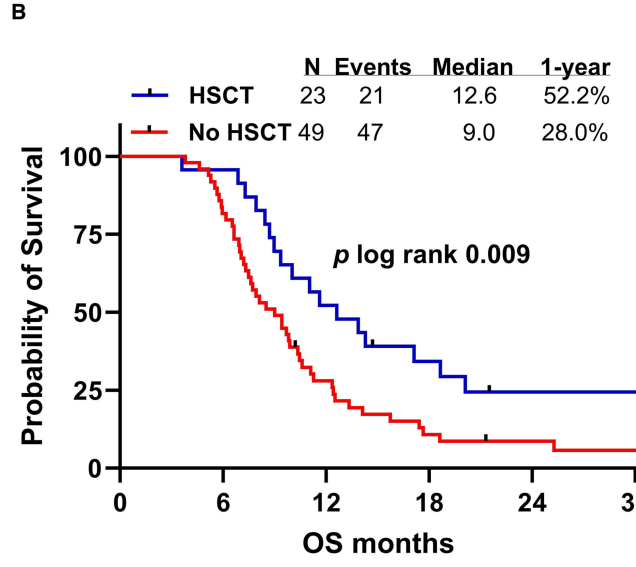

B
N Events Median 1-year
TP53LR 25 24 14.3 64.0%
TP53HR 97 91 9.3 40.0%
p log rank 0.06
Probability of Survival
100
75
50
25
0
OS, mos 0 6 12 18 24 30
No. at risk
TP53LR 25 23 17 8 4 3
TP53HR 97 71 32 15 7 6

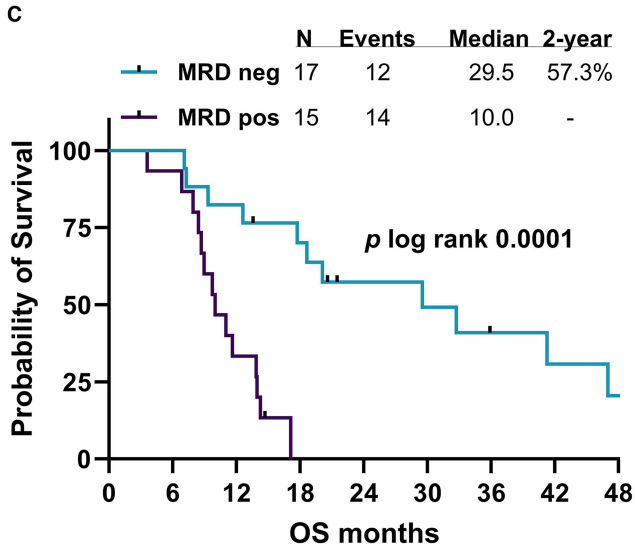

C
N Events Median 1-year
TP53LR 38 33 10.5 44.7%
TP53HR 253 247 5.4 22.1%
p log rank 0.0003
Probability of Survival
100
75
50
25
0
OS, mos 0 6 12 18 24 30
No. at risk
TP53LR 38 30 18 12 7 5
TP53HR 253 117 56 27 13 9

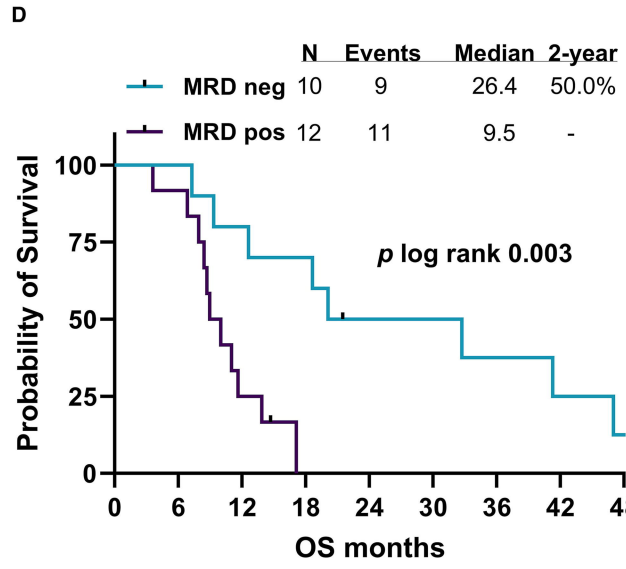

A
N Events Median 1-year
MDS 23 19 17.3 78.3%
AML 23 21 13.9 52.2%
Probability of Survival
100
75
50
25
0
p log rank 0.69
OS, mos 0 6 12 18 24 30
No. at risk
MDS 23 23 13 9 7 6
AML 23 23 18 11 6 4

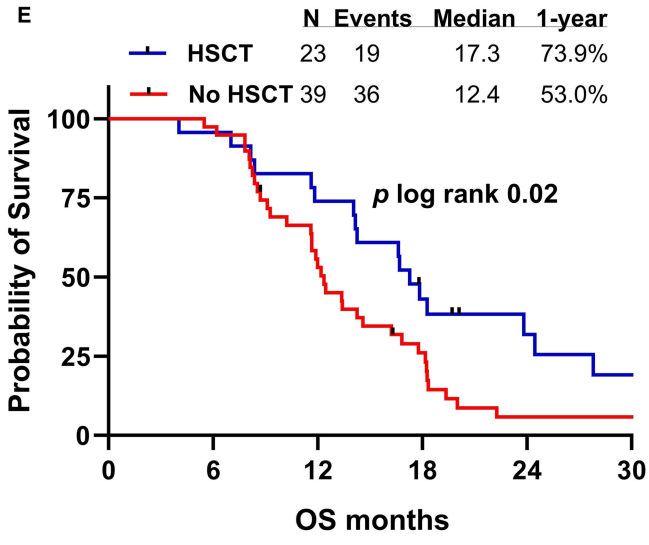

B
N Events Median 1-year
MDS 99 96 9.3 35.0%
AML 99 97 5.3 18.9%
Probability of Survival
100
75
50
25
0
p log rank 0.001
OS, mos 0 6 12 18 24 30
No. at risk
MDS 99 71 35 13 5 5
AML 99 46 20 10 3 1

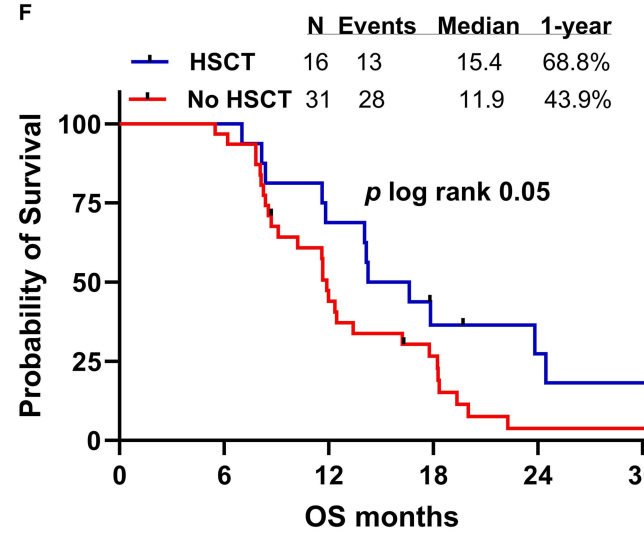

A
N Events Median 1-year
HSCT 32 26 14.0 59.4%
No HSCT 61 58 9.4 30.7%
p log rank 0.001
Probability of Survival
OS months


B
N Events Median 1-year
HSCT 23 21 12.6 52.2%
No HSCT 49 47 9.0 28.0%
p log rank 0.009
Probability of Survival
OS months


C
N Events Median 2-year
MRD neg 17 12 29.5 57.3%
MRD pos 15 14 10.0 -
p log rank 0.0001
Probability of Survival
OS months


D
N Events Median 2-year
MRD neg 10 9 26.4 50.0%
MRD pos 12 11 9.5 -
p log rank 0.003
Probability of Survival
OS months


E
N Events Median 1-year
HSCT 23 19 17.3 73.9%
No HSCT 39 36 12.4 53.0%
p log rank 0.02
Probability of Survival
OS months


F
N Events Median 1-year
HSCT 16 13 15.4 68.8%
No HSCT 31 28 11.9 43.9%
p log rank 0.05
Probability of Survival
OS months

Supplemental Data

Clinical interrogation of *TP53* aberrations and its impact on survival in patients with myeloid neoplasms

Jayastu Senapati[1]*, Sanam Loghavi[2]*, Guillermo Garcia-Manero[1], Guillin Tang[2] Tapan Kadia[1], Nicholas J. Short[1], Hussein A. Abbas[1], Naszrin Arani[3], Courtney D. DiNardo[1], Gautam Borthakur[1], Naveen Pemmaraju[1], Betul Oran[4], Elizabeth Shpall[4], Uday Popat[4], Richard Champlin[4], Sherry Pierce[1], Sankalp Arora[3], Ghayas Issa[1], Musa Yilmaz[1], Keyur Patel[1], Koichi Takahashi[1], Guillermo Montalban-Bravo[1], Danielle Hammond[1], Fadi G. Haddad[1], Farhad Ravandi[1], Hagop M. Kantarjian[1], Naval G. Daver[1]

[1]Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston.

[2]Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston.

[3]Department of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston.

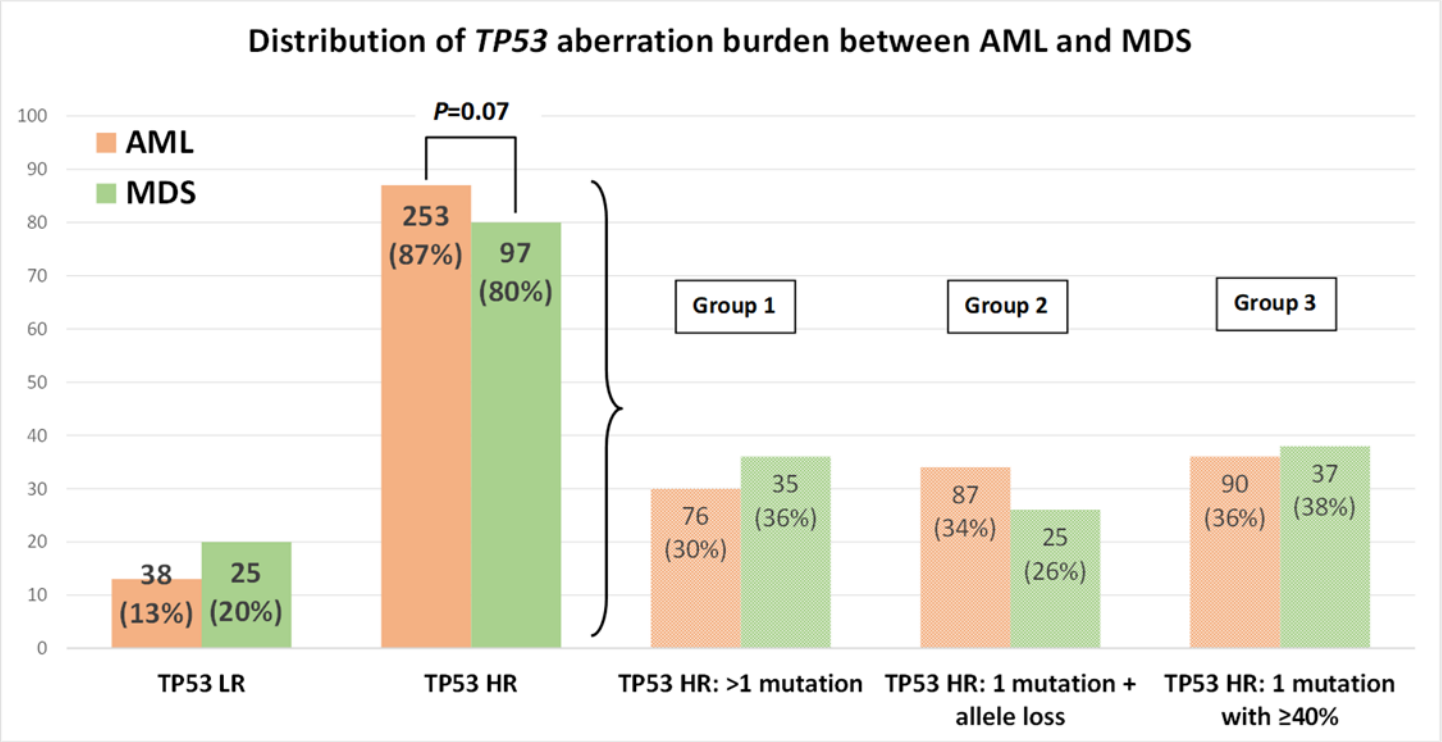
[4]Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston.

* These authors contributed equally

Pages: 22
Figures: 17
Tables: 5

(Sequence of tables and figures in this supplement is according to the appearance in the manuscript)

Figure S1: Types of *TP53* aberration amongst the patients stratified based on the diagnosis of MDS or AML



Abbreviations: LR, low risk; HR, high-risk; VAF, variant allele fraction

Figure S2: Overall survival of patients with multi-hit *TP53* mutations stratified by the sum of VAFs (<50% versus ≥50%)

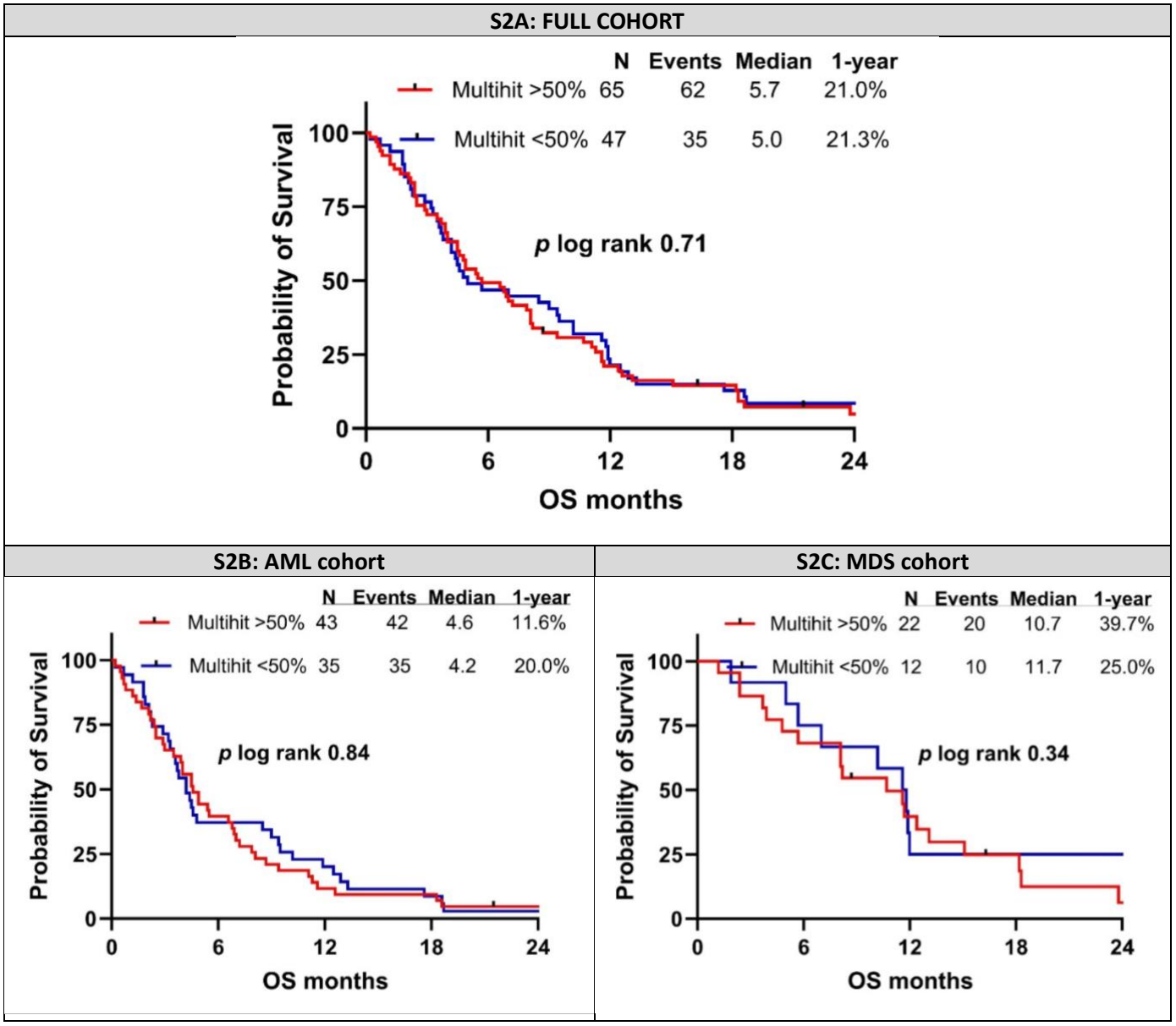


Figure S3: Classification and regression tree (CRT) model predicting the VAF cutoff for inferior survival at 1 year in patients with AML harboring a single-hit *TP53* mutation (n=128); **ROC AUC= 0.68**

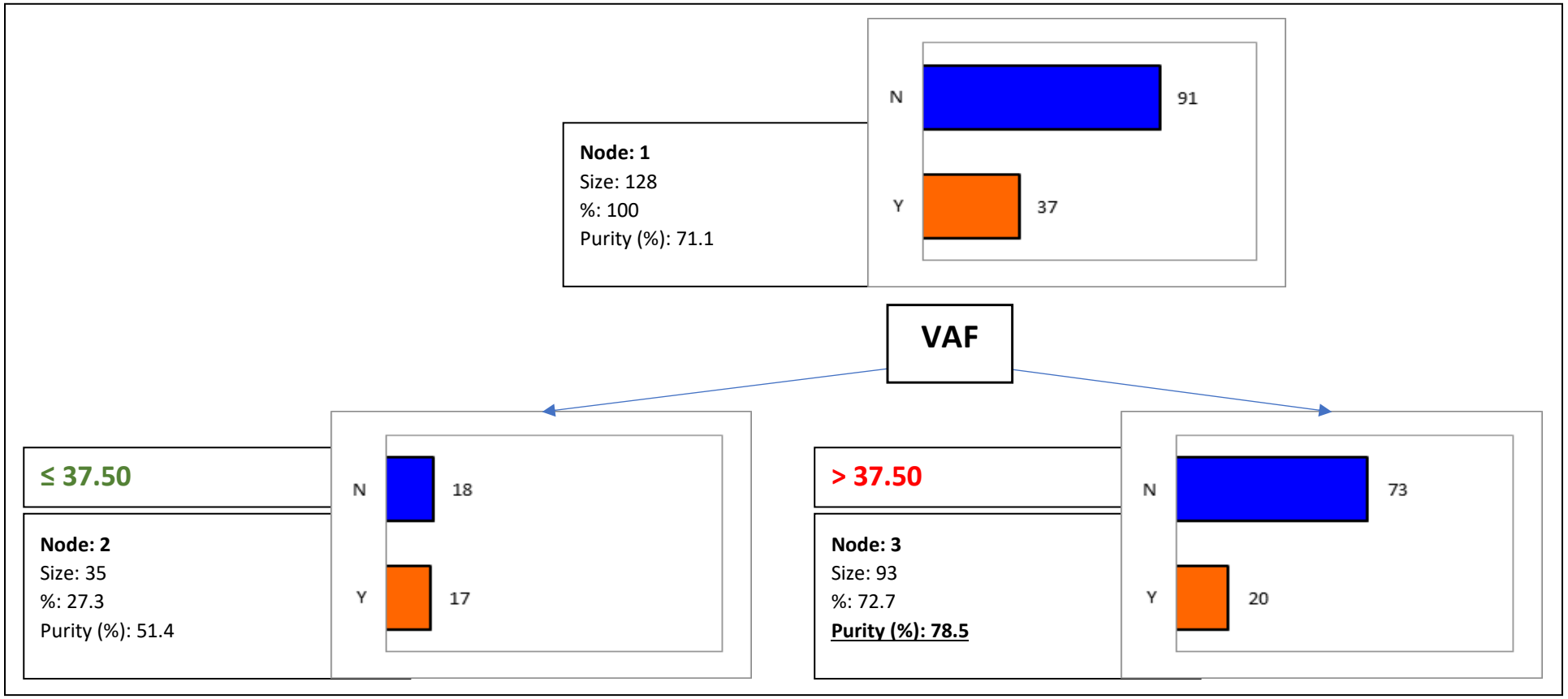


Figure S4: Classification and regression tree (CRT) model predicting the VAF cutoff for inferior survival at 1 year in patients with AML harboring a single-hit *TP53* mutation (n=128); Depth level of 2, **ROC AUC= 0.73**

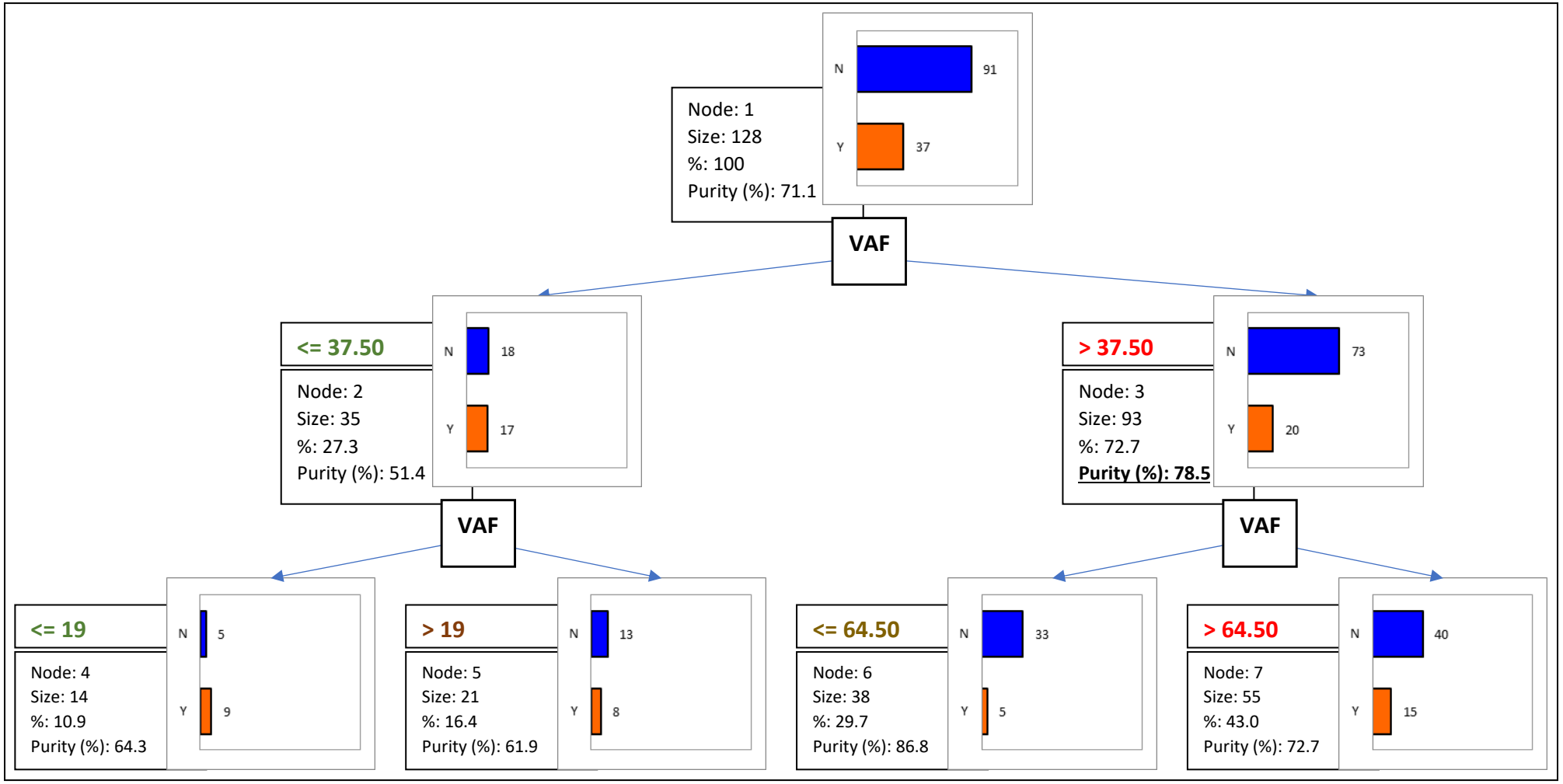


Figure S5: OS of patients with AML harboring a single *TP53* mutation stratified based on VAF cutoffs predicted from the CRT model.

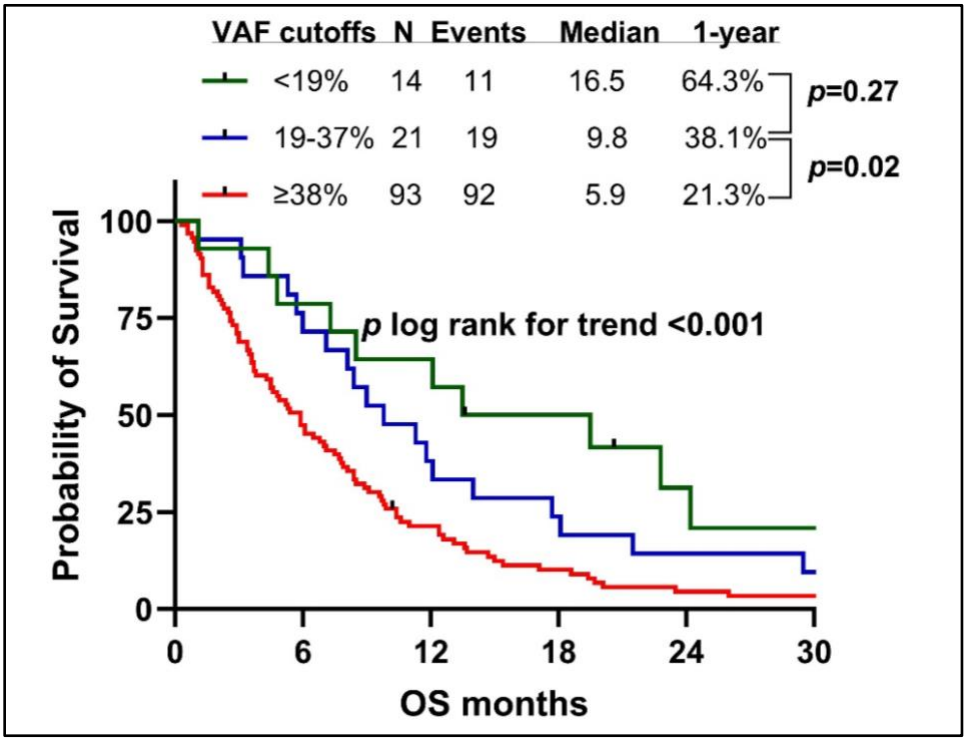


Figure S6: Representation of the type of *TP53* mutation in the full patient cohort

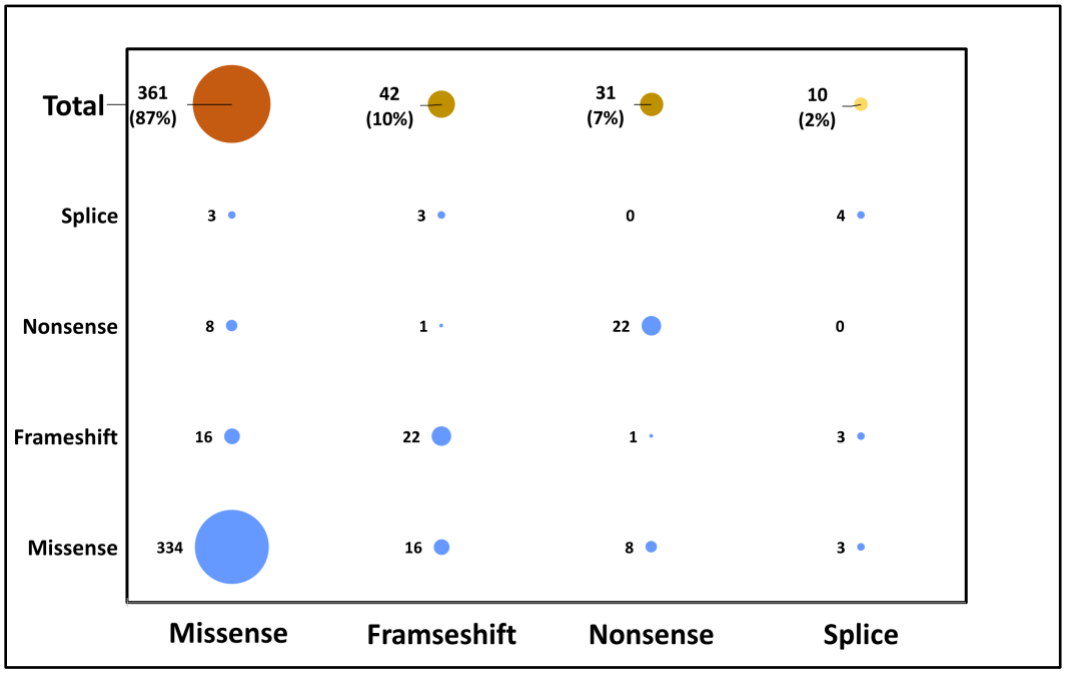


Table S1A-B: Details of treatment regimens

A: AML cohort

With Venetoclax (90/291 [30.9%]); n (%)			
Intensive Chemotherapy (9/90 [10%])		**Low-intensity therapy (81/90 [90%])**	
FLAG/FLAG-IDA	5	Decitabine	58
CLIA	3	CLAD/LDAC	6
MEC	1	AZA	2
		AZA+ APR246	6
		AZA+ Pevenidostat	4
		Aza-Magrolimab	2
		Aza/Decitabine+ FLT3i	2
		Decitabine + GO	1
Without Venetoclax (201/291[69.1%])			
Intensive Chemotherapy (48/201 [23.9%])		**Low-intensity therapy (153/201 [76.1%])**	
CLIA	14	Decitabine*	41
FA/FA-IDA	8	CLAD/LDAC	27
IA + Nivolumab	7	Decitabine + Vosaroxin	13
IA	6	Decitabine + Ruxolitinib	7
CIA	5	Decitabine + SGN-CD33A	2
CPX	4	Decitabine + BP1001	1
		Decitabine+ Clofarabine	1
7+3	2	Guadecitabine (SG110)*	14
CAT/CECA	2	Guadecitabine+ CLAD	7
		AZA*	6
		AZA+ Magrolimab	12
		AZA+ Nivolumab/Pembro	6
		AZA+ Lenalidomide	4
		AZA+ Vorinostat/Pracinostat	4
		AZA+ FLT3i	2
		AZA+ Rigosertib	1
		AZA+ Enasidenib	1
		LDAC + Daunorubicin	1
		LDAC + Omacetaxine	1

Abbreviations: FLAG, fludarabine/intermediate dose cytarabine/G-CSF; IDA, idarubicin; CLIA, cladribine /idarubicin/intermediate dose cytarabine; MEC, mitoxantrone, etoposide, intermediate dose cytarabine; CLAD, cladribine; LDAC, low dose cytarabine; AZA, azacitidine; APR246, eprenetapopt; FLT3i, FMS like tyrosine kinase 3 inhibitor; GO, gemtuzumab ozogamicin; FA, fludarabine. Intermediate dose cytarabine; IA, idarubicin, intermediate dose cytarabine; CIA, clofarabine, idarubicin, intermediate dose cytarabine; 7+3, 7 days of continuous infusion cytarabine and 3 days of daunorubicin; CAT, cyclophosphamide /cytarabine/topotecan; CECA, cyclophosphamide, etoposide, carboplatin; intermediate dose cytarabine; Pembro, pembrolizumab

*These groups denote HMA monotherapy

SGN CD33A: CD33-targeting antibody-drug conjugate using a pyrrolobenzodiazepine dimer

BP1001: Liposomal Grb2 antisense oligonucleotide

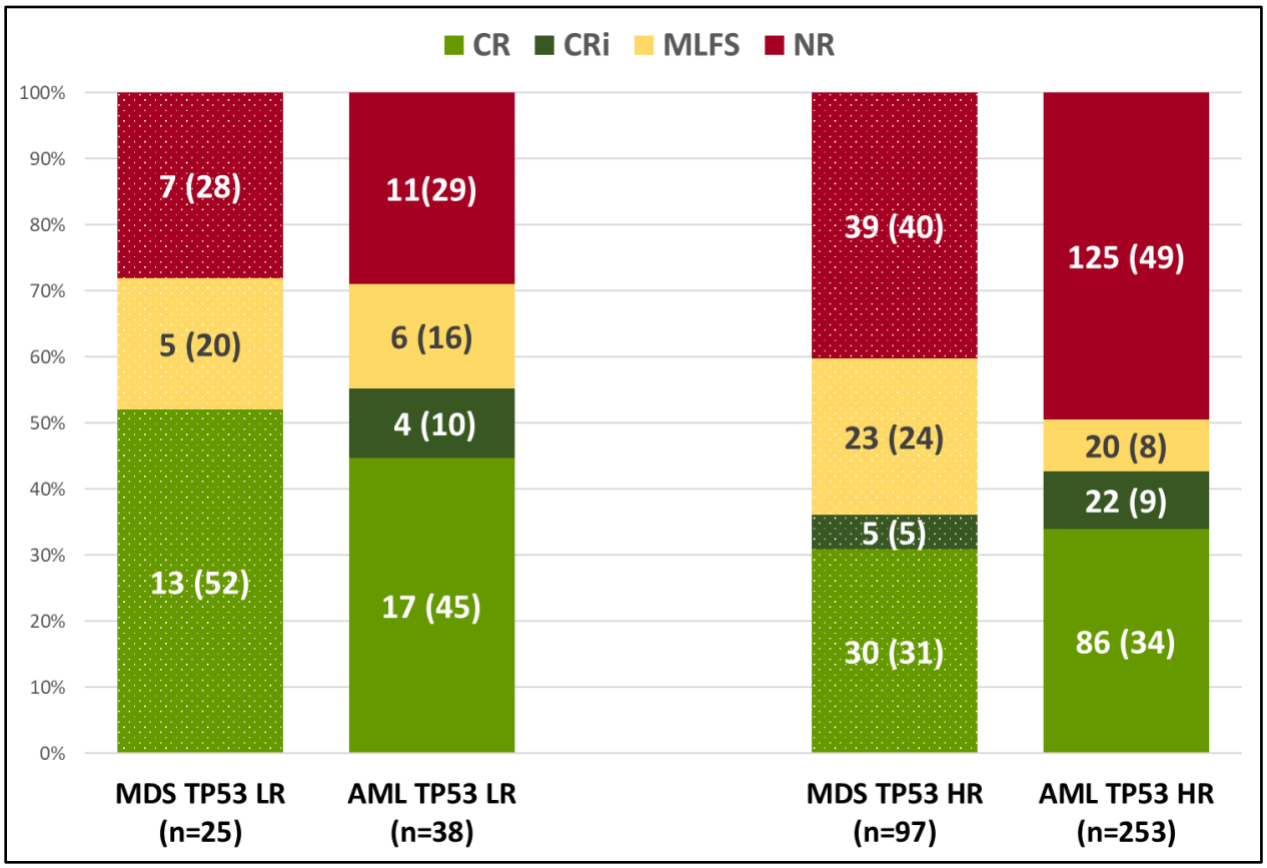
B: MDS cohort

With Venetoclax (7/122 [5.7%])	
Decitabine	6
AZA	1
Without Venetoclax (115/122 [94.3%])	
Decitabine	24
Guadecitabine (SG110)	22
AZA	12
AZA+ IPI/PEMBRO/NIVO	20
AZA+ Magrolimab/other CD47 blocker	8
AZA+ Lenalidomide	6
AZA+ APR 246	6
AZA+ Pracinostat	4
AZA+ CB839	4
AZA+ Lirilumab	2
AZA+ Rigosertib	1
CLAD/LDAC	4
Lenalidomide	1
FF-10501-01	1

Abbreviations: AZA, azacitidine; IPI, ipilimumab; PEMBRO, pembrolizumab; NIVO, nivolumab; APR246, eprenetapopt; CLAD, cladribine; LDAC, low dose cytarabine

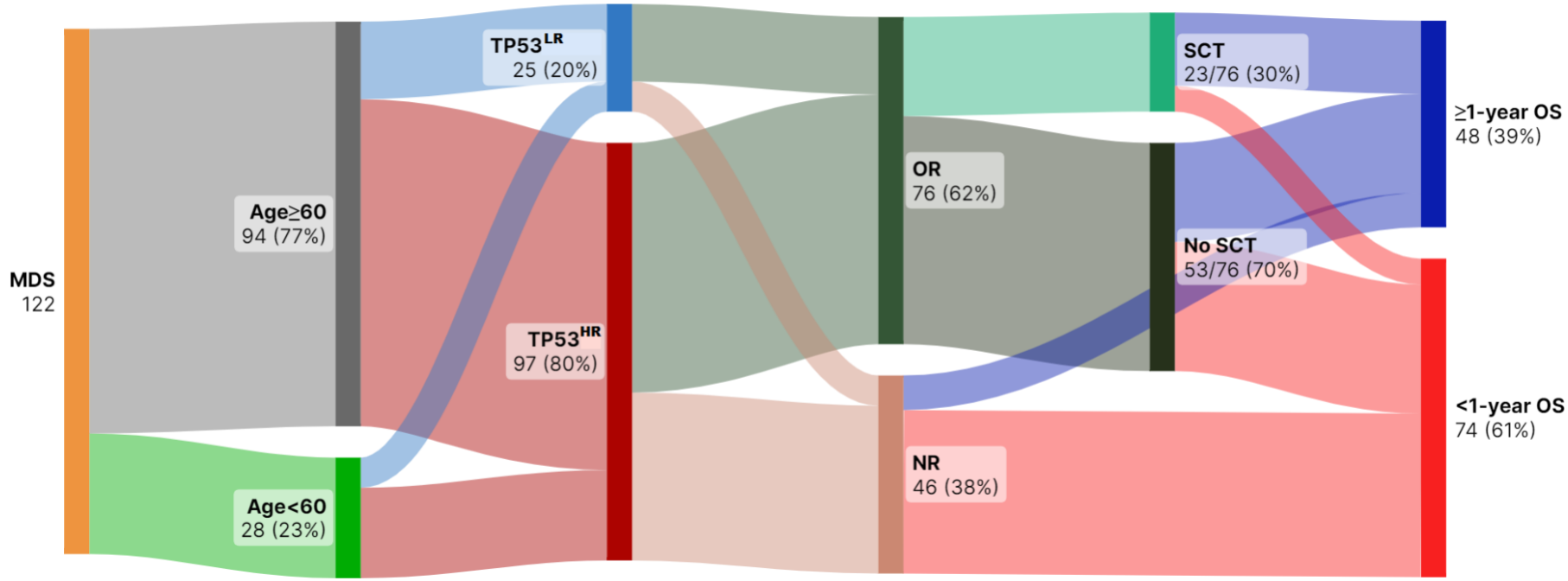
CB839: Glutaminase inhibitor
FF-10501-01: Inosine-5-monophosphate dehydrogenase inhibitor

Figure S7: Response rates in the MDS and AML cohort based on the *TP53* aberration burden.



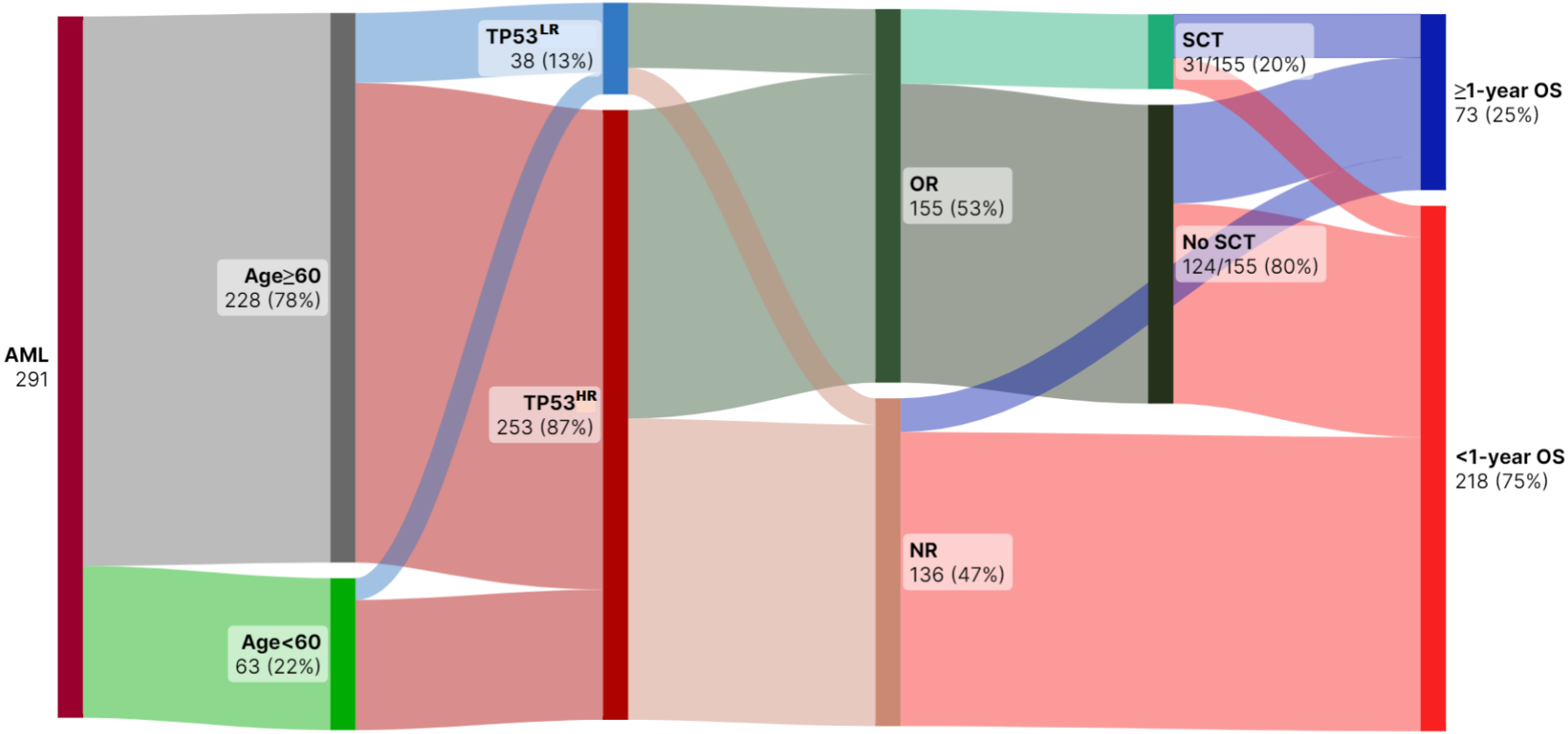
Abbreviations: CR, complete remission; CRi, CR with incomplete counts recovery; MLFS, morphological leukemia free state; NR, no response; TP53 LR, *TP53*^{LR}; TP53 HR, *TP53*^{HR}

Figure S8A: Sankey Diagram of patients with MDS showing age, *TP53* burden, response rates, allogeneic stem cell transplantation and survival at 1 year.



Abbreviations: MDS, myelodysplastic syndrome; OR, overall response; NR, no response, SCT, allogeneic stem cell transplnatation, OS, overall survival

Figure 8B: Sankey Diagram of patients with AML showing treatment intensity, *TP53* burden, response rates, allogeneic stem cell transplantation (SCT)* and survival at 1 year.

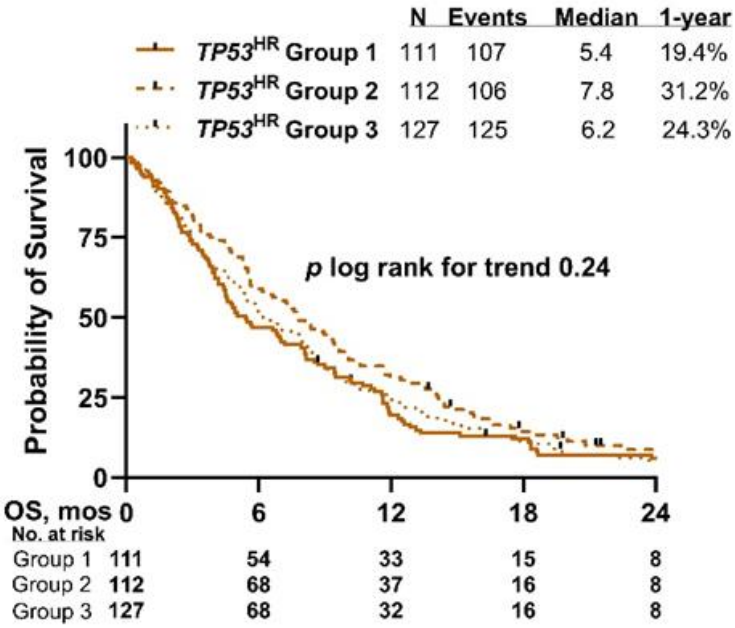


*Total 32 patients with AML underwent SCT, 31 patients after attainment of an OR and one patient with stable disease.

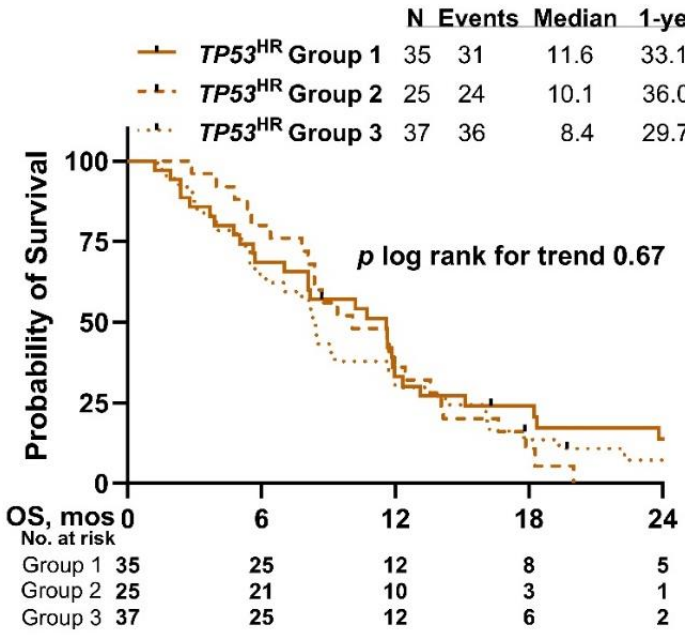
Abbreviations: AML, acute myeloid leukemia; OR, overall response; NR, no response, SCT, allogeneic stem cell transplnatation, OS, overall survival

Figure S9: OS in the *TP53*^{HR} group based on the type of *TP53* aberrations

S9A: OS in the full cohort based on type of *TP53*^{HR} aberration



S9B: OS in the MDS cohort based on *TP53*^{HR} aberration



S9C: OS in the AML cohort based on *TP53*^{HR} aberration

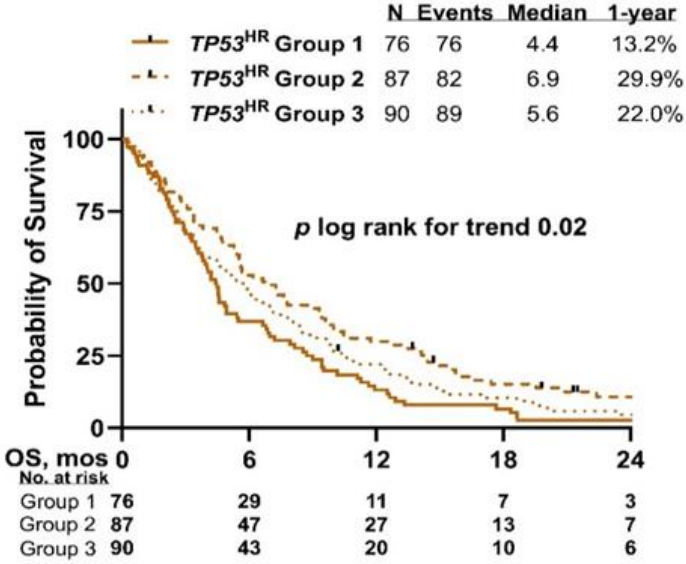
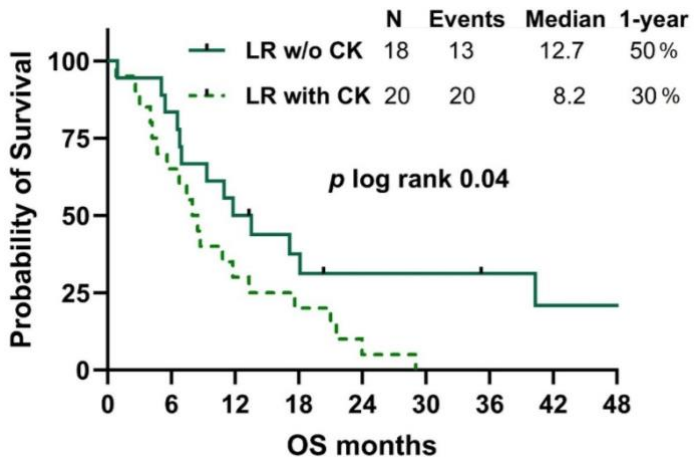
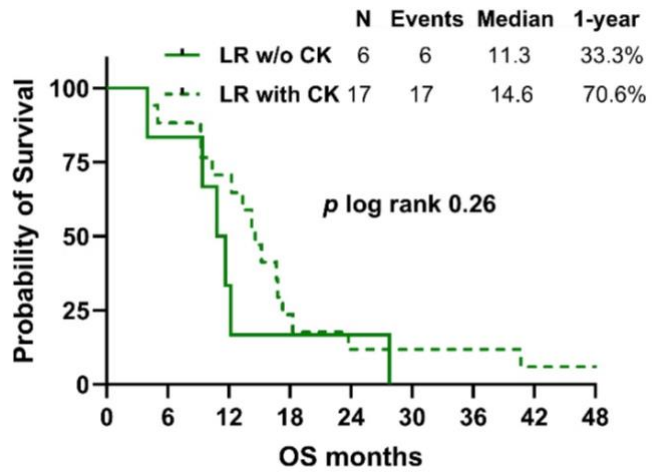


Figure S10: Overall survival (OS) of patients with AML or MDS and *TP53*^{LR} stratified by complex karyotype

S10A: *TP53*^{LR} AML



S10B: *TP53*^{LR} MDS



Abbreviations: LR, *TP53*^{LR}; w/o, without; CK, complex karyotype

Figure S11: Overall survival (OS) of patients with MDS and AML stratified by BM blast percentage

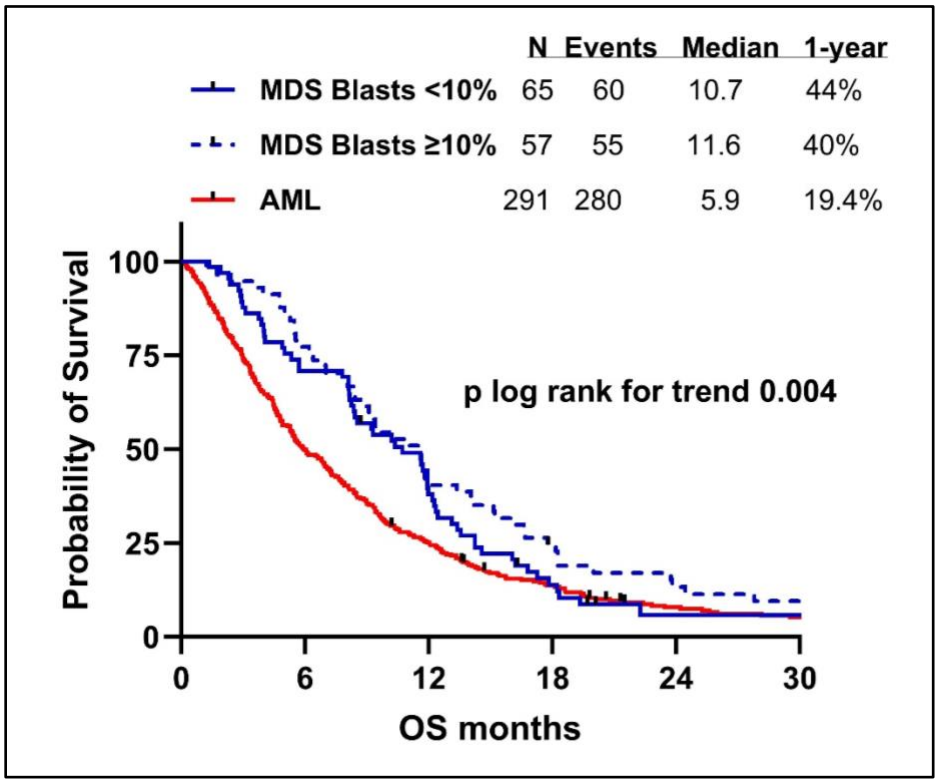


Figure S12: Comparison of OS of patients in the MDS group to the AML group stratified by the *TP53* aberration status.

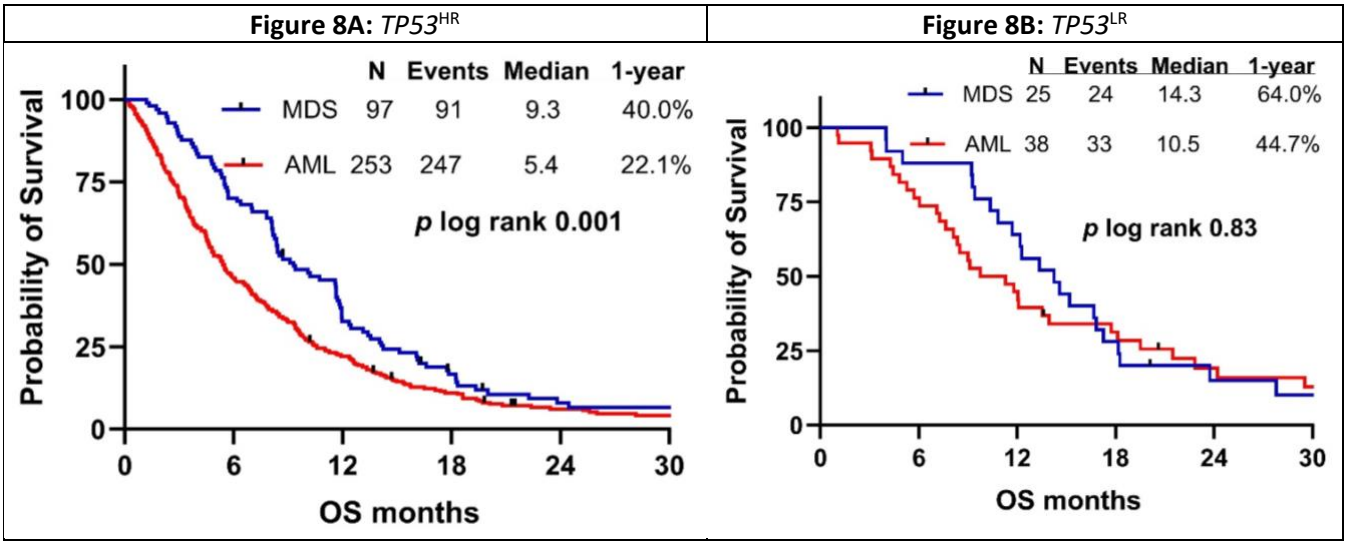


Figure S13: Comparison of OS of patients with MDS versus patients with AML who had *TP53*[HR] and underwent an HSCT.

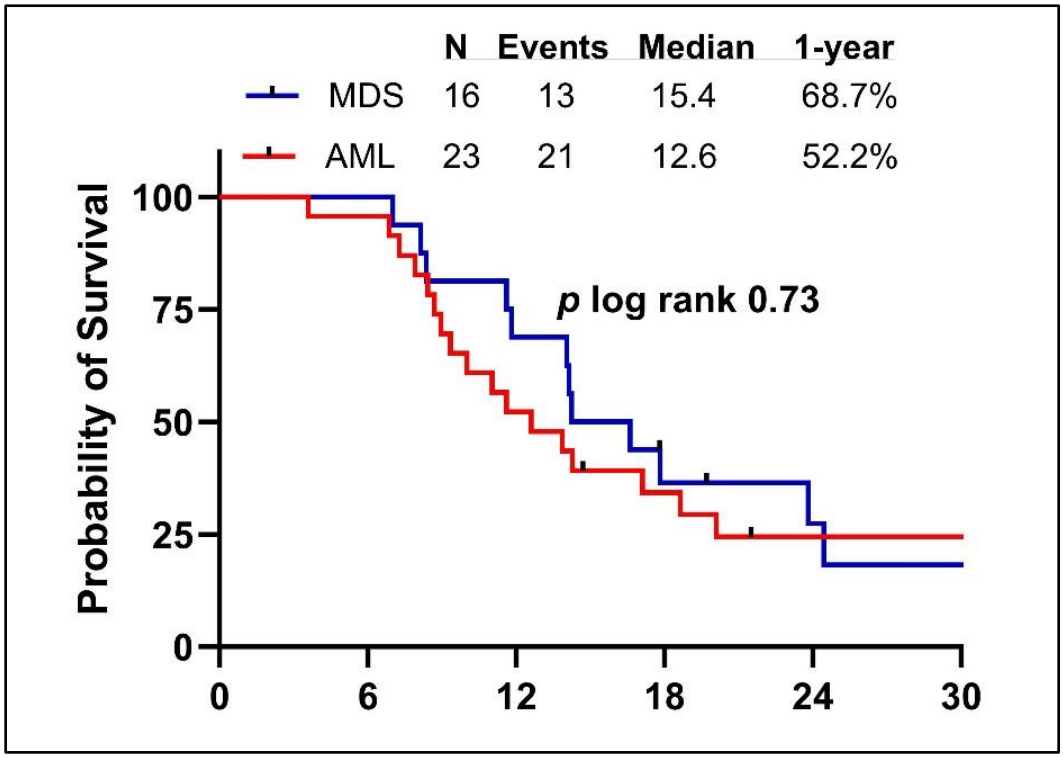


Figure S14: CRT decision tree showing variables affecting survival at 1 year for the full cohort; **ROC AUC 0.69**

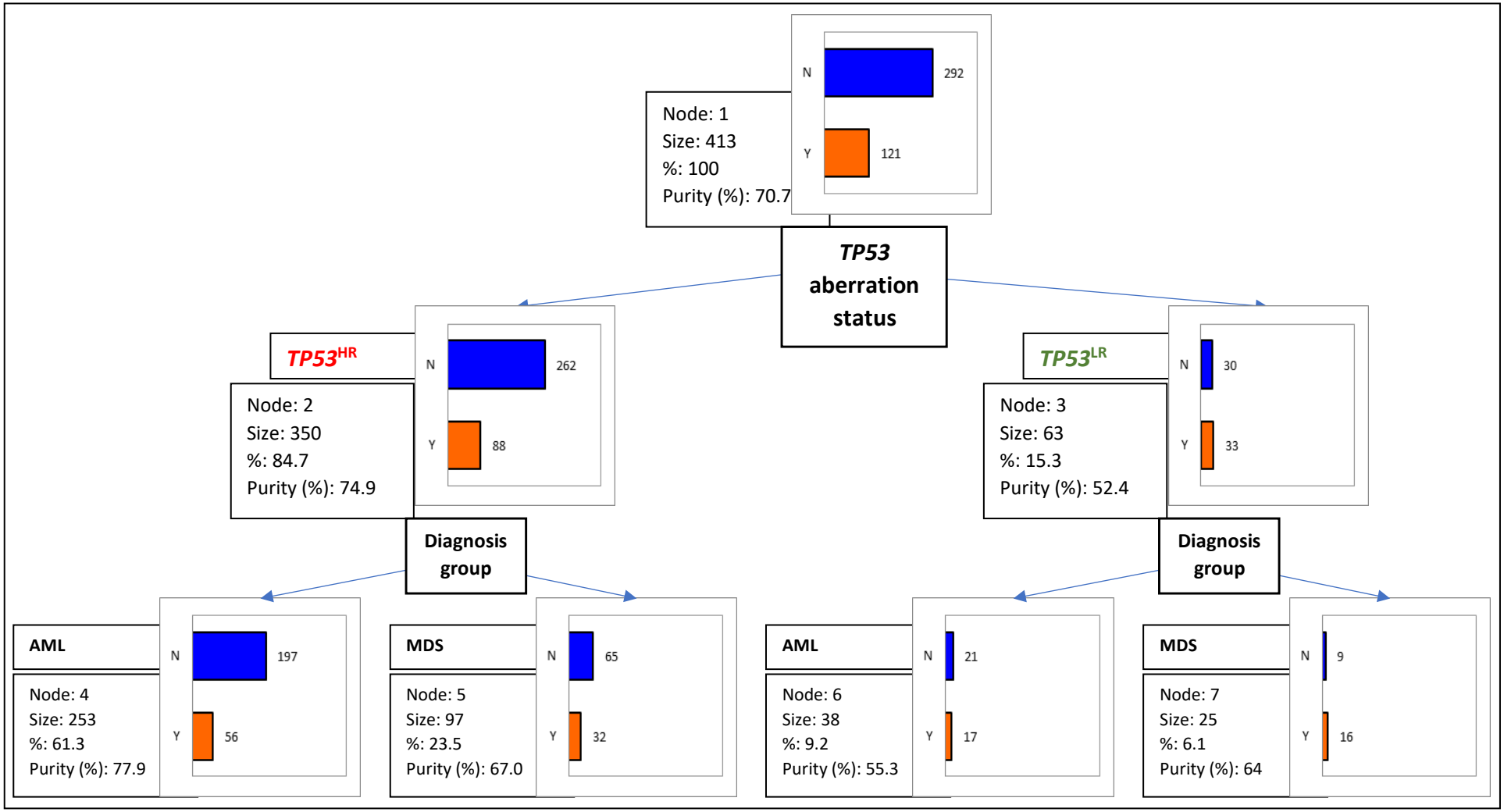


Table S2: Logistic regression analysis of factors affecting overall response in patients with AML.

Univariate Analysis				Multivariate Analysis			
Variable	**Odds ratio**	**95% CI**	**P value**	**Variable**	**Odds ratio**	**95% CI**	**P value**
Age (continuous)	1.01	0.99-1.03	0.35				
Age ≥ 60 years	1.57	0.89-2.77	0.11				
Complex CTG	0.46	0.22-0.91	0.03	**Complex CTG**	0.58	0.26-1.21	0.15
***TP53*^{LR}**	2.40	1.17-5.24	0.02	***TP53*^{LR}**	2.15	0.99-4.95	0.05
Venetoclax	2.08	1.25-3.52	0.005	**Venetoclax**	2.09	1.23-3.59	0.007
Intensive therapy	0.6	0.33-1.07	0.08	**Intensive therapy**	0.69	0.37-1.25	0.22
De novo AML	1.05	0.66-1.67	0.84				

Table S3: Response rates in patients with AML based on treatment intensity.

Full AML Cohort (n=291)										
Treatment intensity	n	Median age [IQR]	Age ≥60 years	P-value	CRc (%)	P-value	ORR (%)	P-value	HSCT (%)	P-value
Low intensity	234	72.2 [66.9-77.5]	213 (91)	<0.001	104 (44)	0.99	130 (56)	0.14	21 (9)	0.03
Intensive	57	56.6 [46.8-60.1]	15 (26)		25 (44)		25 (44)		11 (19)	
AML with *TP53*^{HR} (n=253)										
Low intensity	202	73.2 [67.1-77.7]	185 (91)	<0.001	87 (43)	0.87	107 (53)	0.16	13 (6)	0.01
Intensive	51	57.1 [48.0-60.3]	14 (27)		21 (41)		21 (41)		10 (20)	

Figure S15: Survival outcomes in patients with AML stratified by the treatment intensity.

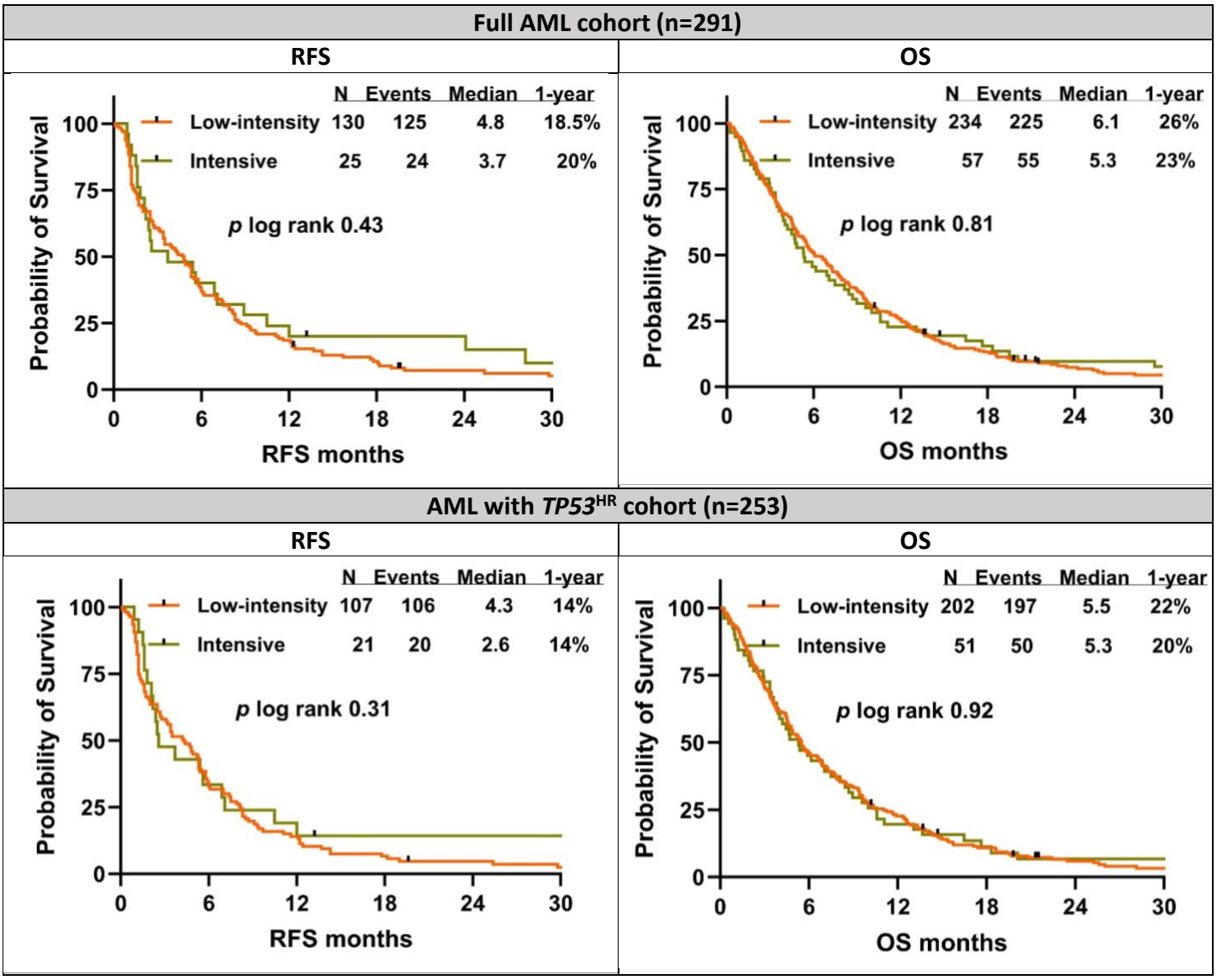


Figure S16: Survival outcomes in patients with AML <60 years of age stratified by the treatment intensity.

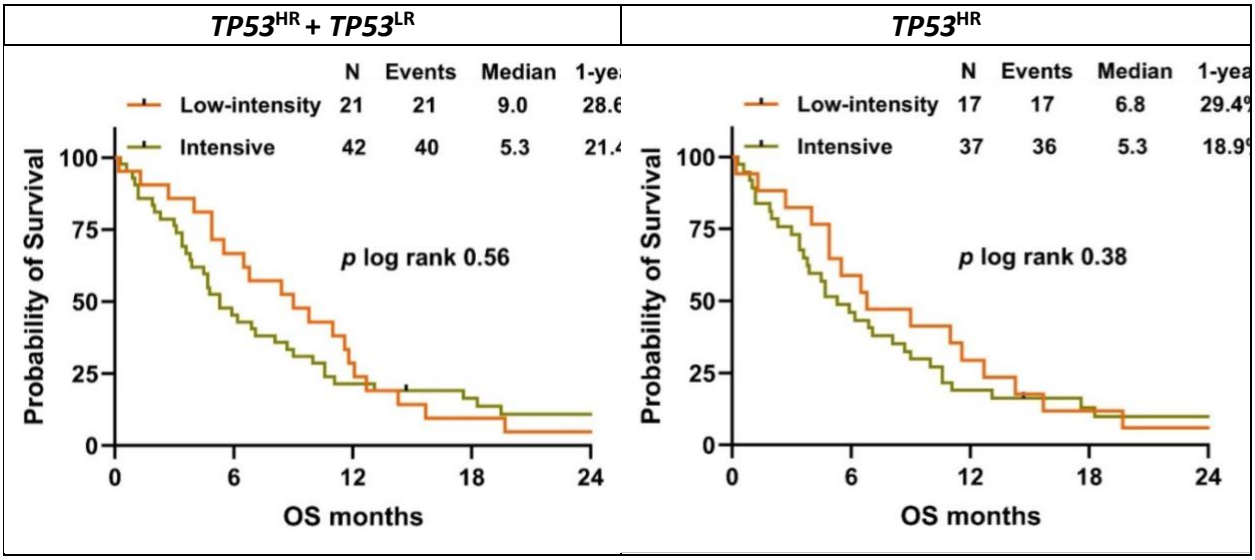


Table S4: Response rates in patients with AML patients based on venetoclax exposure.

Full AML Cohort (n=291)									
Venetoclax	n	Median age [IQR]	Low intensity therapy (%)	CRc (%)	P-value	ORR (%)	P-value	HSCT (%)	P-value
Yes	90	71.3 [64.7-77.5]	81 (90)	49 (54)	0.02	59 (66)	0.005	11 (12)	0.69
No	201	68.8 [59.8-75.7]	153 (76)	80 (40)		96 (48)		21 (10)	
AML with *TP53*^{HR} (n=253)									
Yes	82	72.4 [65.1-77.5]	73 (89)	44 (54)	0.02	52 (63)	0.005	7 (9)	0.99
No	171	69.5 [59.8-75.9]	129 (75)	64 (37)		76 (44)		16 (9)	

Figure S17: Survival outcomes in patients with AML stratified by venetoclax exposure.

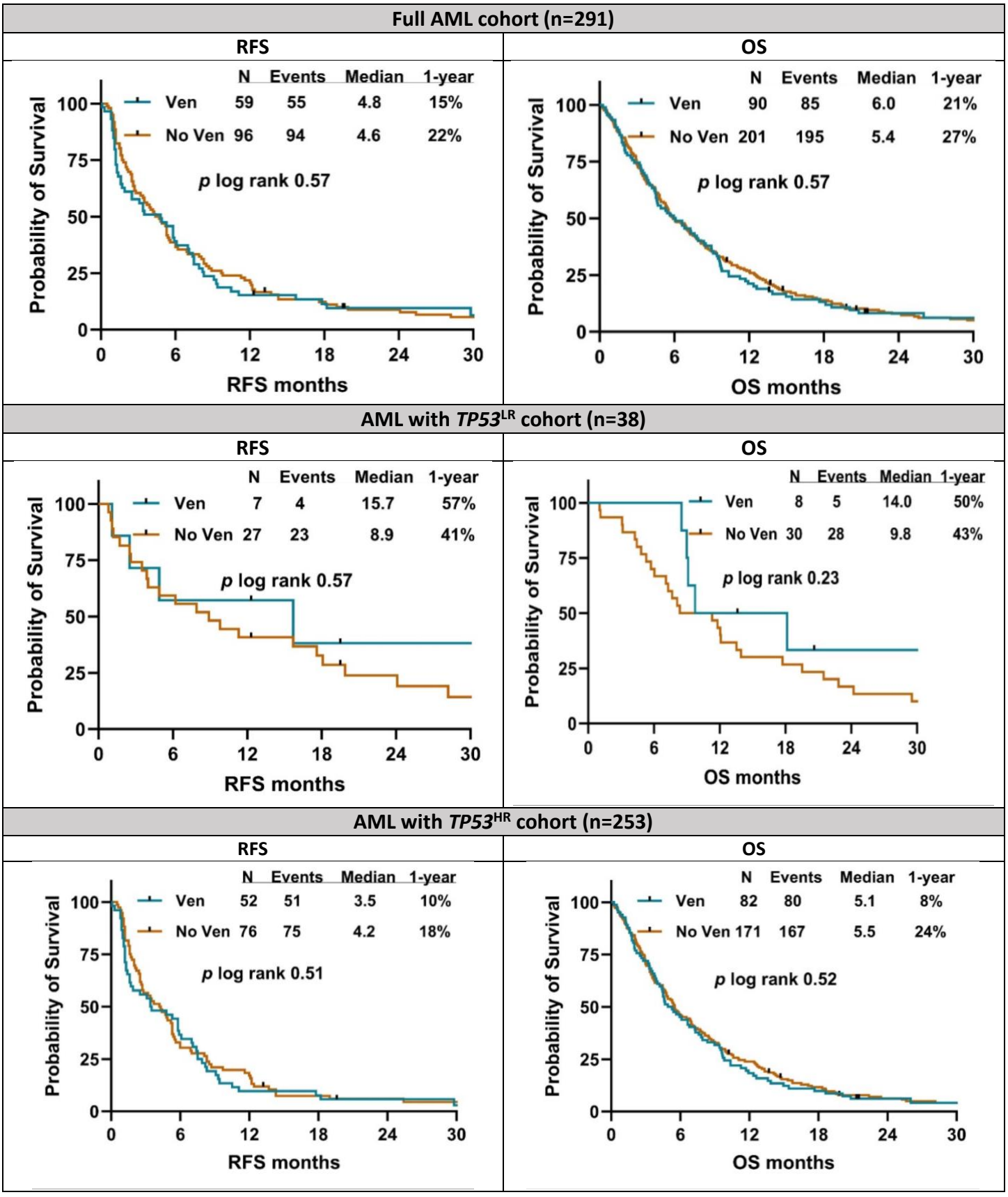


Table S5: Baseline characteristics and outcomes of patients with AML treated with HMA based low-intensity therapy

Variables		HMA monotherapy	HMA+ venetoclax	HMA-non-venetoclax doublets
n		61	75	61
Age (years)		75. 2 [40.8-87.4]	73.6 [37.4-85.6]	70.0 [31.9-82.7]
Age ≥60		52 (85)	72 (96)	53 (87)
***TP53*^{HR}**		51 (84)	63 (84)	44 (72)
Response Rates	CRc ORR	17 (28) 25 (41)	42 (56) 52 (69)	30 (49) 37 (61)
HSCT		2 (3)	7 (9)	6 (10)
Median Follow up (months)		NR	NR	NR
Median OS (months)		5.9	6.1	6.9
Median RFS (months)=ORR		n=25 5.3	n=52 3.5	n=37 4.2

Abbreviations: HMA, hypomethylating agent; CRc, composite complete response; ORR, overall response rate; NR, not reached; OS overall survival; RFS, relapse free revival

Supplemental Methods File

Clinical Interrogation of *TP53* Aberrations and its Impact on Survival in Patients with Myeloid Neoplasms

Jayastu Senapati[1]*, Sanam Loghavi[2]*, Guillermo Garcia-Manero[1], Guillin Tang[2] Tapan Kadia[1], Nicholas J. Short[1], Hussein Abbas[1], Naszrin Arani[3], Courtney D. DiNardo[1], Gautam Borthakur[1], Naveen Pemmaraju[1], Betul Oran[4], Elizabeth Shpall[4], Uday Popat[4], Richard Champlin[4], Sherry Pierce[1], Sankalp Arora[3], Ghayas Issa[1], Musa Yilmaz[1], Keyur Patel[1], Koichi Takahashi[1], Guillermo Montalban-Bravo[1], Danielle Hammond[1], Fadi G. Haddad[1], Farhad Ravandi[1], Hagop M. Kantarjian[1], Naval G. Daver[1]

[1]Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston.

[2]Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston.

[3]Department of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston.

[4]Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston.

* These authors contributed equally

1. Assessment of *TP53* mutations:

Next-generation sequencing (NGS) was performed using our clinically validated myeloid panels, interrogating the entire exonic or hotspot regions of 28, 53 or 81 genes (depending on the time of presentation) frequently mutated in myeloid malignancies, including TP53, as described previously[1,2]. Sequencing libraries were prepared using 250 ng of genomic DNA and respective sequencing libraries were subjected to the Illumina MiSeq (Illumina,Inc., San Diego, CA, USA) sequencer. A minimum sequencing coverage of x250 (bidirectional true paired-end sequencing) was required to allow achieving a lower limit of detection of 2% variant allelic frequency in the background of wild-type sequence.

2. Assessment of *TP53* allelic loss/deletion:

Allelic loss/deletion at the TP53 locus was studied using a combination of conventional karyotyping, FISH and aCGH. Allelic loss/deletion was defined as described previously[3,4] : monosomy 17 (-17); isochromosome i(17)(q10); del(17)(pvar(variable)) with pvar centromeric to p13.1; unbalanced translocations involving 17(p), including der(var)t(var;17)(var;qvar),–17; der(var)t(var;17)(var;pvar), –17 with pvar centromeric to p13.1; der(17)t(17;var)(pvar;var)der(17)t(var;17)(var;pvar) with pvar centromeric to p13.1; der(var)t(var;17)(var;qvar) with dicentric der; der(var)t(var;17)(var;pvar) with pvar centromeric to p13.1 and dicentric der; balanced translocation and 17p13 breakpoint: t(17;var)(p13;var) or t(var;17)(var;p13) in the presence of TP53 deletion by FISH; additive material: add(17)(pvar) in the presence of TP53 deletion by FISH; dicentric chromosome dic(var;17)(var;pvar); and ring chromosome r(17)(pvarqvar) with the presence of TP53 deletion by FISH.