MARS: Mutation-Adjusted Risk Score for Advanced Systemic Mastocytosis

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ABSTRACT

Purpose

To develop a risk score for advanced systemic mastocytosis (AdvSM) patients that integrates clinical and mutation characteristics.

Patients and Methods

The study included 383 AdvSM patients from the 'German Registry on Disorders of Eosinophils and Mast Cells' (training set, n = 231) and several centers for mastocytosis in the USA and Europe - all within the European Competence Network on Mastocytosis (ECNM) (validation set, n = 152). A Cox multivariable model was used to select variables that were predictive of overall survival (OS).

Results

In multivariable analysis, the following risk factors were identified regarding OS: age > 60 years, anemia (hemoglobin < 10 g/dL), thrombocytopenia (platelets < 100 x 10^9/L), presence of one high molecular risk gene mutation (ie, in *SRSF2*, *ASXL1*, and/or *RUNX1*), and of ≥ 2 high molecular risk gene mutations. By assigning hazard ratio (HR)-weighted points to these variables, three risk categories were defined: low-risk (median OS not reached), intermediate-risk (median OS 3.9 years, 95% CI 2.1 to 5.7 years), and high-risk (median OS 1.9 years, 95% CI 1.3 to 2.6 years) (P < .0001). The mutation-adjusted risk score (MARS) was independent of the World Health Organization (WHO) classification and was confirmed in the independent validation set. During a median follow-up of 2.2 years (range 0-23), 63/383 (16%) patients had a leukemic transformation to secondary mast cell leukemia (32%) or secondary acute myeloid leukemia (68%). The MARS was also predictive for leukemia-free survival (P < .0001).

Conclusion

The MARS is a validated five-parameter WHO independent prognostic score that defines three risk groups among patients with AdvSM and may improve upfront treatment stratification for these rare hematologic neoplasms.

INTRODUCTION

Systemic mastocytosis (SM) is characterized by expansion of clonal mast cells that infiltrate various organ systems. The extent of organ infiltration and subsequent organ damage serve as a basis for the World Health Organization (WHO) classification into indolent SM (ISM) and advanced SM (AdvSM). AdvSM compromises patients with SM and an associated hematologic neoplasm (SM-AHN), aggressive SM (ASM), and mast cell leukemia (MCL).[1-4]

SM-AHN (70-80% of all AdvSM patients) is the most heterogeneous and clinically challenging subtype. The AHN usually resembles a myeloid neoplasm, e.g. chronic myelomonocytic leukemia (CMML), myelodysplastic/myeloproliferative neoplasm unclassifiable (MDS/MPN-U), chronic eosinophilic leukemia (CEL) or MDS. In the vast majority of the patients, the phenotypically most important somatic mutation - *KIT* D816V - is detectable in the clonal mast cell compartment as well as in cells derived from the AHN.[5,6]

The WHO classification is most widely used for prognostication and has been validated in multiple studies. In contrast to ISM, AdvSM has a poor prognosis.[7] The overall survival (OS) of AdvSM patients ranges from few months to several years with a median OS of approximately 4 years.[8,9]

A number of clinical, serological, cytomorphological, immunological and molecular parameters have been reported to be of (WHO independent) prognostic significance in patients with AdvSM.[10,11] Recent data, however, have highlighted that the molecular landscape of AdvSM is complex with at least one additional somatic mutation (e.g., in *ASXL1*, *CBL*, *JAK2*, *RUNX1*, *SRSF2*, or *TET2*) being present in >60% of AdvSM patients.[12,13] In more recent studies, several groups examined the prognostic impact of these mutations. The presence and number of additional molecular aberrations, notably in *SRSF2, ASXL*, and/or *RUNX1* (S/A/R), have a strong adverse influence on progression (leukemic transformation) to secondary MCL and/or secondary AML, response to treatment and OS.[8-

[10,13-15] To date, the independent prognostic value of most variables and proposed risk scores have been derived from relatively small sets of patients, and they have not been confirmed or validated.[14]

In this study, we evaluated a large cohort of clinically, morphologically, and genetically well characterized AdvSM patients who were enrolled within the 'German Registry on Disoders of Eosinophils and Mast Cells' with the aim to establish a risk score integrating both clinical and molecular characteristics. The proposed clinical risk score (CRS) and mutation-adjusted risk score (MARS) were subsequently validated in an independent cohort of AdvSM patients derived from several centers within the European Competence Network on Mastocytosis (ECNM).

PATIENTS AND METHODS

Patients

A total of 383 AdvSM patients were included. For the training set, 231 AdvSM patients were recruited within the 'German Registry on Disorders of Eosinophils and Mast Cells' between 2003 and 2018, with a final update performed in November 2018. The diagnosis of AdvSM (SM-AHN, ASM, and MCL) was established according to the WHO classification.[1,4] For the training set, bone marrow (BM) biopsies and BM smears were evaluated by reference pathologists of the ECNM (H-PH and KS). The study design adhered to the tenets of the Declaration of Helsinki and was approved by the institutional review board of the Medical Faculty of Mannheim, Heidelberg University. All patients gave written informed consent.

The validation set included 152 patients derived from multiple centers of excellence for mastocytosis in the USA (Stanford, California, USA) and Europe (Spanish Network on Mastocytosis [REMA], Toledo and Salamanca, Spain; Vienna, Austria; Freiburg, Germany - all members of the ECNM).

Mutational and Cytogenetic Analyses

Molecular analyses were performed at diagnosis of AdvSM (prospectively or retrospectively). Targeted Next-Generation Sequencing (NGS) was either performed by 454 FLX amplicon chemistry (Roche, Penzberg, Germany) or library preparation based on the TruSeq Custom Amplicon Low Input protocol (Illumina, San Diego, CA) and sequencing on the MiSeq instrument (Illumina, San Diego, CA) to investigate mutation status of *KIT* and the following 32 genes: *ASXL1*, *BCOR*, *CALR*, *CBL*, *CSNK1A1*, *DNMT3A*, *ETNK1*, *ETV6*, *EZH2*, *FLT3*, *GATA1*, *GATA2*, *IDH1*, *IDH2*, *JAK2*, *KRAS*, *MLL*, *MPL*, *NPM1*, *NRAS*, *PHF6*, *PIGA*, *PTPN11*, *RUNX1*, *SETBP1*, *SF3B1*, *SRSF2*, *TET2*, *TP53*, *U2AF1*, *ZRSR2*, *WT1*.[12] Subsequent to bcl2fastq and demultiplexing, alignment and variant calling were performed using JSI

SeqNext v4.4.0 (JSI Medical Systems, Kippenheim, Germany) software with default parameters. Only base calls with quality score > 30 were considered for further processing. A median of ~1800 reads were aligned to the target region. All regions below the minimal coverage of 400 reads were rejected and resequenced for higher depth. Variants were called with a variant allele frequency (VAF) cutoff of 3% and each assessed manually for pathogenicity. Mutation assessment was performed using COSMIC (v78), dbSNP (v150), ClinVar (2018-07), gnomAD (r2.0.2 and dbNSFP v3.5). Cytogenetic analysis and reporting were performed according to the International System for Human Cytogenetic Nomenclature criteria using standardized techniques.

Statistical Analyses

All statistical analyses considered clinical and laboratory parameters obtained at time of diagnosis or first referral to our center that in most instances coincided with time of BM biopsy and study sample collection. OS analysis was considered from the date of diagnosis to date of death or last visit. Leukemia-free survival (LFS) was considered from the date of diagnosis to date of death, last visit or progression (leukemic transformation) to secondary MCL or secondary SM-AML. As the MARS reflected the highest C-index, LFS analyses was examined for this score, only. OS probabilities and LFS were estimated with the Kaplan-Meier method and compared by the log-rank test in univariate analysis. For OS, a Cox proportional hazards model with a stepwise selection procedure was used to select covariates, based on their statistical significance ($P < .05$). Significant covariates were confirmed by forward-selection and backward-elimination techniques. Based on of the magnitude of the hazard ratios (HRs) obtained from multivariable analysis, a weighted score was assigned to each significant variable for OS in the learning set. Bonferroni adjustments were made to univariate analysis with no changes the multivariable models. The Wilcoxon-Mann-Whitney

U test was used to compare continuous variables and medians of distributions. Receiver operating characteristic (ROC) curve was used to dichotomize continuous variables to define optimal cut-off value for each variable used in univariate analyses. Harrell's concordance index (C-index, on the basis of ROC) was used to evaluate the ability of the risk scores to predict outcome (C-index measures the goodness-of-fit of a model, with 0.5 indicating no discrimination and 1.0 indicating perfect prediction). For categorical variables, two patient groups were compared with the Fisher's exact test. All tests were two-sided, retaining $P < .05$ as statistically significant.

RESULTS

Characteristics

The characteristics of the training set patients (n = 231) are listed in Table 1. The median age was 69 years with a male predominance (68%). The WHO diagnosis was ASM (n = 30, 13%), SM-AHN (n = 181, 78%), and MCL (± AHN) (n = 20, 9%), respectively. The four most common AHN subtypes were CMML, MDS/MPN-U, CEL, and MDS. The median leukocyte counts, hemoglobin level, and platelet counts were 8.3 x 10^9/L, 10.3 g/dL and 115 x 10^9/L, respectively, and the median serum tryptase level was 168 µg/L (normal value < 11.4). Treatment modalities included midostaurin, cladribine, and sequential midostaurin/cladribine or cladribine/midostaurin in 111 (48%) patients. During a median follow-up of 2.2 years (range 0-23), 118 (51%) patients died. Transformation to secondary MCL (43%) or secondary AML (57%) was observed in 35 (15%) patients (Table 1).

No significant differences were seen between the training and validation (n = 152) sets regarding e.g. gender, hemoglobin, platelets, alkaline phosphatase, leukemic transformation, median follow-up, and number of deaths, respectively (Table 1). Patients in the training set were significantly older (median 69 versus 65 years), ASM was less frequent (13% versus 30%) and SM-AHN was more frequent (78% versus 63%). More patients in the training set were treated with midostaurin or sequential midostaurin/cladribine or cladribine/midostaurin treatment regimens.

Importantly, the median OS and LFS were not significantly different between the training and the validation sets (OS, 3.8 and 4.4 years; LFS 3.3 and 3.5 years; P = .8 and P = .9, respectively; supplementary Figure 1A). In addition, no differences were seen regarding OS within the four most common AHN's (supplementary Figure 3A-B) and between *KIT* positive and *KIT* negative patients (supplementary Table 2).

Gene Mutations

In the training set, the *KIT* mutation status was as follows: *KIT* D816V (n = 214, 93%), other *KIT* mutations (n = 6, 2%), *KIT* mutation negative (n = 11, 5%). The status of additional mutations was assessed in 190/231 (82%) patients. At least one additional mutation was observed in 82% of all patients. The most frequently affected genes (in ≥ 5% of patients) were *TET2* (n = 79, 42%), *SRSF2* (n = 75, 39%), *ASXL1* (n = 42, 22%), *RUNX1* (n = 34, 18%), *JAK2* (n = 23, 12%), *N/KRAS* (n = 17, 9%), *CBL* (n = 17, 9%), *IDH1/2* (n = 9, 5%), *SF3B1* (n = 9, 5%), and *EZH2* (n = 9, 5%). The presence of at least 1 and of ≥ 2 S/A/R mutation(s) was documented in 105 (55%) patients and 43 (23%) patient, respectively (Table 2, Figure 1A-D). An aberrant karyotype was detected in 27/168 (16%) patients.

With the exception of different numbers of patients without *KIT* mutation (5% versus 12%, respectively), no significant differences were observed between the training and the validation sets (e.g., the number of S/A/R positive patients was comparable; Table 2).

Prognostic Impact of the WHO Classification

The WHO classification of AdvSM is of prognostic significance. In the training and the validation sets, the median OS for ASM, SM-AHN and MCL (± AHN) was not reached (training set) and 10.1 years (validation set), 3.6 and 2.9 years, and 0.8 and 0.5 years, respectively. The WHO defined intermediate-risk category of SM-AHN (n = 275, 72%) represents by far the largest group compared to the low-risk category of ASM (n = 77, 20%) and the high-risk category of MCL (n = 31, 8%) (supplementary Figure 1B, supplementary Figure 2A-B).

Prognostic Impact of the S/A/R Gene Panel

Stratification based on the presence and number of high molecular risk gene mutation(s) (i.e. S/A/R) was of significant prognostic impact. In the training and the validation sets, median OS was not reached and 10.1 years, 3.0 years and 4.3 years, and 1.5 years and 1.8 years for no mutation, 1 mutation, and ≥ 2 gene mutations in the S/A/R panel, respectively. The three S/A/R-based risk groups were balanced as followed: low-risk, 154 (47%); intermediate-risk 102 (31%); and high-risk 73 (22%) (Figure 2A-B; supplementary Figure 1C).

Development and Validation of Clinical Risk Score for Advanced SM: CRS

We applied a Cox proportional hazard model using the patients from the 'German Registry on Disorders of Eosinophils and Mast Cells' in the training set (n = 231). In univariate analyses, the model included the following variables: age > 60 years, sex, WHO subtype, hemoglobin < 10 g/dL, platelets < 100 x 10^9/L, mast cell infiltration in BM histology > 30%, serum tryptase > 150 µg/L, albumin < 35 g/dL, alkaline phosphatase > upper normal limit (UNL), and splenomegaly (palpable or radiographic, yes/no). The multivariable analysis identified four independent predictors of survival: age > 60 years (HR 3.2, confidence interval [CI] 1.8-5.9, *P* < .0001), hemoglobin < 10 g/dL (HR 2.0, CI 1.3-3.0, *P* = .002), platelets < 100 x 10^9/L (HR 1.7, CI 1.1-2.6, *P* = .01) and alkaline phosphatase > UNL (HR 1.8, CI 1.1-2.9, *P* = .03). For assignment of individual scores, we divided the HR value of each variable by the median value of the regression coefficients of all variables in the final model (rounded to nearest 0.5 point). Accordingly, a weighted score of 1 was assigned to hemoglobin < 10 g/dL, platelets < 100 x 10^9/L, and alkaline phosphatase > UNL, whereas a score of 1.5 was assigned to age > 60 years. On this basis, we generated the CRS: low-risk, 0 to 1.5; intermediate risk 2 to 2.5; high-risk, 3 to 4.5. The model was then applied to the validation cohort (Table 4).

The median OS for the training set and the validation set was not reached (training set) and 12.2 years (validation set), 3.8 and 4.3 years, 2.6 and 1.8 years, for low-risk (n = 98, 28%), intermediate-risk (n = 111, 32%), and high-risk (n = 136, 39%), respectively (Table 4, Figure 2C-D; supplementary Figure 1D).

Development and Validation of Mutation-Adjusted Risk Score: MARS

To appreciate the value of adding molecular information to the CRS, we applied a Cox proportional hazards model among patients for whom mutation status (including S/A/R gene status) was available (training set, n = 191). The model was started by considering the same variables using in developing the CRS and included the presence and number of high molecular risk gene mutations: zero, one or ≥ 2 S/A/R mutation(s).

Table 3 summarizes the results of univariate and multivariable analyses in the training set. The multivariable model identified five independent predictors of survival: age > 60 (HR 2.4, CI 1.4-5.0, $P < .003$), hemoglobin < 10 g/dL (HR 2.0, CI 1.3-3.0, $P = .002$), platelets < 100 x 10^9/L (HR 1.7, CI 1.1-2.5, $P = .02$), S/A/R 1 mutation (HR 2.5, CI 1.6-4.5, $P < .0001$), and S/A/R ≥ 2 mutations (HR 4.4, CI 2.1-7.3, $P < .0001$). For assignment of individual scores, we divided the HR value of each variable the median value of the regression coefficients of all variables in the final model (rounded to nearest 0.5 point). Accordingly, a weighted score of 1 was assigned to age > 60 years, hemoglobin < 10 g/dL, platelets < 100 x 10^9/L, and S/A/R 1 mutation, whereas a score of 2 was assigned to S/A/R ≥ 2 mutations. These weighted scores were used to generate three risk groups which comprise the MARS: low-risk, 0 to 1; intermediate risk, 2; high-risk, 3 to 5. The model was then applied to the validation cohort. Table 4 describes the OS of the combined training and validation sets for the CRS and the MARS.

The median OS for the training and the validation sets was not reached (training set) and 12.2 years (validation set), 3.9 years and 4.4 years, 1.9 years and 1.9 years, for low-risk (n = 103, 31%), intermediate-risk (n = 86, 26%), and high-risk patients (n = 140, 43%), respectively (Table 4, Figure 2E-F and Figure 2G).

The MARS was also predictive for LFS. The median LFS for the training and the validation sets was not reached (training set) and 11 years (validation set), 3.9 and 3.9 years, and 1.5 and 1.4 years, for low-risk, intermediate-risk, and high-risk, respectively (Figure 2H, supplementary Figure 2C-D, and supplementary Table 1).

Comparison of WHO, CRS and MARS

On basis of ROC curve analyses, the C-index was 0.42 for the WHO classification, 0.73 for the CRS, and 0.81 for the MARS (Figure 1F). For better comparison of the C-index between the four stratification tools (WHO, S/A/R, CRS, and MARS), we included the same samples (with fully available data-set from the training set, n = 190) across all rules. We established a cross table illustrating the distribution of AdvSM patients in the new scoring system (rows Figure 1E) in comparison to the WHO classification (colors within each row in Figure 1E). Figure 1E illustrates significant risk redistributions when using MARS across the WHO classification. Particularly, the large SM-AHN (n = 237, 72% of all patients) cohort defined as intermediate-risk according to the WHO classification was reclassified as low-risk (n = 60, 25%), intermediate-risk (n = 64, 27%), and high-risk (n = 113, 48%) by the MARS. In ASM and MCL (± AHN), 38% (n = 24) and 83% (n = 25) were represented in the intermediate-risk or high-risk MARS categories, respectively. The significant advantages of MARS in comparison to CRS were i) the enhanced stratification regarding OS within all three risk groups, especially of the intermediate-risk and high-risk groups (Figure 2C-D, E-F and supplementary Figure 1D) and ii) the prediction of LFS since S/A/R positivity (included in

the MARS) at initial diagnosis is significantly associated with transformation to secondary MCL and AML. Seventy percent (n = 42) of all patients with leukemic transformation and available S/A/R status (n = 60) had at least one S/A/R mutation at initial diagnosis.

DISCUSSION

In clinical practice, the 2016 WHO classification of SM is widely used for prognostic purposes due to the lack of validated international risk scores. Although it robustly distinguishes indolent SM from AdvSM, its value for stratification within the various subtypes of AdvSM (OS: ASM > SM-AHN > MCL) remains suboptimal for three main reasons: i) the clinical and histological heterogeneity represented by the various subtypes of AdvSM, ii) the imbalance of the various subtypes, with SM-AHN representing 70-80% of patients, and ASM and MCL representing only 20-30% of individuals, and iii) the wide range of survival times within the subtypes of AdvSM, and particularly within the SM-AHN variant between a few months and several years.[7,8,11,16,17] Therefore, the main goal of the current study was to devise and validate a new WHO independent risk score for patients with AdvSM which integrates objective clinical and mutation characteristics.

The current analysis corroborates the prognostic value of the previously identified high molecular risk gene mutations,[8,9,13,14,18] especially the negative impact of S/A/R. The presence and number of gene mutations in the S/A/R panel had a strong adverse impact on OS in both the training set and the validation set. The three genes (S/A/R) are among the top five most frequent mutations observed in AdvSM (but also other myeloid neoplasms)[19-21] and allow a balanced stratification into three risk cohorts.

Next, we established a clinical risk score (CRS) by defining four easily accessible and objective parameters based on multivariable analyses: age > 60 years, anemia (hemoglobin < 10 g/dL), thrombocytopenia (platelets < 100 x 10^9/L), and elevated alkaline phosphatase (> UNL). As illustrated in Figure 2C-D and supplementary Figure 1D, LFS and OS were significantly different among the three risk groups. The prognostic impact of the CRS was confirmed in the validation set. The C-index was comparable with the S/A/R-based stratification (0.73 versus 0.74).

Finally, we combined the clinical and molecular data and generated the MARS. In multivariable analyses, age > 60 years, anemia (hemoglobin < 10 g/dL), thrombocytopenia (platelets < 100 x 10^9/L), 1 S/AR mutation, and ≥ 2 S/A/R mutations were independent predictors for OS. Based on these five parameters, a simple risk scoring system was established for OS. The MARS was confirmed in the validation set and categorizes AdvSM patients into three groups of significant size. OS was not reached, 4.3 and 1.9 years for AdvSM patients with low-risk, intermediate-risk, and high-risk, respectively. According to the C-index (0.81), the MARS improves the prediction of OS as compared to the WHO classification (C-index 0.42) and the CRS (0.73), especially for the intermediate-risk and high-risk groups, and uses clinical and molecular data which are now commonly available. S/A/R positivity at initial diagnosis, which is the backbone of the MARS, is significantly associated with secondary leukemic transformation (MCL and AML) and therefore the MARS is also predictive for LFS.

Some recently published risk scores from our own group and from others also included variables such as anemia, thrombocytopenia, elevated alkaline phosphatase and high molecular risk gene mutations.[10,22] The pivotal strengths of the current analyses include i) indolent SM was excluded in the prognostic models as it has *per se* a nearly normal life expectancy, ii) the highest number of clinically, morphologically and genetically well characterized AdvSM patients ever reported, iii) most patients had access to targeted treatment modalities such as midostaurin, iv) the vast majority of patients of the training set were diagnosed through fully centralized pathology and genetic analyses, and v) the homogenous mutation profile (clinical and outcome characteristics) of the training set and the large and independent validation set (derived from centers with expertise in mastocytosis), particularly regarding the individual frequency of gene mutations in the S/A/R panel.

Although there are no data from clinical trials, the MARS may become useful for guiding selection of, and predicting response to therapies. Previous data have shown that the multikinase/KIT-inhibitor midostaurin has disease modifying activity in AdvSM with sustained responses and more favorable outcome in patients with absence of mutations in the S/A/R gene panel and at least 25% reduction of the *KIT* D816V expressed allele burden after 6 months of therapy.[9,16,23,24] As the MARS low-risk cohort reflects the majority of these patients, midostaurin may be an optimal choice for these individuals. The generally poor prognosis of MARS intermediate- and high-risk patients may predict less robust responses with currently available therapies, including midostaurin monotherapy, highlighting the need for disease-modifying treatments in these higher risk cohorts. [9,16,23-25] Because of the significantly higher rates of leukemic transformation and inferior survival, more intensive treatment, e.g. combination therapies with midostaurin that also target the AHN, or use more potent and selective second generation *KIT* D816V inhibitors, followed by allogeneic stem cell transplantation (SCT) in eligible candidates, should be taken into consideration in these patients. In the largest, yet reported cohort of 57 AdvSM patients undergoing allogeneic SCT, treatment-related mortality was generally similar to other hematological neoplasms. Important details included the superior outcome of myeloablative vs. dose-reduced conditioning and the heterogenous survival within AdvSM, being significantly better in SM-AHN as compared to ASM or MCL, respectively. However, more data is warranted, preferably generated in national and international registries upon the key questions regarding optimal timing, debulking and conditioning strategies.

We conclude that the WHO classification remains the pivotal diagnostic tool for subtyping of SM into indolent SM and AdvSM. The MARS is a WHO independent and complementary tool for the heterogeneous cohort of patients with AdvSM by defining three risk groups based on a five-parameter risk score which may improve upfront treatment

stratification for these rare hematologic neoplasms.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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FIGURE

Figure 1. Mutational profile, categorization of patients according to mutation-adjusted risk score (MARS) of advanced systemic mastocytosis versus the World Health Organization (WHO) classification, and the performance of the scores. Relative frequency distribution of (A) *KIT* mutations, (B) number of affected genes in addition to *KIT*, (C) mutations in addition to *KIT*, and (D) gene mutations in the [a]*SRSF2*, *ASXL1*, *RUNX1* (S/A/R) panel of the training set. (E) colored bars represent the WHO risk stratification (x-axis) in the context of the stratification based on the MARS (represented by the rows). (F) Shown is the C-index (to evaluate the ability of the prognostic scores to predict outcome, 0.5 indicating no discrimination and 1.0 indicating perfect prediction) of WHO based stratification, S/A/R mutation based stratification, clinical risk score (CRS), and MARS. ASM, aggressive SM; MCL, mast cell leukemia; SM-AHN; SM with an associated neoplasm. [b] The MCL cohort included patients with MCL and MCL-AHN.

Figure 2. Overall survival (OS) for the training set (left) and the validation set (right) of advanced systemic mastocytosis (AdvSM) patients. Patients in both sets are grouped by (A-B) [a] *SRSF2*, *ASXL1*, *RUNX1* (S/A/R) mutation based stratification, (C-D) the clinical risk score (CRS), and (E-F) the mutation-adjusted risk score (MARS). (G-H) OS and leukemia-free survival of all AdvSM patients (training + validation) by MARS is shown.

Table 1. Baseline Clinical and Laboratory Characteristics in Training and Validation Sets of Patients With Advanced Systemic Mastocytosis (AdvSM)

Characteristics	Training (n = 231)	Validation (n = 152)	*P*
Age, years			
Median	69	65	.003
Range	24-90	22-92	
Sex, n (%)			
Men	156 (68)	92 (61)	.2
Women	75 (32)	60 (39)	.2
WHO diagnosis, n (%)			
ASM	30 (13)	46 (30)	< .0001
SM-AHN	181 (78)	95 (63)	.001
MCL (± AHN)	20 (9)	11 (7)	.7
AHN subtypes, n (%)			
CMML	57 (29)	22 (23)	.3
MDS/MPN-U	50 (26)	12 (13)	.01
CEL	34 (18)	11 (11)	.2
MDS	30 (16)	17 (18)	.7
Others[a]	22 (11)	34 (35)	.001
Leukemic transformation, n (%)	35 (15)	28 (18)	.4
Secondary MCL (± AHN)	15 (43)	5 (18)	
Secondary SM-AML	20 (57)	23 (82)	
Time to transformation, years			
Median	1.6	1.6	
Range	0.2-5.9	0.1-11.1	
Hemoglobin, g/dL			
Median	10.3	10.7	.3
Range	5.7-20.5	4-18.1	
< 10 g/dL, n (%)	100 (46)	59 (40)	.4
Leukocytes, x 10^9/L			
Median	8.3	7.4	.4
Range	1.3-124.0	0.6-191.0	
Platelets, x 10^9/L			
Median	115	125	.7
Range	5-958	6-486	
< 100 x 10^9/L, n (%)	94 (43)	62 (42)	.8
Mast cell infiltration in BM histology, (%)			
Median	30	20	.7
Range	5-100	5-90	
Serum tryptase, µg/L			
Median	168	159	.7
Range	15-1854	2-2036	
Albumin, g/L			
Median	37	40	.6
Range	16-48	26-57	
Alkaline phosphatase[b], U/L			
Median	180	155	.3
Range	35-1928	28-1074	
> UNL, n (%)	128 (65)	85 (61)	.5
Splenomegaly[c], n (%)	171 (74)	83 (60)	.007
Treatment modalities, n (%)			
Midostaurin	56 (24)	17 (12)	.001
Cladribine	20 (9)	23 (15)	.07
Midostaurin + cladribine[d]	35 (15)	8 (5)	.003
Follow-up, years			
Median	2.2	2.1	.7
Range	0-23	0-23	
Death, n (%)	118 (51)	76 (50)	

Abbreviations: AHN, associated hematologic neoplasm; ASM, aggressive systemic mastocytosis; BM, bone marrow; CEL, chronic eosinophilic leukemia; CMML, chronic myelomonocytic leukemia; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; MDS/MPN-U, MDS/MPN unclassifiable; MCL, mast cell leukemia; UNL, upper normal limit; WHO, World Health Organization.

[a] acute myeloid leukemia, primary mylofibrosis, polycthaemia vera, essential thrombocythemia, chronic myeloid leukemia, indolent lymphoma, and myeloma

[b] data available in n = 197 (training) and n = 140 (validation), respectively

[c] palpable or radiographic

[d] sequential midostaurin/cladribine or cladribine/midostaurin treatment

Table 2. Genetic Characteristics in Training and Validation Sets of Patients With Advanced Systemic Mastocytosis (AdvSM)

Characteristics	Training (n = 231)	Validation (n = 152)	*P*
Driver mutation, n (%)			
KIT D816V	214 (93)	126 (88)[b]	.1
Other *KIT* mutations	6 (2)[a]	-	
No *KIT* mutations	11 (5)	18 (12)	.009
Additional somatic mutations[c], n (%)			
TET2	79 (42)	58 (42)	1.0
SRSF2	75 (39)	45 (32)	.2
ASXL1	42 (22)	24 (17)	.3
RUNX1	34 (18)	32 (23)	.3
JAK2	23 (12)	2 (9)[d]	1.0
N/KRAS	17 (9)	5 (6)[e]	.6
CBL	17 (9)	8 (10)[e]	.8
IDH1/2	9 (5)	6 (7)[e]	.4
SF3B1	9 (5)	9 (8)[f]	.3
EZH2	9 (5)	8 (7)[f]	.4
S/A/R[g] mutation(s), n (%)	105 (55)	70 (50)	.4
≥ 2 S/A/R mutations	43 (23)	30 (22)	.9
Aberrant karyotype[h], n (%)	27 (16)	19 (22)	.2

[a] *KIT* D816H, n = 3; *KIT* D816Y, n = 2; *KIT* F522C, n = 1
[b] *KIT* status available in n = 144
[c] Most frequently affected genes (in ≥ 5% of patients); data available in n = 190 (training) and n = 139 (validation)
[d] data available in n = 23
[e] data available in n = 82
[f] data available in n = 115
[g] ≥ 1 gene mutation(s) in *SRSF2*, *ASXL1* and/or *RUNX1* (S/A/R) panel
[h] data available in n = 168 (training) and n = 85 (validation)

Table 4. Clinical Risk Score (CRS) and Mutation-Adjusted Risk Score (MARS) in Patients With Advanced Systemic Mastocytosis (AdvSM) – Proposal for Scoring Systems and Overall Survival (OS)

Characteristics	CRS	MARS	Category (n) (score range)	CRS	Category (n) (score range)	MARS
	Prognostic Points	Prognostic Points		Median (range) OS (years)		Median (range) OS (years)
Age > 60 years	1.5	1	Low (98)	N.R.	Low (103)	N.R.
Hemoglobin < 10 g/dL	1	1	(0-1.5)		(0-1)	
Platelets < 100 x 10^9/L	1	1	Intermediate (111)	3.9	Intermediate (86)	4.3
Alkaline phosphatase > UNL	1	-	(2-2.5)	(2.7-5.1)	(2)	(3.2-5.4)
S/A/R[a] (1 mutation)	-	1	High (136)	2.5	High (140)	1.9
S/A/R[b] (≥ 2 mutations)	-	2	(3-4.5)	(1.8-3.1)	(3-5)	(1.6-2.3)

Abbreviations: N.R., not reached; UNL, upper normal limit
[a] 1 gene mutation in *SRSF2*, *ASXL1* and/or *RUNX1* (S/A/R) panel
[b] ≥ 2 gene mutations in the S/A/R panel

Table 3. Univariate and Multivariable Overall Survival (OS) Analysis in Training Set Based on Clinical and Molecular Characteristics (Mutation-Adjusted Risk Score, MARS) in Patients With Advanced Systemic Mastocytosis (AdvSM)

Characteristics	MARS					
	Univariate			Multivariable		
	HR	95% CI	*P*	HR	95% CI	*P*
Age > 60 years	3.4	2.0-5.8	< .0001	2.4	1.4-5.0	.003
Sex (men vs. women)	1.7	1.1-2.5	.02			
WHO						
SM-AHN vs. ASM	2.3	1.3-4.0	.004			
MCL vs. SM-AHN	2.9	1.5-5.8	.002			
MCL vs. ASM	3.4	2.0-5.9	< .0001			
Hemoglobin < 10 g/dL	2.4	1.6-3.5	< .0001	2.0	1.3-3.0	.002
Platelets < 100 x 10^9/L	2.4	1.6-3.5	< .0001	1.7	1.1-2.5	.017
Mast cell infiltration[a] > 30%	1.3	0.8-1.9	.3			
Serum tryptase > 150 µg/L	1.7	1.1-2.5	.02			
Albumin < 35 g/L	1.9	1.3-3.0	.002			
Alkaline phosphatase > UNL	2.6	1.6-4.1	< .0001			
Splenomegaly	2.0	1.0-4.2	.05			
S/A/R[b] (1 mutation)	4.3	2.7-6.9	< .0001	2.5	1.6-4.5	< .0001
S/A/R[c] (≥ 2 mutations)	7.6	3.5-9.9	< .0001	4.4	2.1-7.3	< .0001
Aberrant karyotype	1.5	0.9-2.5	.1			

Abbreviations: AHN, associated hematologic neoplasm; ASM, aggressive systemic mastocytosis; BM, bone marrow; CI, confidence interval; MCL, mast cell leukemia; NR, not reached; vs., versus; UNL, upper normal limit; WHO, World Health Organization

[a] Mast cell infiltration in bone marrow histology

[b] 1 gene mutation in *SRSF2*, *ASXL1* and/or *RUNX1* (S/A/R) panel

[c] ≥ 2 gene mutations in the S/A/R panel

Figure 1

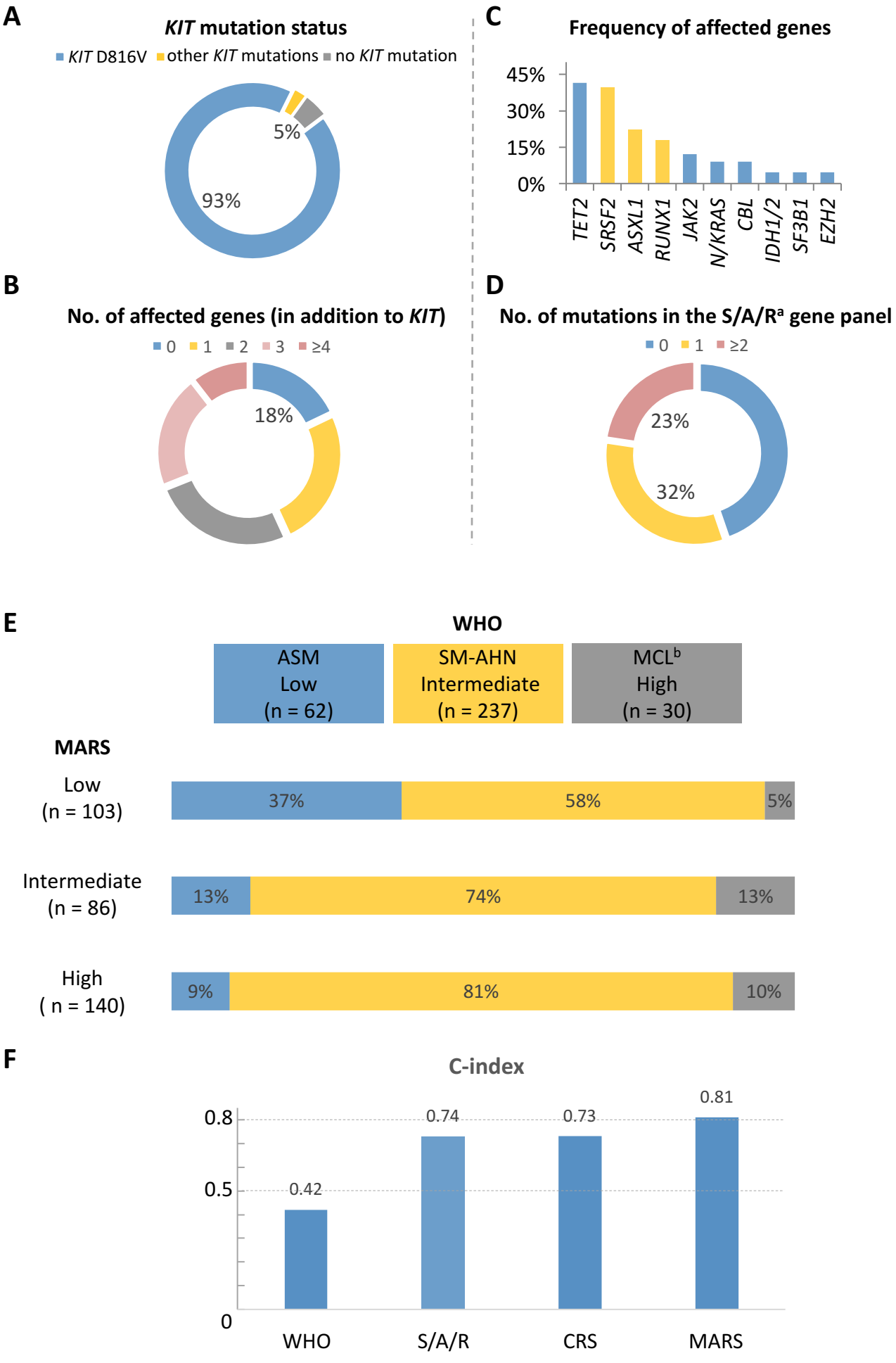
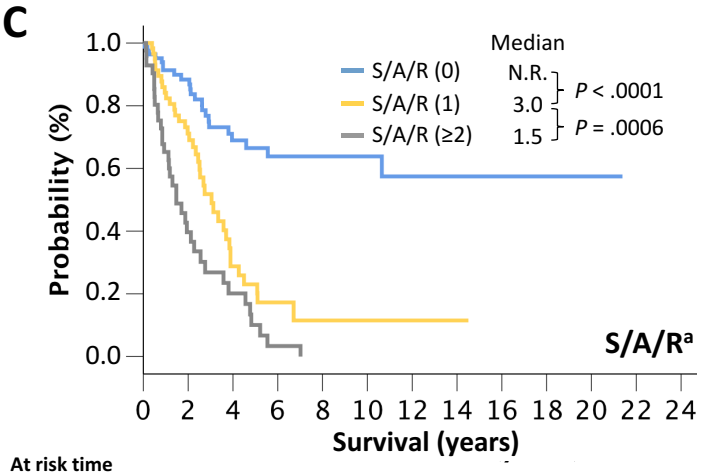

A
KIT mutation status
KIT D816V
other KIT mutations
no KIT mutation
5%
93%
C
Frequency of affected genes
45%
30%
15%
0%
TET2
SRSF2
ASXL1
RUNX1
JAK2
N/KRAS
CBL
IDH1/2
SF3B1
EZH2
B
No. of affected genes (in addition to KIT)
0
1
2
3
≥4
18%
D
No. of mutations in the S/A/R[a] gene panel
0
1
≥2
23%
32%
E
WHO
ASM
Low
(n = 62)
SM-AHN
Intermediate
(n = 237)
MCL[b]
High
(n = 30)
MARS
Low
(n = 103)
37%
58%
5%
Intermediate
(n = 86)
13%
74%
13%
High
(n = 140)
9%
81%
10%
F
C-index
0.8
0.5
0
0.42
0.74
0.73
0.81
WHO
S/A/R
CRS
MARS

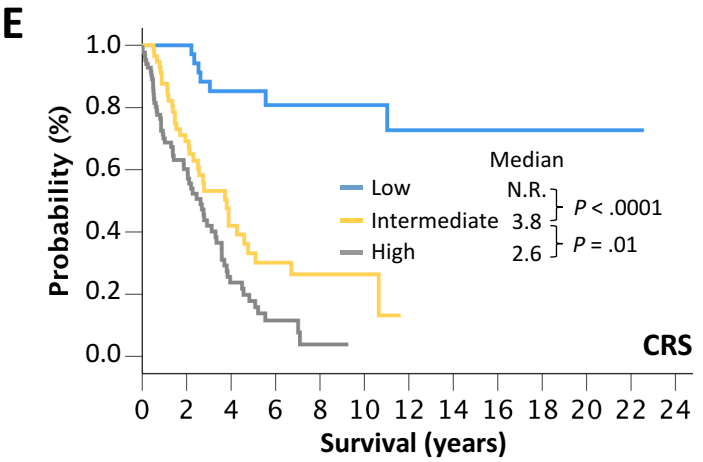
Figure 2

Training



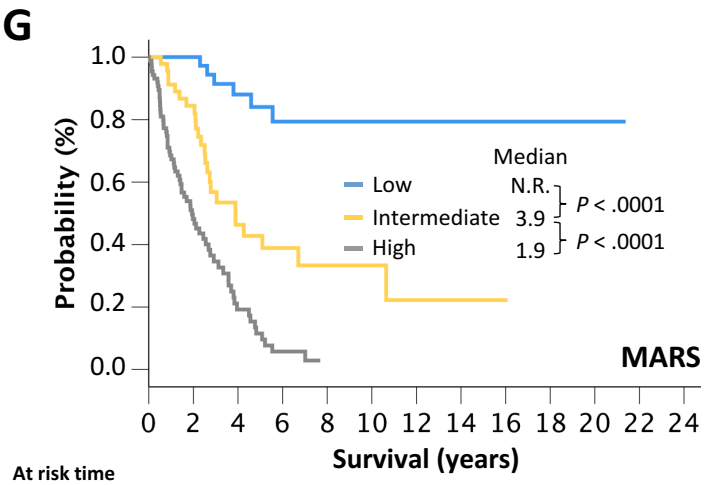
At risk time

S/A/R (0)	85	57	33	21	14	11	6	5	4	2	2
S/A/R (1)	62	34	10	5	2	1	1	1			
S/A/R (≥2)	43	13	6	1							



At risk time

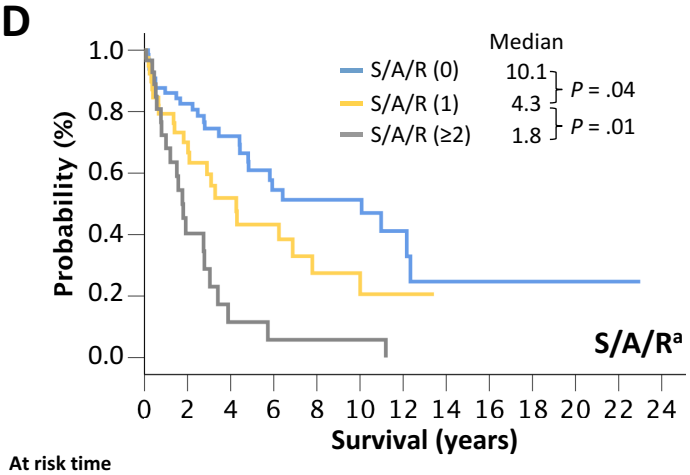
Low	48	35	25	17	13	10	7	6	4	3	3	1
Intermediate	63	34	15	9	4	3						
High	85	39	13	4	1							



At risk time

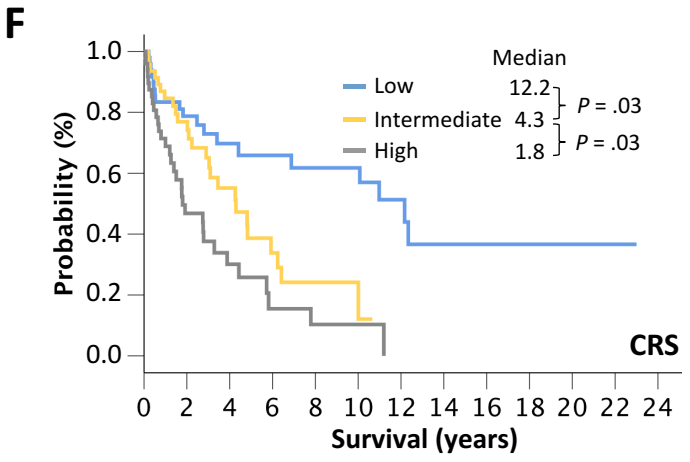
Low	53	37	26	16	12	9	5	4	3	2	2
Intermediate	48	35	13	8	4	3	2	2	1		
High	89	32	10	3							

Validation



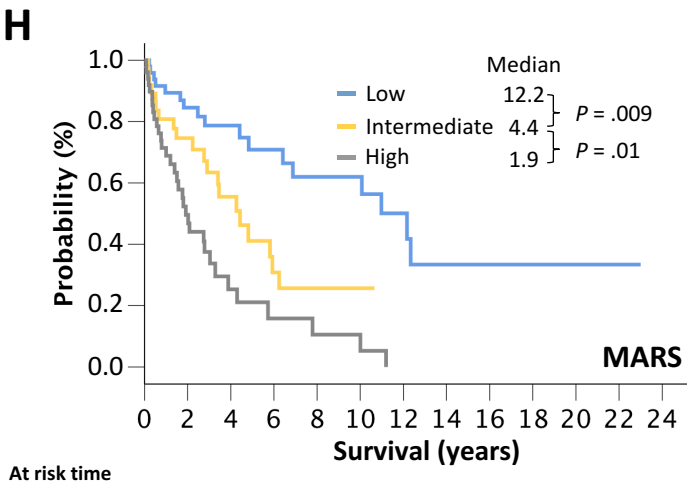
At risk time

S/A/R (0)	69	47	27	17	14	12	6	3	3	3	1	1
S/A/R (1)	40	21	13	9	5	4	1					
S/A/R (≥2)	30	8	2	1	1	1						



At risk time

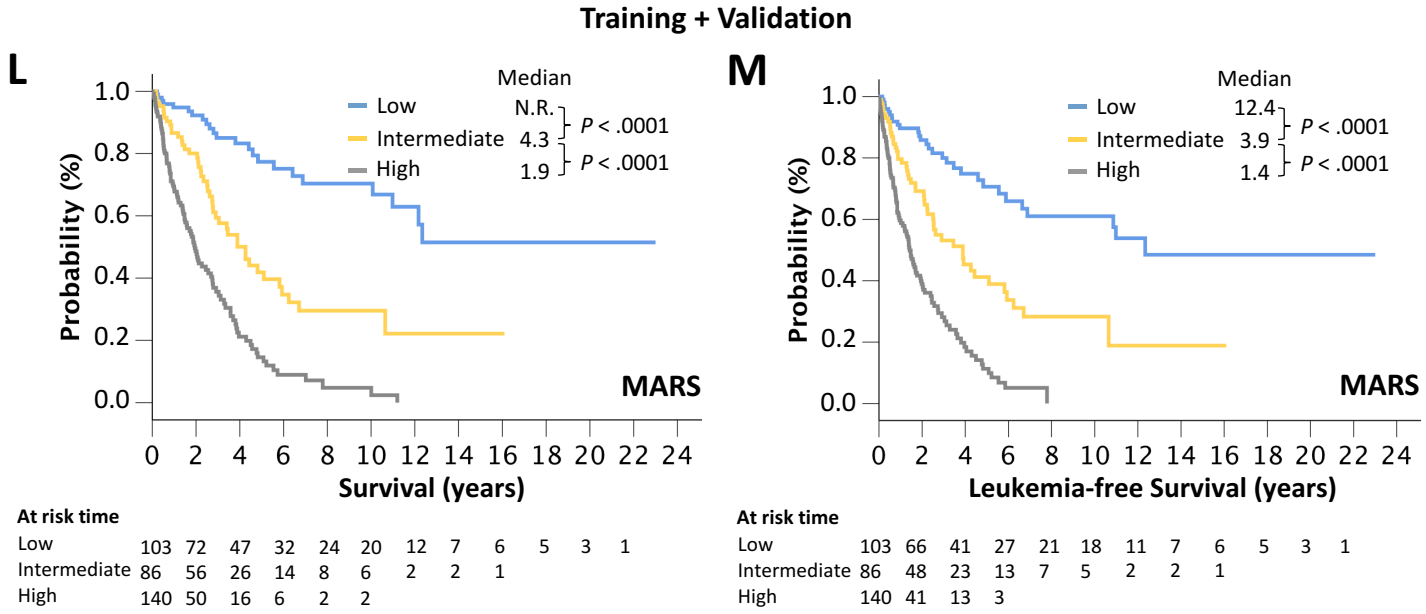
Low	50	33	19	16	14	13	8	4	4	4	1	1
Intermediate	48	28	15	7	3	2						
High	51	17	7	3	2	2						



At risk time

Low	50	35	21	16	12	11	7	3	3	3	1	1
Intermediate	38	21	13	6	4	3						
High	51	18	6	3	2	2						

Figure 2



At risk time

Low	103	72	47	32	24	20	12	7	6	5	3	1
Intermediate	86	56	26	14	8	6	2	2	1			
High	140	50	16	6	2	2						

At risk time

Low	103	66	41	27	21	18	11	7	6	5	3	1
Intermediate	86	48	23	13	7	5	2	2	1			
High	140	41	13	3								

Supplementary Table 1. Clinical Characteristics and Outcome Stratified in Low-, Intermediate-, and High-risk According to the Mutation-Adjusted Risk Score (MARS) for Patients With Advanced Systemic Mastocytosis (Including Both, Training and Validation Sets)

Characteristics	MARS			*P* 1 vs. 2	*P* 1 vs. 3	*P* 2 vs. 3
	Low-risk (1) (n = 103)	Intermediate-risk (2) (n = 86)	High-risk (3) (n = 140)			
WHO diagnosis, n (%)						
ASM	38 (37)	11 (13)	13 (9)	< .0001	< .0001	.5
SM-AHN	60 (58)	64 (74)	113 (81)	.02	< .0001	.3
MCL (± AHN)	5 (5)	11 (13)	14 (10)	.07	.02	.5
Mast cell infiltration[a], (%)						
Median	20	30	30	.8	.6	.9
Range	5-100	5-100	5-95			
Serum tryptase, µg/L						
Median	105	168	188	.08	.001	.1
Range	2-1970	4-2036	5-1854			
Alkaline phosphatase, U/L						
Median	107	151	234	.046	< .0001	< .0001
Range	28-639	35-1928	35-1279			
Treatment modalities, n (%)						
Midostaurin	19 (18)	11 (13)	32 (23)	.3	.4	.08
Cladribine	13 (13)	12 (14)	14 (10)	.8	.5	.4
Midostaurin + Cladribine[b]	6 (6)	11 (13)	24 (17)	.1	.01	.5
Death, n (%)	23 (22)	44 (51)	98 (70)			
Leukemia-free survival, years						
Median	12.4	3.9	1.4	< .0001	< .0001	< .0001
95% CI	-	2.4-5.5	1.1-1.7			
Overall survival, years						
Median	NR	4.3	1.9	< .0001	< .0001	< .0001
95% CI		3.2-5.4	1.6-2.3			

Abbreviations: AHN, associated hematologic neoplasm; ASM, aggressive systemic mastocytosis; BM, bone marrow; CI, confidence interval; MCL, mast cell leukemia; NR, not reached; vs., versus; UNL, upper normal limit; WHO, World Health Organization

[a] in bone marrow histology

[b] sequential midostaurin/cladribine or cladribine/midostaurin treatment

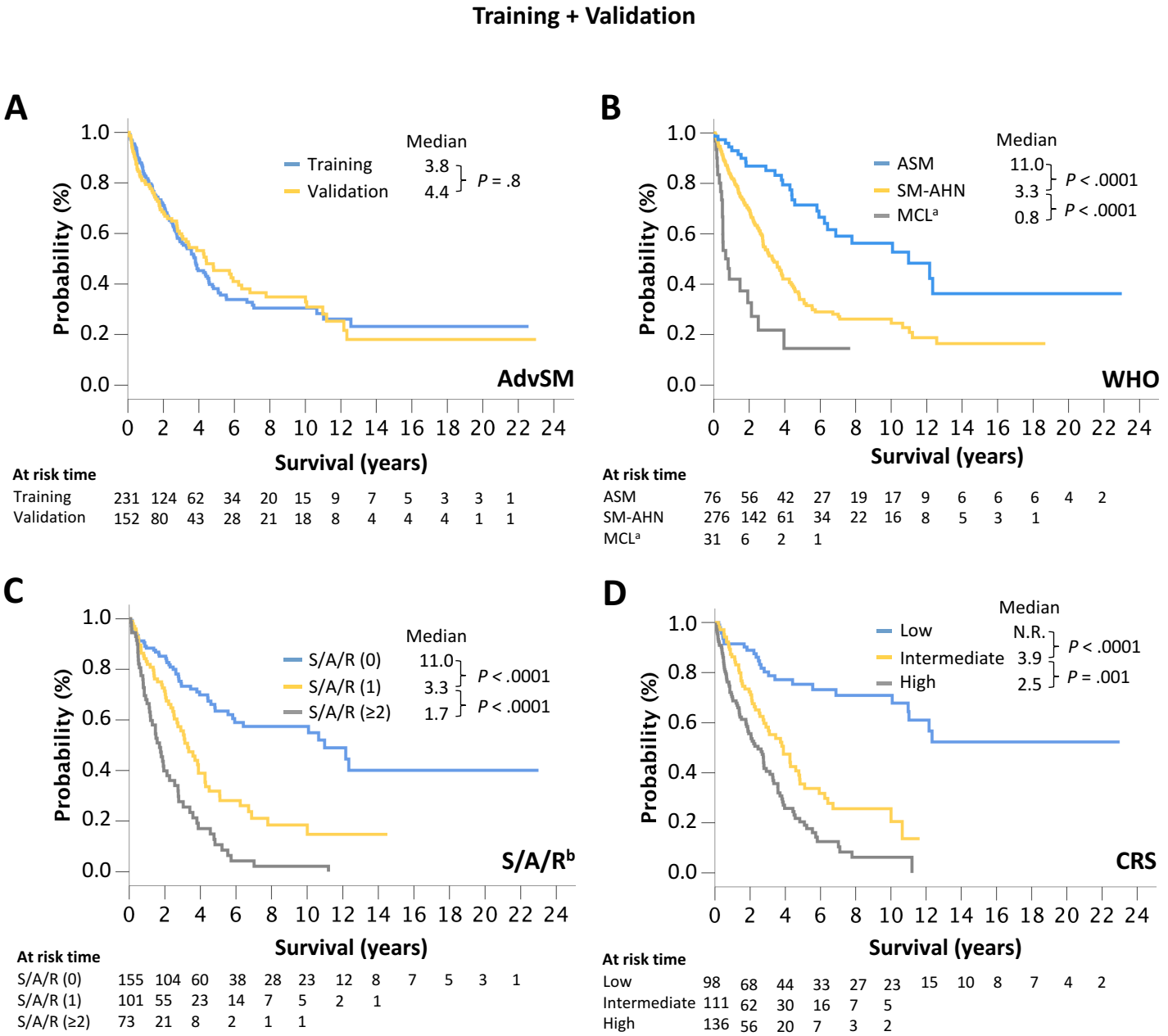
Supplementary Table 2. Comparison Between *KIT* Positive and *KIT* Negative Patients With Advanced Systemic Mastocytosis (AdvSM) Regarding Baseline Clinical, Laboratory, and Genetic Characteristics

Characteristics	*KIT* positive (n = 346)	*KIT* negative (n = 29)	*P*
Age, years			
Median	67	60	.001
Range	24-90	22-85	
Sex, n (%)			
Men	226 (65)	16 (55)	.3
Women	120 (35)	13 (45)	.3
WHO diagnosis, n (%)			
ASM	73 (21)	4 (14)	.5
SM-AHN	250 (72)	17 (59)	.1
MCL (± AHN)	23 (7)	8 (28)	.001
Leukemic transformation, n (%)	55 (16)	4 (14)	1.0
Hemoglobin, g/dL			
Median	10.4	11.0	.5
Range	4-20.5	7.4-15.1	
< 10 g/dL, n (%)	147 (44)	8 (30)	.2
Platelets, x 10^9/L			
Median	116	128	.9
Range	5-958	18-486	
< 100 x 10^9/L, n (%)	141 (43)	11 (41)	.8
Mast cell infiltration in BM histology, (%)			
Median	30	25	.8
Range	5-100	5-80	
Serum tryptase, µg/L			
Median	170	55	.06
Range	4-2036	2-926	
Alkaline phosphatase, U/L			
Median	179	91	.002
Range	28-1928	52-377	
Additional somatic mutations[a], n (%)	242 (81)	11 (46)	< .0001
S/A/R mutation(s), n (%)	164 (55)	4 (17)	< .0001
Overall survival			
Median, years	3.9	4.3	
95% CI	3.1-4.6	3.1-5.4	

Abbreviations: AHN, associated hematologic neoplasm; ASM, aggressive systemic mastocytosis; BM, bone marrow; MCL, mast cell leukemia; WHO, World Health Organization.

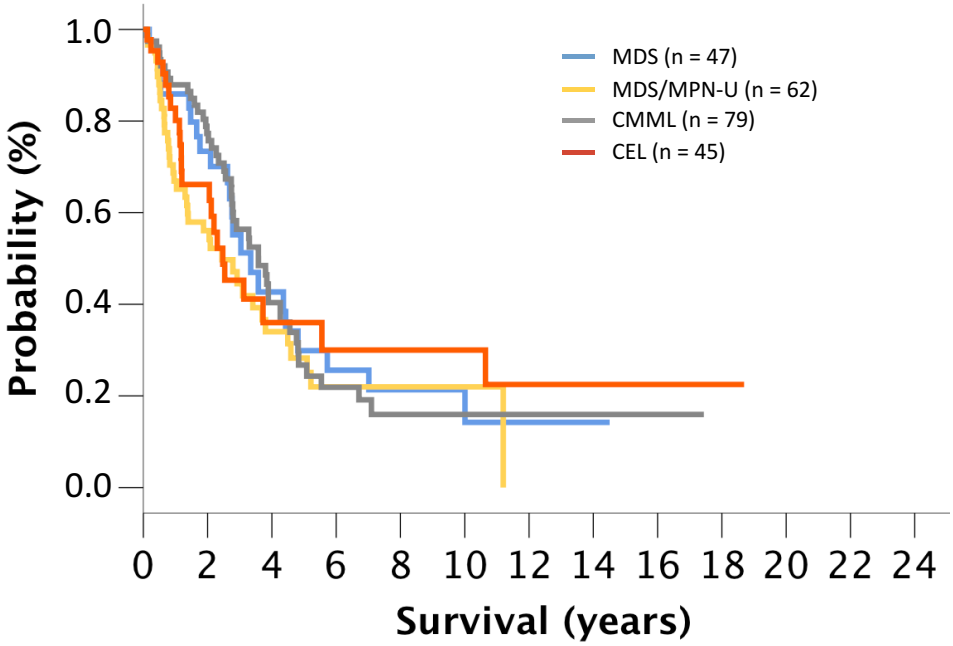
[a] data available in n = 298 (*KIT* positive) and n = 24 (*KIT* negative)

Supplementary Figure 1



Supplementary Figure 1. Overall survival (OS) and leukemia-free survival (LFS) of all advanced systemic mastocytosis (AdvSM) patients (training + validation sets) grouped by (A) AdvSM (comprises all AdvSM subtypes, aggressive SM [ASM], SM with an associated hematologic neoplasm [SM-AHN], and mast cell leukemia [MCL]), (B) World Health Organization (WHO) based stratification, (C) *SRSF2*, *ASXL1*, *RUNX1* (S/A/R) mutation based stratification, and (D) the clinical risk score (CRS). [a] The MCL cohort included patients with MCL-AHN.

Supplementary Figure 2



Supplementary Figure 2. Overall survival of 233 advanced systemic mastocytosis (AdvSM) patients with SM and an associated hematologic neoplasm (SM-AHN; myelodysplastic syndrom, MDS, n = 47; MDS/myeloproliferative neoplasm unclassifable, MDS/MPN-U, n = 62; chronic myelomonocytic leukemiam CMML, n = 79; chronic eosinophilic leukemia CEL, n = 45).