Multidimensional assessment of patient condition and mutational analysis in peripheral blood, as tools to improve outcome prediction in myelodysplastic syndromes: a prospective study of the Spanish MDS Group

Fernando Ramos[1,2],MD MPH PhD, Cristina Robledo[3], PhD, Arturo Pereira[4], MD PhD, Carmen Pedro[5], MD, Rocío Benito[3], Ph.D, Raquel de Paz[6], MD PhD, Mónica del Rey[3], PhD, Andrés Insunza[7], MD, Mar Tormo[8], MD PhD, María Díez-Campelo[9], MD PhD, Blanca Xicoy[10], MD, Eduardo Salido[11], MD, Javier Sánchez-del-Real[1], MD, Leonor Arenillas[12], MD, Lourdes Florensa[12], MD PhD Elisa Luño[13], MD PhD, Consuelo del Cañizo[9], MD PhD, Guillermo F. Sanz[14], MD PhD and Jesús María Hernández-Rivas[3,9], MD PhD; on behalf of the Spanish Group for Myelodysplastic Syndromes (GESMD).

[1]Department of Hematology, Hospital Universitario de León, and [2]Institute of Biomedicine (IBIOMED, León); [3]Unidad de Diagnóstico Molecular y Celular del Cáncer, IBSAL, IBMCC-Centro de Investigación del Cáncer (USAL-CSIC), Salamanca; [4]Department of Blood Transfusion, Hospital Clínic i Provincial, Barcelona; [5]Department of Hematology, Hospital del Mar, Barcelona; [6]Department of Hematology, Hospital Universitario La Paz, Madrid; [7]Department of Hematology, Hospital Universitario Marqués de Valdecilla, Santander; [8]Department of Hematology & Oncology, Hospital Clínico de Valencia; [9]Department of Hematology, Hospital Universitario de Salamanca; [10]Institut Català d'Oncologia-Hospital Universitari Germans Trias i Pujol, Badalona, Josep Carreras Leukemia Research Institute; [11]Department of

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1002/ajh.24813

Hematology, Hospital Universitario La Arrixaca, Murcia; [12]Department of Pathology, Hospital del Mar, Barcelona; [13]Department of Hematology, Hospital Universitario Central de Asturias, Oviedo; and [14]Hospital Universitari i Politècnic La Fe, Valencia, Spain.

CORRESPONDING AUTHOR:

Fernando Ramos MD. Servicio de Hematología, Hospital Universitario de León. Altos de Nava s/n, E-24008 León (Spain). E-mail: mail@fernandoramosmd.es. Phone:+34 987 237 400/Ext. 43628. Fax: +34 987 277 227.

RUNNING TITLE:

Lee index and mutational analysis in MDS

KEYWORDS

Myelodysplastic syndromes, prognosis, comorbidity, geriatric assessment, Lee index, mutational analysis

COUNTS:

Figures 3, Tables 1, Abstract 247 words, Text 2747 words, References 49.

- We have evaluated the relative contribution of patient condition and mutational analysis as tools to improve the IPSS-R for estimating overall survival and the risk of leukemic transformation in MDS patients in a prospective cohort of 200 consecutive MDS patients.

- Patient condition, assessed by the multidimensional Lee index, and patient mutational status in peripheral blood by NGS can improve the prediction of clinical outcomes of patients with MDS already stratified by IPSS-R.

ABSTRACT

The International Prognostic Scoring System and its revised form (IPSS-R) are the most widely used indices for prognostic assessment of patients with myelodysplastic syndromes (MDS), but can only partially account for the observed variation in patient outcomes. This study aimed to evaluate the relative contribution of patient condition and mutational status in peripheral blood when added to the IPSS-R, for estimating overall survival and the risk of leukemic transformation in patients with MDS.

A prospective cohort (2006-2015) of 200 consecutive patients with MDS were included in the study series and categorized according to the IPSS-R. Patients were further stratified according to patient condition (assessed using the multidimensional Lee index for older adults) and genetic mutations (peripheral blood samples screened using next-generation sequencing). The change in likelihood-ratio was tested in Cox models after adding individual covariates.

The addition of the Lee index to the IPSS-R significantly improved prediction of overall survival [hazard ratio (HR) 3.02, 95% confidence interval (CI) 1.96–4.66, $p<0.001$), and mutational analysis significantly improved prediction of leukemic evolution (HR 2.64, 1.56–4.46, $p<0.001$). Non-leukemic death was strongly linked to patient condition (HR 2.71, 1.72–4.25, $p<0.001$), but not to IPSS-R score ($p=0.35$) or mutational status ($p=0.75$). Adjustment for exposure to disease-modifying therapy, evaluated as a time-dependent covariate, had no effect on the proposed model's predictive ability.

In conclusion, patient condition, assessed by the multidimensional Lee index, and patient mutational status can improve the prediction of clinical outcomes of patients with MDS already stratified by IPSS-R.

INTRODUCTION

Approximately 25% of the patients diagnosed with MDS die from progression to acute myeloid leukemia (AML), whereas the rest will have a non-leukemic death (NLD), including the 25% who die from MDS-unrelated causes.[1] Since its publication in 1997,[2] the International Prognostic Scoring System (IPSS) has been the widest-used prognostic index in MDS patients, and a revised form (IPSS-R) was published in 2012.[3] The IPSS-R was derived from a large retrospective patient series, incorporates refined cytogenetic stratification[4] and takes into account depth of cytopenia. In addition, enumerating bone marrow blast from nonerythroid cellularity and using a unique cutoff of 3.5 has been shown to improve its prognostic performance.[5-7]. However, neither the IPSS nor the IPSS-R have faced the problem of death from MDS-unrelated causes.

The term “patient condition” is defined here as the general status of the typically old MDS patient, assessed multidimensionally according to demographic, behavioral, nutritional, comorbid and functional parameters. The Comprehensive Geriatric Assessment captures most of this factors;[8] however, many patients diagnosed with MDS are <65 years and cannot therefore be considered elderly, and the term “older adults” seems more appropriate. The 12-item Lee index[9] is one of the best-performing multidimensional prognostic indices for older adults[10] , used to predict 4- and 10-year mortality.[9,11] Developed from a population of community-dwelling adults aged >50 years, the index incorporates age, gender, smoking habit,[12] body mass index, comorbidity and functional performance into a single tool suitable for daily practice. A modification of this index predicts 5-year mortality.[13] Although it cannot substitute a Comprehensive Geriatric Assessment in the oldest adults[14], the

index has been adequately validated and can also be applied to patients aged between 50-65 years old.

Mutational analysis of bone marrow (BM) at diagnosis has been demonstrated to contribute to survival prediction, independently of the IPSS in several retrospective patient series[15-18] and also, recently, independently of the IPSS-R in a prospective patient series.[19] There is however a lack of prospective studies analyzing the relative contribution of mutational status and patient condition to outcome prediction in patients already stratified according to the IPSS-R. Futhermore, mutational analysis of peripheral blood (PB) samples from MDS patients, a variant of the so-called "liquid biopsy",[20] has not been performed on a sufficiently large population of MDS patients. A template for a molecular upgrade of the IPSS-R ("IPSS-R molecular") derived from mutational analysis of BM data was recently proposed,[21] but did not take into account patient condition. In 2016, authors of the European Haematology Association Roadmap for European Hematology Research concluded that studies analyzing the relationship between genotype, gender, age and comorbidities are required to improve ther prognostic/predictive models for MDS.[22]

The aim of this study was to evaluate the relative contribution of patient condition and mutational analysis in peripheral blood, as tools to improve the IPSS-R for estimating overall survival (OS) and the risk of leukemic transformation (LT) in MDS patients. Our hypothesis was that both of them may add prognostic information to the IPSS-R.

METHODS

PATIENTS AND CLINICAL METHODS

A total of 266 consecutive patients diagnosed with MDS (according to French-American-British criteria)[23] were prospectively recruited from eight Grupo Español de Síndromes Mielodisplásicos (GESMD) sites (June 2006–October 2010). MDS diagnosis, classification and cytogenetic evaluation were performed following GESMD recomendations.[24] All procedures were performed in accordance with the Declaration of Helsinki 1975. Written informed consent was obtained from all patients, and Institutional Review Boards at each hospital approved the study.

Patients were reclassified according to the World Health Organization (WHO) 2008 criteria[25] and prognosis categorized according to IPSS-R.[3] Out of 266 patients, 200 primary MDS patients (Figure 1) were enrolled and followed up until August 2015 (median 3.6 years; range 0.1–7.7). Sixty-six patients were excluded because of CMML diagnosis, doubtful MDS, secondary MDS, cytogenetic analysis was not available, absence of adequate metaphases for cytogenetic analysis, or consent revocation. Fifty-five patients received disease-modifying therapeutic strategies: frontline azacitidine (AZA) (n=32), frontline intensive chemotherapy (IC) (n=5), frontline AZA +IC rescue (5), and allogeneic hematopoietic cell transplantation (HCT) (n=13; 5 frontline, 1 post-frontline AZA, 6 post-frontline IC, and 1 after frontline AZA and rescue with IC).

Patient condition was evaluated using the Lee index;[9] IPSS-R 'differentiating variables for survival' were also analyzed (age, Eastern Cooperative Oncology Group performance status [ECOG PS], serum lactate dehydrogenase [LDH] at diagnosis, ferritin, and beta-2 microglobulin).[3]

Unprocessed PB samples collected at diagnosis from every patient at each study site was sent unprocessed at ambient temperature to a central facility for

nucleic acid extraction and were kept frozen at -80º C until mutational analysis performed.

MUTATIONAL ANALYSIS

The mutational analysis by next-generation sequencing (NGS) was carried out for the 159 (79.5%) patients whose DNA was of adequate quality, following procedures described elsewhere.[26] A list of genes analyzed in all patients is shown in Table S1. Given the difficulties to develop a robust molecular profile for outcomes prediction, the patients were instead stratified according to the modified score of Bejar et al.[21] In short, patients were stratified into 3 groups according to the presence/absence of mutations in the *SF3B1* gene and the presence/absence of any mutations in the genes *TP53*, *CBL*, *EZH2*, *RUNX1*, *U2AF1 or ASXL1*; favorable: *SF3B1* mutation only; unfavorable: wildtype *SF3B1* + mutation in any of the other six genes; intermediate: all other patients. All assays were performed blinded to the study end points, by technicians who were not involved in patient management.

STATISTICAL ANALYSIS

Continuous and ordinal variables were summarized by their median and interquartile range (IQR), and the Mann-Whitney's test was used to analyze differences in mean rank. Categorical variables were described by counts and relative frequencies, and the Fisher's exact test was used to analyze differences in distribution between patient subsets. Patient condition was assessed using the Lee index original quartiles;[9] however, the second and third quartiles were merged into a common intermediate category (clear overlap in OS). Whenever possible, other covariates were dichotomized according to clinically relevant

cut-offs (upper limit of normal range for LDH; ferritin >500 ng/mL; beta-2 microglobulin >3 mg/L). Cases with missing values in one covariate were excluded in all the tests where that covariate was used.

OS was defined as the time from diagnosis to death (as a result of all causes), last follow-up or study closing date, and was plotted using the Kaplan-Meier product-limit method. Cumulative incidence of LT was calculated from diagnosis to progression to AML (> 20% blast cells in PB or BM), last follow-up or closing date using the method described by Coviello & Boggess (in the context of the competitive risk of death unrelated to leukemia).[27] Patients treated by allogeneic HCT were censored at the time of transplant.

The log-rank test was used to check the association between categorical predictors and OS. The proportional hazards assumption was verified and all covariates analyzed by testing the change in likelihood-ratio in Cox models following addition of each individual covariate to the IPSS-R. At least 10 events per predictor was considered an appropriate number for multivariable analysis. Finally, we opted for straightforward models that included only IPSS-R and the best additional covariates. The increased discriminating power of the expanded prognostic models on OS prediction, versus IPSS-R alone, was evaluated by the Harrell's C index and the R^2 explained variation.[28,29] The independent effect of the covariates on the incidence of LT and non-leukemic death (NLD) in the framework of competing risks was analyzed, according to the method by Fine and Gray.[30] Statistical analyses were performed using SPSS release 19.0 (SPSS Inc., Chicago, IL, USA) and Stata, Release 11 (StataCorp LLC, College Station, TX, USA).

RESULTS

PATIENT CHARACTERISTICS AND OVERALL PREDICTED OUTCOME

The baseline clinical characteristics of the 200 patients enrolled in the study are shown in Table S2. A total of 47/200 (23.5%) patients progressed to AML, 117 (58.5%) died (76 without AML and 41 after LT), 13 (6.5%) patients were lost to follow-up; and 70 (35%) patients were censored (alive) at the study closing date. Median OS was 4 years (95% confidence interval [CI] 2.9–5.0; see Figure S1a) and median time from diagnosis to AML transformation was 1 year (range 0.05–5.4 [IQR 0.5–2.5]). Cumulative incidence of LT at 1, 3 and 4 years — considering AML-unrelated death as a competing risk — was 11% (95% CI 9–16%), 19% (95% CI 14–27%) and 23% (95% CI 17–30%), respectively (Figure S2).

MUTATIONAL ANALYSIS

Of the 159 patient samples included in the mutational analysis, 144 (90.6%) contained ≥1 mutation [median (IQR): 2 (0–7)]. For additional details see Tables S3 and S4. Mutations were found in 75/111 (67.6%) genes analyzed. The most frequently mutated gene was *SF3B1* (23.3%) followed by *TET2* (22.6%), *DNMT3A* (15.7%), *SRSF2* (15.1%), *ASXL1* (13.2%) and *RUNX1* (13.2%). The relative mutation frequency of genes included in the genetic score was *SF3B1 (*23.3%), *RUNX1* (13.2%), *ASXL1 (*13.2%), *TP53 (*6.3%), *U2AF1 (*5.0%), *EZH2* (3.1%) and *CBL (*1.9%). To facilitate future inter-series comparison, patients were reclassified according to the 2016 revision of the WHO classification[31], taking into consideration the eventual presence of *SF3B1* mutations in patients with 5–15% ring sideroblasts (Table S5).

OVERALL SURVIVAL PREDICTION

IPSS-R adequately predicted OS in univariate analysis in our series (HR 2.12, CI 95% 1.76-2.53, p<0.001; Figure S1b), but Lee index and the mutational analysis according to the Bejar's 2015-modified molecular score also showed a statistically significant univariate impact (Figure 2 and Table I). The addition of Lee index to IPSS-R, significantly improved the model's prognostic power (as measured by the likelihood ratio test) whereas mutational analysis did not have such an effect (Table I). The increased discriminating power of the expanded prognostic models on OS compared with IPSS-R alone is shown in Table S6.

Several "differentiating variables for survival" also added prognostic information for OS (although not for LT) independently of the IPSS-R but with lower adjusted HRs (Tables S7 and S8). Furthermore, none of them improved significantly the prognostic power of the combination of IPSS-R and Lee index. More specifically, the addition of age to IPSS-R and Lee index did not significantly improve the prognostic power of the model (p=0.06).

PREDICTION OF LEUKEMIC TRANSFORMATION AND NON-LEUKEMIC DEATH

Both IPSS-R (standardized hazard ratio [SHR] 2.23, 95% CI 1.75–2.84, p<0.001) and mutational analysis (p<0.001) could be used to predict cumulative incidence of LT by univariate analysis, whereas the Lee index could not (p=0.71) (Figures S3 and S4, Table I). The addition of mutational analysis to the IPSS-R improved LT prediction (p<0.001; Table I). The risk of LT was lower with advancing age, as assessed by univariate analysis (HR 0.71, 95% CI 0.55–0.91, p=0.007).

As expected, patient-related covariates (such as the Lee index) did not contribute to prediction of LT as assessed by multivariable analysis, but did predict NLD (p<0.001, HR 2.71, 95% CI 1.72–4.25). In contrast, IPSS-R and mutational analysis contributed to prediction of LT but not NLD (p=0.35, HR 1.10, 95% CI 0.90–1.35 and p=0.75, SHR 1.05, 95% CI 0.77–1.45; respectively).

Finally, adjustment of the above models for exposure to disease-modifying therapeutic strategies had no effect on their prognostic power, as measured by changes in the HR (data not shown).

CLINICO-BIOLOGICAL CORRELATES

There was a significant correlation between the Lee index score, and ECOG PS and serum beta-2 microglobulin levels (rho +0.49, p<0.001; and rho +0.42, p<0.001; respectively), but not with the IPSS-R score (rho -0.13, p=0.061). In contrast, there was a significant association between IPSS-R category and both the original molecular score[12] and the modified molecular score[18] (p=0.004, and p=0.023, respectively; chi-squared for trend). We did not find any statistically significant association between patient condition and mutational status (p=0.28, Table S9).

Transfusion dependence was not associated with the Lee index score (p=0.18). However, it was associated with ECOG PS (20.0%, 36.3%, 36.8% and 64.3%, for the 0, 1, 2 and 3+4 subsets, respectively; p=0.002) and IPSS-R (2%, 31.2%, 39.4%, 68.0% and 66.7% for the very low, low, intermediate, high and very high subsets, respectively; p<0.001).

DISCUSSION

In agreement with prospective studies published in 2016, we confirmed the prognostic power of the IPSS-R in terms of OS[19,32] and LT[32] in patients with MDS. Addition of patient condition to the IPSS-R, as measured by the multidimensional Lee index (including age, gender, smoking habit, body mass index, comorbidity and functional performance), improved OS prediction, whereas addition of mutational status improved LT prediction. This may be particularly useful for patients classified in the lower- and intermediate-risk categories of the IPSS-R, and therefore a new integrative prognostic algorithm for patients with MDS is proposed (Figure 3).

Within the series, patient age was a predictor of shorter OS and a lower risk of LT, as assessed by univariate analysis. According to the data presented, NLD acted as a mediator for both, acting directly on the OS and behaving as a competing risk event for LT.

The IPSS-R and the WHO Classification-Based Prognosis Scoring System (WPSS) are used to predict both OS and LT across the whole spectrum of primary MDS.[3,33,34] In recent years, other prognostic scoring systems for patients with lower-risk MDS[35,36], as well as for patients with secondary and previously-treated MDS[37], have been published, most of which include age and/or ECOG PS as prognostic variables. We hypothesize that that incorporation of patient condition and mutational status in PB might also increase the prognostic power of these indices.

ECOG PS is one of the most commonly used covariates in prognostic models for cancer patients; however, it does not take into account comorbidity, which is also a relevant predictor of OS in patients with MDS. [38-45] Comorbidity was not analyzed during IPSS or IPSS-R development, but was evaluated in later

studies in retrospective cohorts. In our study, the multidimensional Lee index, categorized in three strata (score <6, 6-13, and >13) adequately predicted OS in a prospective cohort of MDS patients. Interestingly, the Lee index did not correlate with transfusion dependence whereas PS did. This result could be related to the fact that the Lee index evaluates functional performance of patients prior to MDS diagnosis, whereas ECOG PS evaluates the functional impact of MDS itself.

An interesting observation from our data is the differential contribution of the patient condition and mutational analysis to prognosis assessment of patients with MDS. Although IPSS-R is a potent prognostic tool for OS, its effect appears to occur predominantly through its implication in LT, since in our hands it did not predict NLD (though the problem of small numbers may be an issue). In our study, the Lee index improved the predictive power of the IPSS-R in terms of OS, through its power to predict NLD, thus overcoming mutational analysis in this regard.

Mutational analysis by NGS is a promising tool for MDS prognosis assessment and, when performed using PB, may be particularly useful in patients with prominent marrow fibrosis.[26] It is also an ideal tool for studying clonal evolution, especially in older and debilitated MDS patients who would otherwise require repeated BM punctures. In this study, mutational analysis was performed on DNA isolated from the whole cellular fraction of PB, replicating previously published data on the independent prognostic value of mutational analysis. Disease-modifying therapeutic strategies may have acted as a potential confounding factor,[46-49] but after adjusting for it we did not detect any relevant change in the hazard and sub-hazard estimations.

In conclusion, this clinico-biological study in a prospective cohort has demonstrated the independent value of multidimensional evaluation of patient condition and screening for mutations in PB to predict the outcome of patients with MDS. Both variables may be potentially useful add-ons for patients already stratified by the IPSS-R, especially in the very low/low and intermediate-risk categories.

ACKNOWLEDGEMENTS

We would like to thank Cristina Diez Tascón, Xana-Inés Lomas Iglesias, Sara González Briones and Irene Rodriguez Iglesias for technical assistance, and Ariadna Cases for administrative support. We also acknowledge the support of the PETHEMA Foundation for the activities of the Grupo Español de Síndromes Mielodisplásicos. Language assistance was provided by Bethany Degg (FireKite) and was supported by Celgene.

AUTHORSHIP CONTRIBUTIONS

F.R. conceived the study and designed the research. F.R., C.P., R.P., A.I., M.T., M-D-C., B.X. and E.S. recruited the patients and collected the data. L.A., L.F. and C.C. took responsibility for the MDS diagnostic process. F.R. and J.S-R revised the WHO classifications of each patient included. C.R., R.B., and M.R. performed the mutational analysis. E.L. revised the cytogenetic analysis of each patient included. F.R. assembled the data, performed the initial statistical analysis and wrote the draft. A.P. reviewed the survival analysis. G.F.S. and J.M.H-R. supervised the study. All authors interpreted the data, revised and approved the final manuscript.

REFERENCES

1. Nomdedeu M, Pereira A, Ramos F, Valcárcel D, Costa D, Arnan M, et al., and on behalf of the Spanish MDS Group. Excess Mortality in the Myelodysplastic Syndromes. Am J Hematol. 2017;92:149-154.

2. Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, et al. International Scoring System for evaluating prognosis in myelodysplastic syndromes. Blood. 1997;89:2079-2088. Erratum in: Blood. 1998;91:1100.

3. Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Solé F, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood. 2012;120:2454-2465.

4. Schanz J, Tüchler H, Solé F, Mallo M, Luño E, Cervera J, et al. New comprehensive cytogenetic scoring system for primary myelodysplastic syndromes (MDS) and oligoblastic acute myeloid leukemia after MDS derived from an international database merge. J Clin Oncol. 2012;30:820-829.

5. Arenillas L, Calvo X, Luño E, Senent L, Alonso E, Ramos F, et al. Considering Bone Marrow Blasts From Nonerythroid Cellularity Improves the Prognostic Evaluation of Myelodysplastic Syndromes. J Clin Oncol. 2016;34:3284-3292.

6. Calvo X, Arenillas L, Luño E, Senent L, Arnan M, Ramos F, et al.; for the Grupo Español de Síndromes Mielodisplásicos (GESMD). Enumerating bone marrow blasts from nonerythroid cellularity improves outcome prediction in myelodysplastic syndromes and permits a better definition of the intermediate

risk category of the revised international prognostic scoring system (IPSS-R). Am J Hematol. 2017; DOI 10.1002/ajh.24732.

7. Pfeilstöcker M, Tuechler H, Sanz G, Schanz J, Garcia-Manero G, Solé F, et al. Time-dependent changes in mortality and transformation risk in MDS. Blood. 2016 ;128:902-910.

8. Hamaker ME, Prins MC, Stauder R. The relevance of a geriatric assessment for elderly patients with a haematological malignancy: a systematic review. Leuk Res. 2014;38:275-283.

9. Lee SJ, Lindquist K, Segal MR, Covinsky KE. Development and validation of a prognostic index for 4-year mortality in older adults. JAMA. 2006;295:801-808. Erratum in: JAMA. 2006;295:1900.

10. Yourman LC, Lee SJ, Schonberg MA, Widera EW, Smith AK. Prognostic indices for older adults: a systematic review. JAMA. 2012;307:182-192.

11. Cruz M, Covinsky K, Widera EW, Stijacic-Cenzer I, Lee SJ. Predicting 10-year mortality for older adults. JAMA. 2013;309:874-876.

12. Mishra A, Rollison DE, Brandon TH, Al Ali NH, Corrales-Yepez M, Padron E, et al. Impact of tobacco usage on disease outcome in myelodysplastic syndromes. Leuk Res. 2015;39:673-678.

13. Schonberg MA, Davis RB, McCarthy EP, Marcantonio ER. Index to predict 5-year mortality of community-dwelling adults aged 65 and older using data from the National Health Interview Survey. J Gen Intern Med.2009;24:1115-1122.

14. Pallis AG, Fortpied C, Wedding U, Van Nes MC, Penninckx B, Ring A, et al. EORTC elderly task force position paper: approach to the older cancer patient. Eur J Cancer. 2010;46:1502-1513.

15. Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G, et al. Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med. 2011;364:2496-2506.

16. Papaemmanuil E, Gerstung M, Malcovati L, Tauro S, Gundem G, Van Loo P, et al.; Chronic Myeloid Disorders Working Group of the International Cancer Genome Consortium. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2013;122:3616-3627; quiz 3699.

17. Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, Nagae G, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia. 2014;28:241-247.

18. Wu L, Song L, Xu L, Chang C, Xu F, Wu D, et al. Genetic landscape of recurrent ASXL1, U2AF1, SF3B1, SRSF2,and EZH2 mutations in 304 Chinese patients with myelodysplastic syndromes. Tumour Biol. 2016;37:4633-4640.

19. Nazha A, Narkhede M, Radivoyevitch T, Seastone DJ, Patel BJ, Gerds AT, et al. Incorporation of molecular data into the Revised International Prognostic Scoring System in treated patients with myelodysplastic syndromes. Leukemia. 2016;30:2214-2220.

20. Karachaliou N, Mayo-de-las-Casas C, Molina-Vila MA, Rosell R. Real-time liquid biopsies become a reality in cancer treatment. Ann Transl Med. 2015;3:36. doi: 10.3978/j.issn.2305-5839.2015.01.16

21. Bejar R, Papaemmanuil E, Haferlach T, Garcia-Manero G, Maciejewski JP, Sekeres MA, et al. Somatic Mutations in MDS Patients Are Associated with Clinical Features and Predict Prognosis Independent of the IPSS-R: Analysis of Combined Datasets from the International Working Group for Prognosis in MDS-Molecular Committee. Blood. 2015;126(23): 907.

22. Engert A, Balduini C, Brand A, Coiffier B, Cordonnier C, Döhner H, et al; EHA Roadmap for European Hematology Research. The European Hematology Association Roadmap for European Hematology Research: a consensus document. Haematologica. 2016;101:115-208.

23. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, Sultan C. Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. Br J Haematol. 1976;33:451-458.

24. Spanish Group for Myelodysplastic Syndromes (GESMD). Spanish Society of Haematology and Haemotherapy (SEHH). Spanish guidelines for the diagnosis and treatment of myelodysplastic syndromes and chronic myelomonocytic leukaemia. Haematologica (Spanish edition) 2012; 97(Suppl.5): 6-10. Available at http://gesmd.es/ pdfs/guias_smd Haematologia_Guias_SMD.pdf

25. Swerdlow S, Campo E, Harris NL, eds. WHO classification of tumours of haematopoietic and lymphoid tissue. Lyon, France: IARC; 2008.

26. Ramos F, Cristina Robledo C, Izquierdo-García FM, Suárez-Vilela D, Rocío Benito R, Fuertes M, et al.; Spanish Group for Myelodysplastic Syndromes (GESMD). Bone marrow fibrosis in myelodysplastic syndromes: a prospective evaluation including mutational analysis. Oncotarget. 2016;7:30492-30503.

27. Coviello V, Boggess M. Cumulative incidence estimation in the presence of competing risks. The Stata Journal. 2004; 4:103-112.

28. Harrell FE Jr, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med.1996;15:361-387.

29. Burnham KP, Anderson DR. Multimodel inference: understanding AIC and BIC in Model Selection. Sociol Method Res. 2004; 33: 261-304.

30. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc. 1999; 94:496-509.

31. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127:2391-2405.

32. de Swart L, Smith A, Johnston TW, Haase D, Droste J, Fenaux P, et al. Validation of the revised international prognostic scoring system (IPSS-R) in patients with lower-risk myelodysplastic syndromes: a report from the prospective European LeukaemiaNet MDS (EUMDS) registry. Br J Haematol. 2015;170:372-383.

33. Malcovati L, Germing U, Kuendgen A, Della Porta MG, Pascutto C, Invernizzi R, et al. Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol. 2007;25:3503-3510.

34. Della Porta MG, Tuechler H, Malcovati L, Schanz J, Sanz G, Garcia-Manero G, et al. Validation of WHO classification-based Prognostic Scoring System

(WPSS) for myelodysplastic syndromes and comparison with the revised International Prognostic Scoring System (IPSS-R). A study of the International Working Group for Prognosis in Myelodysplasia (IWG-PM). Leukemia. 2015; 29:1502-1513.

35. Garcia-Manero G, Shan J, Faderl S, Cortes J, Ravandi F, Borthakur G, et al. A prognostic score for patients with lower risk myelodysplastic syndrome. Leukemia. 2008; 22:538-543.

36. Valcárcel D, Sanz G, Ortega M, Nomdedeu B, Luño E, Diez-Campelo M, et al. Grupo Español de Síndromes Mielodisplásicos (GESMD). Use of newer prognostic indices for patients with myelodysplastic syndromes in the low and intermediate-1 risk categories: a population-based study. Lancet Haematol. 2015;2:e260-6. doi:10.1016/S2352-3026(15)00067-8.

37. Kantarjian H, O'Brien S, Ravandi F, Cortes J, Shan J, Bennett JM, et al. Proposal for a new risk model in myelodysplastic syndrome that accounts for events not considered in the original International Prognostic Scoring System. Cancer. 2008; 113:1351-1361.

38. Zipperer E, Pelz D, Nachtkamp K, Kuendgen A, Strupp C, Gattermann N, et al. The hematopoietic stem cell transplantation comorbidity index is of prognostic relevance for patients with myelodysplastic syndrome. Haematologica. 2009; 94:729-732.

39. Sperr WR, Wimazal F, Kundi M, Baumgartner C, Nösslinger T, Makrai A, et al. Comorbidity as prognostic variable in MDS: comparative evaluation of the HCT-CI and CCI in a core dataset of 419 patients of the Austrian MDS Study Group. Ann Oncol. 2010; 21:114-119.

40. Naqvi K, Garcia-Manero G, Sardesai S, Oh J, Vigil CE, Pierce S, et al. Association of comorbidities with overall survival in myelodysplastic syndrome: development of a prognostic model. J Clin Oncol. 2011; 29:2240-2246.

41. Breccia M, Federico V, Latagliata R, Mercanti C, D'Elia GM, Cannella L, et al. Evaluation of comorbidities at diagnosis predicts outcome in myelodysplastic syndrome patients. Leuk Res. 2011;35:159-162.

42. Della Porta MG, Malcovati L, Strupp C, Ambaglio I, Kuendgen A, Zipperer E, et al. Risk stratification based on both disease status and extra-hematologic comorbidities in patients with myelodysplastic syndrome. Haematologica. 2011;96:441-449.

43. van Spronsen MF, Ossenkoppele GJ, Holman R, van de Loosdrecht AA. Improved risk stratification by the integration of the revised international prognostic scoring system with the myelodysplastic syndromes comorbidity index. Eur J Cancer. 2014;50:3198-3205.

44. Bammer C, Sperr WR, Kemmler G, Wimazal F, Nösslinger T, Schönmetzler A,et al. Clustering of comorbidities is related to age and sex and impacts clinical outcome in myelodysplastic syndromes. J Geriatr Oncol. 2014;5:299-306.

45. Balleari E, Salvetti C, Del Corso L, Filiberti R, Bacigalupo A, Bellodi A, et al. Age and comorbidities deeply impact on clinical outcome of patients with myelodysplastic syndromes. Leuk Res. 2015;39:846-852.

46. Silverman LR, McKenzie DR, Peterson BL, Holland JF, Backstrom JT, Beach CL, et al; Cancer and Leukemia Group B. Further analysis of trials with

azacitidine in patients with myelodysplastic syndrome: studies 8421, 8921, and 9221 by the Cancer and Leukemia Group B. J Clin Oncol. 2006; 24:3895-3903.

47. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, Giagounidis A, et al.; International Vidaza High-Risk MDS Survival Study Group. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. Lancet Oncol. 2009;10:223-232.

48. Nachtkamp K, Kundgen A, Strupp C, Giagounidis A, Kobbe G, Gattermann N, et al. Impact on survival of different treatments for myelodysplastic syndromes. Leuk Res. 2009; 33:1024-1028.

49. Abáigar M, Ramos F, Benito R, Díez-Campelo M, Sánchez-del-Real J, Hermosín L, et al. Prognostic impact of the number of methylated genes in myelodysplastic syndromes and acute myeloid leukemias treated with azacytidine. Ann Hematol. 2013;92:1543-1552.

CONFLICT OF INTEREST DISCLOSURES

The authors declared no conflicts of interest to declare in relation to the work described here. The sponsors were not involved in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

GRANT SUPPORT

This study was supported in part by research funding from Celgene S.L., Madrid, Spain; Fundación Española de Hematología y Hemoterapia (FEHH) (grant to MDR); Red Temática de Investigación Cooperativa en Cáncer (RTICC), Instituto de Salud Carlos III (ISCIII), Spanish Ministry of Economy and Competitiveness and the European Regional Development Fund (ERDF) “Una manera de hacer Europa” (grant numbers RD12/0036/0069, RD12/0036/0044);Junta de Castilla y León (grant numbers BIO/SA47/13, GRS 994/A/14, GRS 1033/A/14, GRS 1043/A/16); and European Union Seventh Framework Programme [FP7/2007-2013] (grant number 306242-NGS-PTL).

FIGURE LEGENDS

Figure 1. **Patient disposition**

AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; Dx., diagnosis; MDS, myelodysplastic syndromes; IPSS-R, revised International Prognostic Scoring System; Lee index, patient condition as measured by Lee index; MA, mutational analysis; NA, not available; NGS, Next-Generation Sequencing; NLD, non-leukemic death; PB, peripheral blood; yr, year; w/o, without.

Figure 2**. Overall projected survival (95% confidence interval) as a function of patient condition**

Patient condition (age, gender, smoking habit, body mass index, comorbidity, and functional performance) was evaluated by using Lee index for older adults[9]. In this study, the Lee index was categorized into three strata (score <6; 6–13; and >13).

Figure 3**. An integrative algorithm for predicting outcome of MDS patients**

The treatment algorithm proposes: 1. The categorization of patients according to IPSS-R score (enumerating BM blasts from nonerythroid cellularity and using a unique IPSS-R cutoff of 3.5 has been shown to improve IPSS-R prognostic power)[5-7]; 2. NGS mutational analysis of BM or peripheral blood (the technology is expected to improve over time with improved understanding of gene interactions); 3. Further categorization by the Lee index (other indices may also

be used in daily practice[10,11,13,38-43]). Other high-risk features include: severe neutropenia, severe thrombocytopenia, marrow fibrosis grade 2–3, high scores in other dedicated tools[26,35–37].

The dashed line for patients with a Lee index score >13 indicates that this subset of patients might not obtain a definite survival benefit when treated by current therapeutic strategies, despite the prognostic significance of the Lee index score for OS.

BM, bone marrow; IPSS-R, revised International Prognostic Scoring System; MDS, myelodysplastic syndromes; NGS, next-generation sequencing; OS, overall survival.

TABLE LEGENDS

Table I. **Impact of patient condition and mutational status in PB on overall survival and leukemic transformation (unadjusted as well as adjusted for IPSS-R)**

Cat.: categories. Lee's score categories: favorable (lower than 6), intermediate (6-13), unfavorable (greater than 13). Mutational status[21]: patients are stratified into 3 groups according to the presence/absence of mutations in the *SF3B1* gene and the presence/absence of any mutations in the genes *TP53*, *CBL*, *EZH2*, *RUNX1*, *U2AF1 or ASXL1*. Those patients harboring only a mutation in *SF3B1*, but not in the other 6 genes are assigned to the the favorable group, those who have wildtype *SF3B1* and a mutation in any of the other 6 genes are

assigned to the unfavorable group, and the rest are allocated to the intermediate group. PB, Peripheral blood. HR, Hazard ratio. SHR, Standardized hazard ratio. CI 95%, Confidence interval 95%. Adjusted, refers to the values when introducing both IPSS-R and a single additional covariate in the model. IPSS-R categories used for the adjustment: Very low, Low, Intermediate, High, Very high.

		Overall Survival			
	n	**Unadjusted**		**Adjusted for IPSS-R**	
Covariate		*HR (CI 95%)*	*p*-value	*HR (CI 95%)*	*p*-value
Lee index[9] (3 cat.)	199	2.99 *(1.98-4.52)*	<0.001	3.02 *(1.96-4.66)*	<0.001
Mutational status in PB[21] (3 cat.)	159	1.49 *(1.13-1.98)*	0.005	1.11 *(0.80-1.54)*	0.17
		Leukemic transformation			
	n	**Unadjusted**		**Adjusted for IPSS-R**	
Covariate		*SHR (CI 95%)*	*p*-value	*HR (CI 95%)*	*p*-value
Lee index[9] (3 cat.)	199	0.86 *(0.39-1.89)*	0.71	-	-
Mutational status in PB [21] (3 cat.)	159	2.64 *(1.56-4.46)*	<0.001	2.25 *(1.27-3.99)*	<0.001

Table II

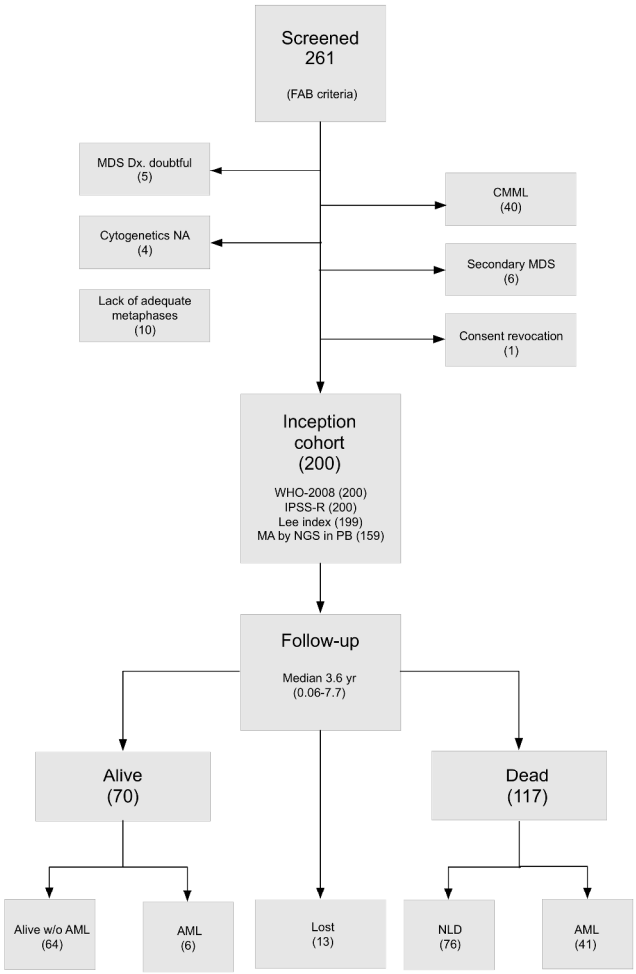

Figure 1. Patient disposition

297x420mm (300 x 300 DPI)

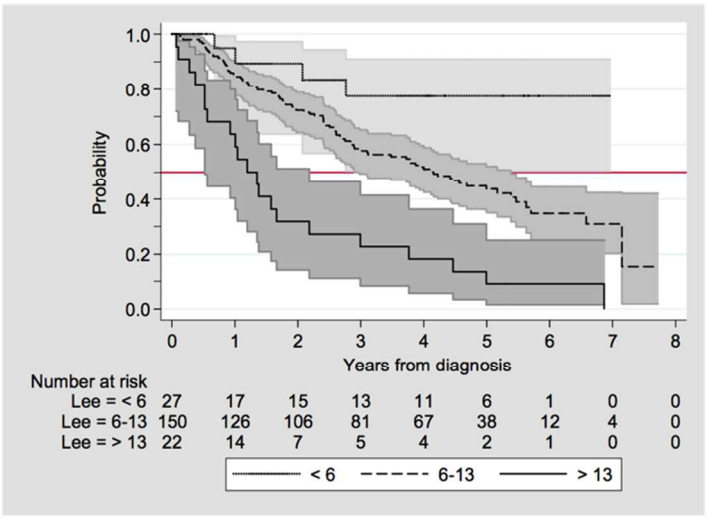

Figure 2. Overall projected survival (95% confidence interval) as a function of patient condition

338x190mm (72 x 72 DPI)

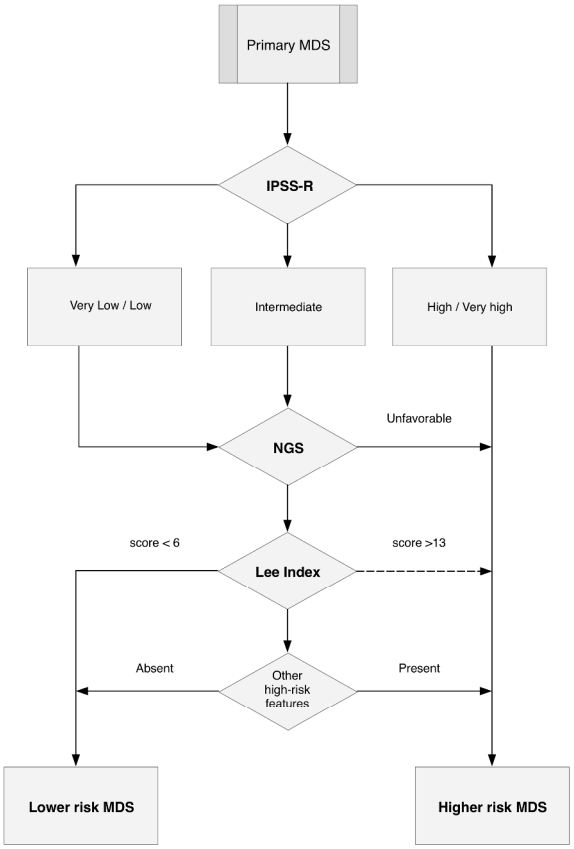

Figure 3. An integrative algorithm for predicting outcome of MDS patients

297x420mm (300 x 300 DPI)