Received: 1 December 2024 | Accepted: 25 October 2025

DOI: 10.1002/hem3.70293

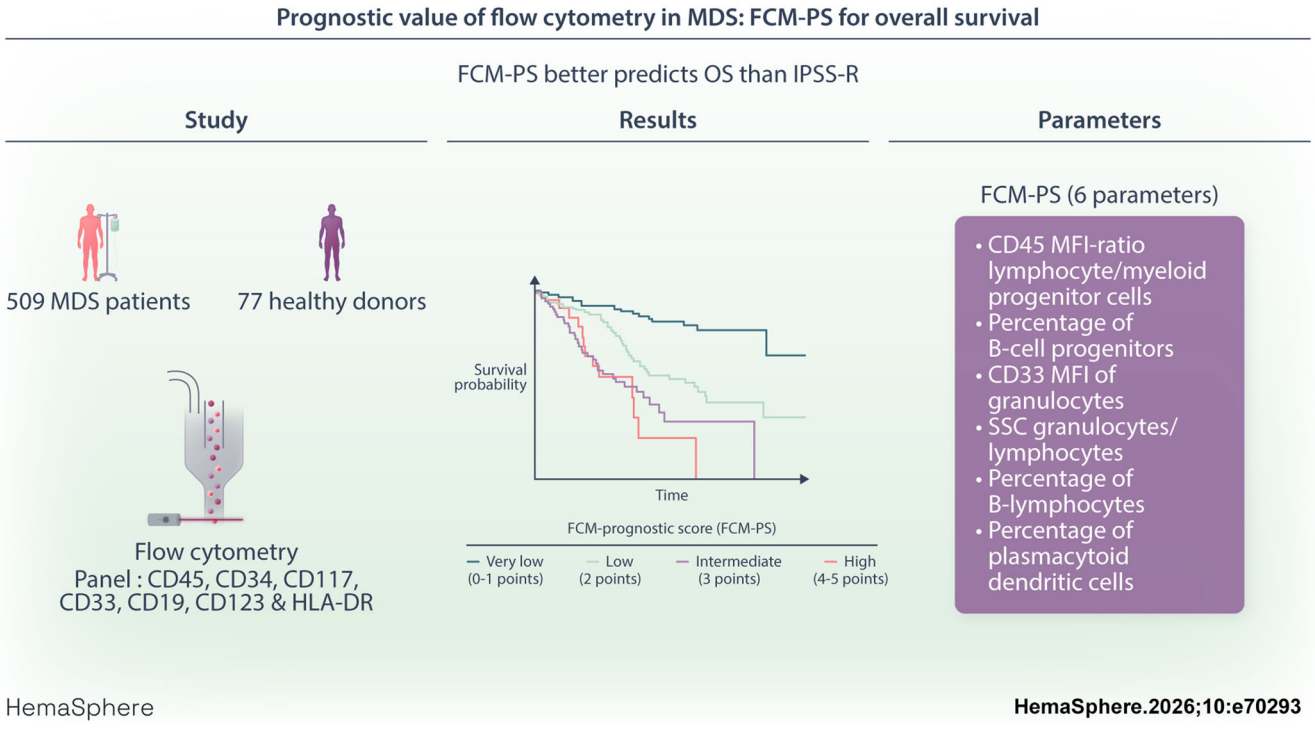
HemaSphere eha

ARTICLE

Prognostic value of flow cytometry in myelodysplastic neoplasms (MDS): Composition of a FCM-prognostic score (FCM-PS) for overall survival

Aida Santaolalla[1,^] | Uta Oelschlaegel[2,^] | Susann Winter[2] | Shirin Jamshidi[3] | Theresia M. Westers[4] | Katja Sockel[2] | Martin Bornhäuser[2] | Rosa Andres Ejarque[3] | Farzin Farzaneh[3] | Antonella Poloni[5,6] | Anne-Sophie Kubasch[7] | Mieke Van Hemelrijck[1] | Arjan A. van de Loosdrecht[4,^^] | Uwe Platzbecker[3,7,^^] | Shahram Kordasti[3,5,8,^^]

Graphical Abstract



Received: 1 December 2024 | Accepted: 25 October 2025

DOI: 10.1002/hem3.70293

ARTICLE

HemaSphere eha

Prognostic value of flow cytometry in myelodysplastic neoplasms (MDS): Composition of a FCM-prognostic score (FCM-PS) for overall survival

Aida Santaolalla[1,^] | Uta Oelschlaegel[2,^] | Susann Winter[2] | Shirin Jamshidi[3] | Theresia M. Westers[4] | Katja Sockel[2] | Martin Bornhäuser[2] | Rosa Andres Ejarque[3] | Farzin Farzaneh[3] | Antonella Poloni[5,6] | Anne-Sophie Kubasch[7] | Mieke Van Hemelrijck[1] | Arjan A. van de Loosdrecht[4,^^] | Uwe Platzbecker[3,7,^^] | Shahram Kordasti[3,5,8,^^]

Correspondence: Aida Santaolalla (aida.santaolalla@kcl.ac.uk) and Shahram Kordasti (shahram.kordasti@kcl.ac.uk)

Abstract

Flow cytometry (FCM) is a co-criterion in myelodysplastic neoplasms (MDS) diagnostics, currently not used for prognostication. This study aimed to develop an FCM-score predicting overall survival (OS) in MDS to improve early clinical patient prognostication. FCM of bone marrow samples was performed for diagnostic purposes in 509 therapy-naïve MDS patients and 77 healthy donors. The following methodology was used: (1) uni- and multivariate Cox proportional hazards regression and Kaplan–Meier curves for OS to assess FCM-parameters' prognostic value; (2) receiver operating characteristic (ROC) curves to test the prognostic superiority of FCM-parameters versus established FCM-scores and clinical risk-scores; and (3) development of a FCM-prognostic score (FCM-PS) based on six FCM-parameters with independent prognostic impact. The final FCM-PS included aberrancies of progenitor cells (increased CD45 mean fluorescence intensity [MFI]-ratio of lymphocytes and myeloid progenitor cells, decreased % of lymphatic progenitor cells), granulopoiesis (increased CD33 MFI, decreased sideward scatter [SSC]-ratio of granulopoiesis and lymphocytes), lymphocytes (increased % of B-lymphocytes), and plasmacytoid dendritic cells (increased %). FCM-PS outperformed established scores for OS (hazard ratio [HR] 4.08 [95% CI 2.54–6.55] vs. Ogata-score: 2.44 [1.61–3.70], International Prognostic Scoring System-Revised [IPSS-R]: 2.37 [1.61–3.49], International Prognostic Scoring System-Molecular [IPSS-M]: 0.816 [0.303–2.196]). Patients in the FCM-PS low score category showed significantly better OS ($P < 0.0001$). Further, FCM-PS allowed discrimination within IPSS-R area under the curve [AUC]: 0.70 vs. 0.62) and IPSS-M (AUC: 0.75 vs. 0.48) subgroups. Validation of the prognostic FCM-PS in an independent patient cohort confirmed good discrimination performance (AUC: 0.70). We introduce a unique, easy-to-use prognostic FCM-PS score (panel: CD45/CD34/CD117/CD33/CD19/CD123/HLA-DR) for OS in MDS, allowing refined risk stratification for IPSS-R subgroups.

[1]Translational Oncology & Urology Research (TOUR), School of Cancer and Pharmaceutical Sciences, King's College, London, United Kingdom
[2]Department of Internal Medicine I, University Hospital Carl Gustav Carus, Faculty of Medicine Carl Gustav Carus, TU Dresden, Dresden, Germany
[3]School of Cancer and Pharmaceutical Sciences, King's College, London, United Kingdom
[4]Department of Hematology, Cancer Center Amsterdam, Amsterdam University Medical Centers, Location VU University Medical Center, Amsterdam, The Netherlands
[5]Department of Clinical and Molecular Sciences, Università Politecnica delle Marche, Ancona, Italy
[6]Hematology Department, AOU delle Marche, Ancona, Italy
[7]Medical Clinic and Policlinic 1, Hematology and Cellular Therapy, University Hospital Leipzig, Leipzig, Germany
[8]Haematology Department, Guy's and St Thomas' NHS Trust, London, United Kingdom
^Aida Santaolalla and Uta Oelschlaegel are joint first authors and contributed equally to this work.
^^Arjan A. van de Loosdrecht, Uwe Platzbecker, and Shahram Kordasti are joint senior authors and contributed equally to this work.

INTRODUCTION

Myelodysplastic neoplasms (MDS) are a heterogeneous group of hematological disorders, characterized by various peripheral cytopenias and cytomorphological dysplasia, and predominantly affecting the older population. With the ageing demographics in developed countries, the incidence of MDS is expected to rise significantly in the coming decades, potentially leading to a substantial increase in healthcare costs.[1] A crucial aspect of clinical management in MDS patients involves accurately determining the risk of disease progression, given that up to around one-third of the patients will progress to acute myeloid leukemia (AML), depending on their risk profile.[2] The introduction of routine somatic mutation analysis has greatly enhanced prognostic prediction in MDS, a development epitomized by the International Prognostic Scoring System-Molecular (IPSS-M).[3,4] Nevertheless, molecular tests are yet to become universally available due to costs and/or technical reasons. Furthermore, the turnaround time for these tests can be prolonged, delaying crucial clinical decisions. Thus, there is an unmet need for a rapid, accurate, and cost-effective method to predict the prognosis in MDS patients, ensuring timely and efficient patient care.

Flow cytometry (FCM) of the bone marrow (BM) is part of the integrated diagnostic approach for MDS according to the classification of the World Health Organization (WHO) and the European-LeukemiaNet (ELN) International MDS Flow working group recommendations.[5–7] Diagnostic FCM-scoring systems like Ogata-score, flow-cytometric scoring system (FCSS), and integrated flow-score (iFS) are routinely utilized to investigate dyspoiesis in myelomonocytic and erythroid progenitors and maturing cells.[8–12] Yet, the full prognostic value of these scores is less clear and often studied in small patient cohorts, using limited marker panels, cell types, or FCM-scores as a single parameter.[10,13–23] Molteni et al. analyzed only the flow cytometric blast count and found significant differences in overall survival (OS) of MDS patients with low/intermediate IPSS (International Prognostic Scoring System).[13] Della Porta et al. validated the Ogata-score, mostly analyzing progenitor cells, as a diagnostic tool and added its prognostic value as a single parameter predicting OS and leukemic transformation.[14] The use of a combination of the Ogata-score with two additional parameters (percentage of mast cells and the abnormal expression of CD71 on nucleated erythroid cells [NEC]) was described to have an implication on event-free survival.[15] The analysis of maturing granulo- and monocytopoiesis, described as diagnostic FCSS-score and Diff-score, showed also prognostic influence on outcome.[16–19] Furthermore, combined analysis of Ogata-score and FCSS-score revealed a prognostic impact on OS.[20,21] Matarraz et al. included also the NEC in the analysis and found several 2-parameter-combinations with an independent impact on OS.[22] Alhan et al., analyzing also FCSS-score plus NEC, refined OS prediction for MDS patients within the IPSS-R (International Prognostic Scoring System-Revised) low-risk category by developing a 3-parameter MDS flow cytometric score.[23] The iFS as a singular parameter, including the analysis of progenitor cells, granulo- and monocytopoiesis and NEC for diagnostic purposes, was significantly associated with an inferior OS, while the prognostic influence of the other FCM-scores (e.g., FCSS, Ogata-score) analyzed in parallel was less pronounced.[11]

Diagnostic FCM-scoring systems such as the Ogata-score, the FCSS, and the RED score[24] were initially developed to improve recognition of dysplasia and aid in the differential diagnosis of MDS. Several of these indices, however, were subsequently shown to also carry prognostic information. For instance, Guarnera et al. demonstrated that the integration of immunophenotypic aberrancies such as abnormal expression of CD15, CD56, and CD38 with mutational profiling refines risk stratification in MDS.[25] Similarly, Li et al. reported that specific disorders of maturation and differentiation of monocytes in patients with MDS, quantified by multiparametric FCM, can independently predict adverse clinical outcome. Additionally, they have shown that CD300 e expression was significantly associated with the clinical stage and disease progression of MDS.[26] Johansson et al. highlighted the prognostic value of the flow cytometric myeloid progenitor (MyP) count.[27]

Other groups have also refined flow-based prognostic models by introducing novel indices or extending established scores. For instance, Majcherek et al. showed that adding aberrant granulocytic markers such as CD11b/HLA-DR and CD11b/CD13 to the Ogata-score increases diagnostic sensitivity.[28] Park et al. incorporated markers of ineffective erythropoiesis and demonstrated their predictive value for response to erythropoiesis-stimulating agents (ESA).[24] Verigou et al. proposed the Dysmyelopoiesis Index (DMI), which quantifies granulocytic maturation defects and showed strong correlations with IPSS, WHO classification-based prognostic scoring system, and IPSS-R, thereby providing independent prognostic information.[29] An early seminal study by our collaborators described the creation of a numerical flow-score based on aberrant antigen expression in the myelomonocytic lineage, including CD5, CD7, and CD56 on CD34+ myeloid blasts, which predicted transfusion dependence and progression to AML independently of cytogenetics and IPSS.[30] Finally, a comprehensive review emphasized that none of the available FCM-based methods alone have sufficient sensitivity for reliable diagnosis, and that combining lineage-specific scores such as the Ogata and RED scores substantially increases sensitivity.[31]

The present study aims to enhance FCM-parameters' prognostic value in MDS by developing a composite, but easy-to-use and cost-effective FCM-score for OS that could be implemented at the time of diagnosis. This involves leveraging a comprehensive antigen panel, using parameters incorporated in known FCM diagnostic scores and targeting myeloid and lymphoid progenitors, maturing cells of the granulo- and monocytopoiesis, and NEC as well as lymphocytes, basophils, eosinophils, plasmacytoid dendritic cells (pDC), and mast cells in therapy-naïve MDS patients.

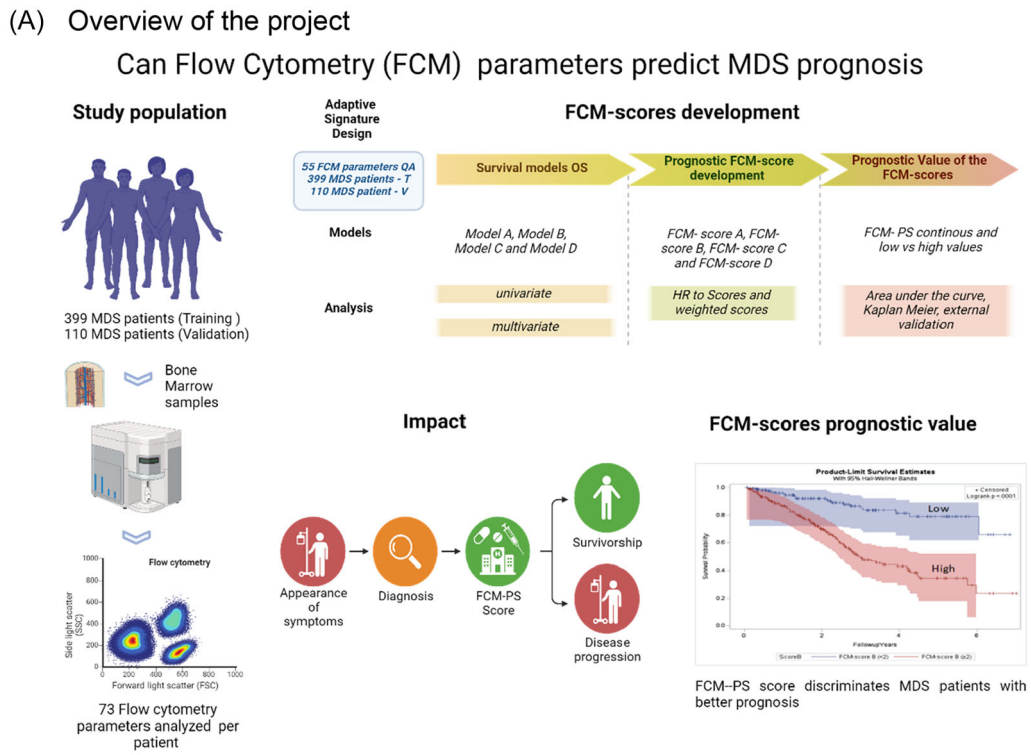
MATERIALS AND METHODS

Study design

This is an observational study that includes an experimental and a computational component. An overview of the study design, methodology, and results is illustrated in Figure 1A/B. Initially, BM samples from therapy-naïve patients in the training and the validation cohort were analyzed for diagnostic purposes using the standard FCM-parameters.[32] This was followed by a computational phase, where the FCM-parameters were utilized as prognostic factors for predicting OS. Four FCM-scores for MDS were developed using an adaptive signature design,[33,34] commonly used to design and validate predictive classifiers when classes of patients will present with future differential prognosis (further explanation is given below in the Methods section). Finally, the best performing FCM-score (FCM-PS) was selected, tested, and validated for OS prognostication in MDS.

Study population

Our study includes two independent cohorts (Table 1), a training cohort that comprises 399 therapy-naïve MDS and chronic myelomonocytic leukemia (CMML) and MDS/MPN (MDS/myeloproliferative neoplasm) with *SF3B1* mutation and thrombocytosis patients



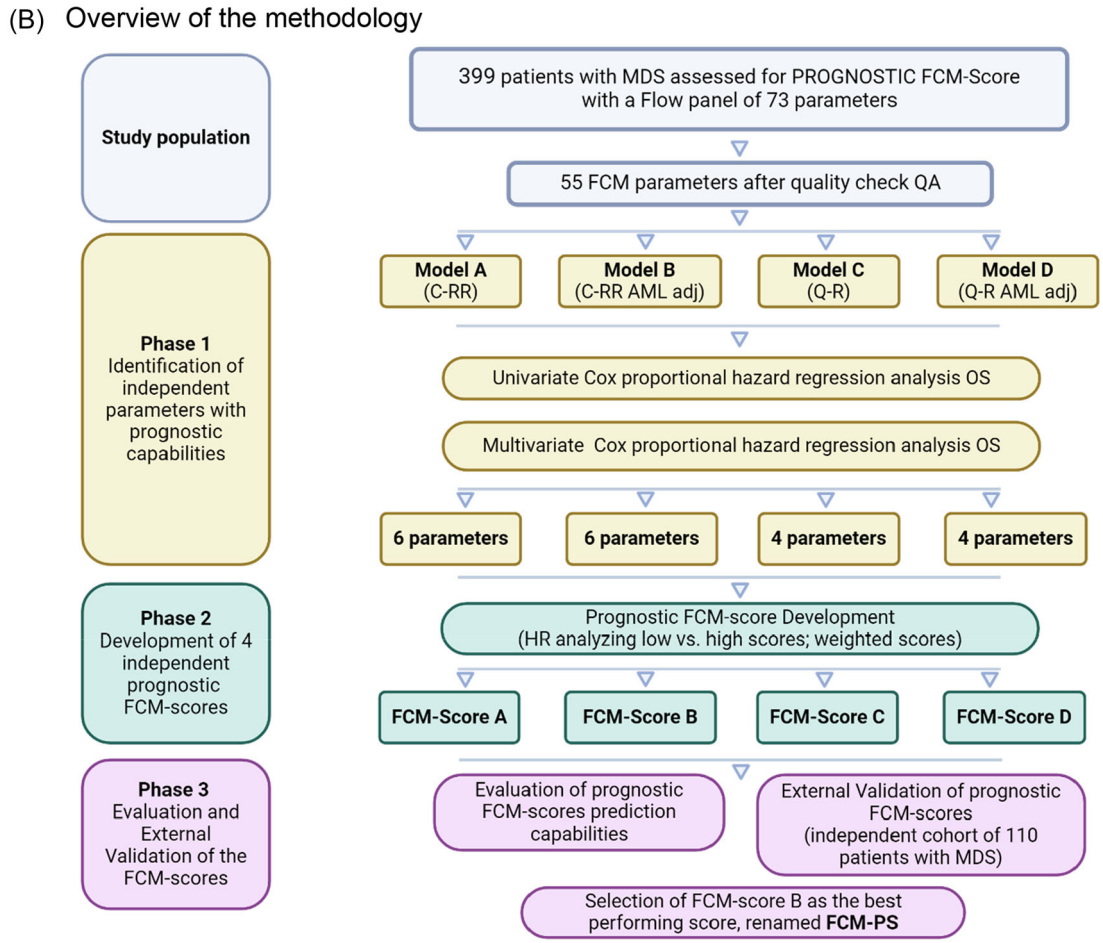


FIGURE 1 **Study design of the project. (A)** Overview of the project and **(B)** Overview of the methodology followed for the development of prognostic flow cytometry (FCM)-scores for overall survival (OS) in myelodysplastic neoplasms (MDS). Abbreviations: AML, adj analysis adjusted for transformation of MDS to acute myeloid leukaemia; C-RR, clinical reference ranges; FCM, flow cytometry; FCM-PS, flow cytometry prognostic score; HR, hazard ratio; MDS, myelodysplastic neoplasms, QA, quality assessment; Q-R, quartile ranges; OS, overall survival; T, training cohort; V, validation cohort.

TABLE 1 Patient characteristics by study population.

	Training cohort (*n* = 399)	Validation cohort (*n* = 110)	P-value[a]
WHO 2022 diagnosis (n, %)[b]			**2.22e−16**
MDS-LB	147 (36.8)	34 (30.9)	
MDS-LB-RS	21 (5.3)	22 (20.0)	
MDS-*SF3B1*	16 (4.0)	15 (13.6)	
MDS-IB1	92 (23.1)	21 (19.1)	
MDS-IB2	72 (18.0)	10 (9.1)	
MDS-5q	9 (2.3)	8 (7.3)	
CMML-1	23 (5.8)	0	
CMML-2	16 (4.0)	0	
MDS/MPN with *SF3B1* and thrombocytosis	3 (0.75)	0	
Age (mean, SD)	70.3 (12.2)	66.6 (11.2)	**0.003**
Gender (n, %)			0.528
Male	249 (62.4)	72 (65.4)	
Female	150 (37.6)	38 (34.6)	
IPSS-R (n, %)	**(*n* = 399)**	**(*n* = 103)**	0.079
Very low	41 (10.3)	13 (12.6)	
Low	147 (36.8)	46 (44.7)	
Intermediate	118 (29.6)	26 (25.2)	
High	68 (17.0)	10 (9.7)	
Very high	25 (6.3)	8 (7.8)	
IPSS-M (n, %)	**(*n* = 84)**		
Very low	4 (4.8)	n.d.	
Low	27 (32.1)		
Moderate low	12 (14.3)		
Moderate high	11 (13.1)		
High	18 (21.4)		
Very high	12 (14.3)		
mTP53 (n, %)	**(*n* = 122)**	**(*n* = 76)**	**0.002**
Yes	15 (12.3)	4 (5.3)	
No	107 (87.7)	72 (94.7)	

Abbreviations: CMML, chronic myelomonocytic leukemia; IB, increased blasts; IPSS-M, International Prognostic Scoring System-Molecular; IPSS-R, International Prognostic Scoring System-Revised; LB, low blasts; MDS, myelodysplastic neoplasms; MPN, myeloproliferative neoplasms; m*TP53*, mutated *TP53* gene; n.d., not done; RS, ring sideroblasts; WHO, World Health Organization.

[a]Statistically significant differences in the variables between cohorts are highlighted in bold.

[b]The included patients were diagnosed between 2012 and 2019. That is why we do not have the information on abnormal partitioning of peripheral blood monocyte subsets necessary to classify the new WHO2022 category of oligomonocytic CMML. Mutational profiling for the *TP53* gene was available only in a subset of patients, including 3/122 patients who would fit in the WHO2022 category of "MDS with biallelic *TP53* inactivation."

from the University Hospital Dresden from clinical diagnosis, followed up from 2012 to 2019, and a validation cohort that consists of 110 MDS patients from Amsterdam University Medical Centre (UMC), 70 of whom were treatment-naïve from clinical diagnosis and 40 refractory to ESA before lenalidomide treatment (HOVON89 trial).[35] FCM investigation of BM samples was part of the standard diagnostic procedure for MDS in both cohorts, as recommended by the ELN International MDS Flow Cytometry working group.[32,36] Patients were categorized according to the WHO2022 classification.[5] Moreover, information on survival and progression to AML, and standard diagnostic FCM-scores including FCSS, Ogata, iFS, and the Revised International Prognostic Score System (IPSS-R)[8-11,37] were available for both cohorts. In the training cohort, *TP53* mutation status was assessed in 122 patients, and a comprehensive molecular characterization, allowing to calculate the IPSS-M according to Bernard et al.,[3] was available for 84 patients. In addition, an FCM investigation was performed for 77 healthy, age-matched donors (HD) from the University Hospital Dresden, who underwent hip replacement (NCT02867085; EK240102007).

Experimental component of the study design

FCM-analysis

The two participating institutions performed a harmonized preparation, measurement, and analysis approach according to the ELN International MDS Flow Cytometry working group.[32,36] The antibody panel, staining, acquisition, and analysis were performed as described previously.[10,11] For more information on the panel, see also Table S1. Thus, cell preparation was performed within 24 h after BM aspiration. Before staining, erythrocytes were removed by bulk lysis using BD Pharm-Lyse (BD Biosciences, San Jose, CA), followed by two washing steps with phosphate-buffered saline (PBS) (Life Technologies, Carlsbad, CA). For surface labeling, cells were incubated with monoclonal antibodies in the dark according to the recommendations of the manufacturer. Subsequently, cells were washed twice and resuspended in 500 µL PBS. Samples were stored at 4°C and measured within 1 h using a FACS Canto II cytometer (BD Biosciences). Daily quality control included a performance check with CS&T beads and an 8-color-control using StatusFlow Whole Blood Control (Biozol, Eching, Germany). Photomultiplier voltages were defined using Rainbow Calibration Particles. For compensation, the automated compensation tool of the FACSDiVa software was used. At least 200,000 events were acquired per tube.

For data analysis, a hierarchical gating strategy according to the ELN International MDS Flow working group was applied[32] (Figure S1). Briefly, after exclusion of doublets (A) and debris (B), all $CD45^{+}$ leukocytes were gated (C). Thereafter, the main BM cell lineages including (D, E) progenitor cells, (C) granulopoiesis ($CD45^{dim}SSC^{int/high}$), monocytopoiesis ($CD45^{high}SSC^{int}$), lymphocytes ($CD45^{dim}SSC^{high}$), and (I, J) NEC $CD45^{neg/dim}$ were gated. Further cell compartments including (F) basophils ($CD123^{+}HLA\text{-}DR^{neg}$), (F, G) pDC ($CD123^{high}HLA\text{-}DR^{+}$), (K) mast cells ($CD117^{high}CD45^{dim}$), and (M, N) eosinophils ($CD11b^{high}CD13^{high}CD16^{neg}CD45^{high}$) were analyzed.

Overall, 73 FCM-parameters were investigated by manual analysis based on recent ELN recommendations,[32] including the following cell types: myeloid/lymphoid progenitor cells (20 parameters), granulopoiesis (16), monocytopoiesis (17), nucleated red cells (10), and other cells (lymphocytes, basophils/eosinophils/mast cells, pDC) (10). These panels facilitated the implementation of MDS diagnostic scores like the Ogata-score (compatible with MDS when ≥2 points), FCSS (≥3 points), and iFS (C).[8-11] Initial quality checks of the 73 FCM-parameters included the following analyses: parameters were tested for normal distribution, Pearson correlation analysis was conducted, and missing data were assessed with the exclusion of parameters if there were ≥30% missing data per FCM-parameter. Eighteen FCM-parameters from the initial panel were excluded, mainly due to redundancy or missing data. The 55 parameters included in model development are highlighted in bold in Table 2.

TABLE 2 Flow cytometry (FCM)-parameters included in the prognostic score development (73 parameters).

Progenitor cells	Granulopoiesis	Monopoiesis	Nucleated erythroid cells	Extra cell compartment
CD34 (%)	**Lympho/Granulo ratio (%)**	**Lympho/Mono ratio (%)**	**% NEC**	**Mast cells (%)**
SSC-ratio (myProgC/Lympho)	**SSC-ratio (Granulo/Lympho)**	**SSC-ratio (Mono/Lympho)**	**CD71 (MFI)**	**pDC (%)**
CD45 (MFI-ratio: Lympho/myProgC)	**CD33 (MFI)**	**CD45 (MFI-ratio: Mono/Lympho)**	**CD71 (CV)**	**Baso (%)**
CD33 (MFI)	**CD15 (MFI)**	**CD33 (MFI)**	**CD36 (MFI)**	**Eos (%)**
CD34 (MFI)	**CD34 (%)**	**CD13 (MFI)**	**CD36 (CV)**	**CD19 (%)**
CD13 (MFI)	**CD10 (%)**	**CD15 (MFI)**	**CD105 (%)**	**CD19CD10 (%)**
CD123 (MFI)	**CD36 (%)**	**CD14 (MFI)**	**CD105 (MFI)**	**CD3 (%)**
HLA (MFI)	**CD13CD11bCD16 (pattern)**	**HLA (MFI)**	**CD235a (MFI)**	CD56dim (%)
lyProgC (%)	**CD56 (%)**	**CD16**	CD71dim (%)	CD56++ (%)
CD11b (%)	CD45 (MFI-ratio: Granulo/Lympho)	**CD14dimCD36 (%)**	CD71 (pattern)	CD56+CD3+ (%)
CD15 (%)	HLA-DR (%)	**CD34 (%)**		
CD71+CD117+ (%)	CD71 (%)	**CD2 (%)**		
CD2 (%)	CD2 (%)	**CD5 (%)**		
CD5 (%)	CD5 (%)	**CD7 (%)**		
CD7 (%)	CD7 (%)	**CD56 (%)**		
CD56 (%)	CD117 (%)	CD11b (MFI)		
CD117 (MFI)		CD36 (MFI)		
CD117 (%)				
CD34+CD38− (%)				
CD33 (%)				

Note: Final 55 parameters used for the composition of the FCM-prognostic scores after quality assessment are highlighted in bold.

Abbreviations: Baso, basophils; CV, coefficient of variation; Eos, eosinophils; FCM, flow cytometry; Granulo, granulopoiesis; Lympho, lymphocytes; lyProgC, lymphatic progenitor cells; MFI, mean fluorescence intensity; Mono, monopoiesis; myProgC, myeloid progenitor cells; NEC, nucleated erythroid cells; pDC, plasmacytoid dendritic cells; SSC, sideward scatter.

Computational component of the study design

Following a data-driven approach, an ad hoc adaptive signature methodology[33,34] was performed to develop the composite prognostic FCM-PS for MDS (Figure 1B). The computational methodological component of the project consisted of three consecutive phases. In a nutshell, *Phase 1* comprised the initial identification of independent parameters with prognostic capabilities, following four different prediction models in the training cohort. This led to the development of four independent prognostic FCM-scores in *Phase 2*. These FCM-scores were composites of the predictors identified in each of the four initial prediction models. Finally, in *Phase 3*, we evaluated the prognostic value of the four independent prognostic FCM-scores in the validation cohort to conclude with the identification of the best FCM-score (FCM-PS) for OS prognostication in MDS.

Prognostic FCM-PS score development (Phase 1 and Phase 2)

In *Phase 1*, four prediction models with different initial settings were investigated in the training cohort using Cox proportional hazard regression analysis. The endpoint assessed was OS, and the four models were censored for patients who were lost to follow-up and patients who underwent a stem cell transplantation. The assumption of proportional hazards was tested in each of the models.

Initially, univariate Cox proportional hazards regression analysis for OS using the log-rank likelihood test was performed for each of the independent 55 FCM-parameters to assess parameters with survival prediction capabilities (Table 2). The initial settings defining the prediction models were the following: laboratory-specific clinical reference ranges (C-RR in Models A, B) and quartile ranges (Q-R in Models C, D) were used to categorize the FCM-parameters and to pinpoint parameters with optimal separation in Cox regression. Moreover, Models B and D were further adjusted for patients who developed AML during the course of the disease (C-RR AML adj, Q-R AML adj).

The parameters that presented prediction capabilities independently in each of the four model approaches were then included in multivariate Cox proportional hazards regression analysis using the log-rank likelihood test (one hazard regression per model: multivariate Models A–D). Only the parameters with the best performance in the univariate analysis were included in the multivariate analysis, meaning parameters able to predict better or worse survival based on the hazard ratios (HRs). Parameters with best performance included in the multivariate analysis are listed in Table S2.

In *Phase 2*, we developed the prognostic FCM-scores (one FCM-score per model: multivariate scores FCM-A to D) using the best performance parameters identified in the multivariate analyses in *Phase 1* (Models A–D). Only parameters able to predict better or worse survival based on the HRs in the multivariate analysis were considered for the composite prognostic FCM-scores (Table S2). We then explored two distinct scoring approaches to incorporate the

identified *Phase 1* parameters: a *non-weighted* calculation, where each parameter identified in the scores and present in a given FCM patient sample was counted as "1 point"; and a *weighted* approach, where the parameter's weights were based on the HR resulting from the multivariate analyses as follows: a FCM-parameter with a HR less than 2 ($1 < HR < 2$) in a given FCM sample was scored 1 point, with a HR equal or larger than 2 and less than 3 ($2 \leq HR < 3$) was given 2 points, and with a HR equal or larger than 3 ($HR \geq 3$) was given 3 points. The scores per parameter were then summed to obtain a final score per patient sample (Supplementary Methodology: S1 to see a calculation example).

Relevant scripts are provided in the Supplementary Information: S2.

Performance and validation of the prognostic FCM-score (Phase 3)

In *Phase 3*, diverse statistical analyses were conducted to assess the performance of the four developed FCM-scores A to D (*non-weighted and weighted*). Thus, multivariate proportional Cox regression analysis for OS, C-index expressing the prediction potential, receiver operating characteristic (ROC) curve, and Kaplan–Meier curves were performed to test the independent prognostic value of the developed FCM-scores. After conducting and interpreting the above-mentioned analyses, the best performing score was identified and named FCM-PS (FCM-prognostic score).

Therefore, the following analyses were only performed in FCM-PS to test further its prediction capabilities. Initially, to understand the risk gradient associated with the FCM-PS score, the distribution of the values of the FCM-PS in the training population was visualized against the HR for OS for each FCM-PS category calculated, considering the average patient in the population as the reference. The performance of the new FCM-PS was then compared with known prognostic factors, including age, sex, and IPSS-R for the full training cohort, and IPSS-M in a subset of the training cohort ($n = 84$ patients), as well as with published diagnostic FCM-scores (Ogata-, FCSS-, and iFS-score)[8-11] for the full training cohort. To further understand the prognostic capabilities of the FCM-PS, we also performed a multivariate proportional Cox regression analysis for transformation to AML in the training cohort, given that this dataset had sufficient events to perform the analysis. The validation cohort did not have enough events to perform this analysis. Furthermore, Kaplan–Meier curves were performed for different risk categories of the new FCM-PS (very low risk [0–1 points in the score], low risk [2 points in the score], intermediate risk [3 points in the score], high risk [4–5 points in the score]).

The FCM-PS was then further explored in reliability analyses including the following: (A) comparison of the distribution of the new FCM-PS score among the full training MDS cohort and the healthy donors using chi-square test; (B) comparison of the distribution of *TP53* mutations (wild type vs. mutated) among the FCM-PS using chi-square test and Fisher's exact test (given the small sample size of 122 patients); and (C) comparison of the distribution of patients with and without a *TP53* mutation (biallelic or VAF > 50% only) and/or a complex karyotype among the FCM-PS for the training cohort (17 vs. 382 patients) using chi-square test and Fisher's exact test.

Finally, the FCM-PS was validated in an independent cohort of 110 MDS patients, replicating the above-described methodology, to test its independent prognostic value and to ensure the generalizability of the results. The following methods were used: multivariate Cox proportional hazard analysis, C-index expressing the prediction potential, Kaplan–Meier curves, and ROC curve.

Statistical analyses have been performed using SAS software (version 9.4).

RESULTS

Study population characteristics for both the training and validation cohort are detailed in Table 1. The mean age of the training cohort is higher than that of the validation cohort (mean age [SD]: 70.3 years [12.2] vs. 66.6 years [11.2]) (P-value = 0.003) and the proportion of patients in the IPSS-R higher-risk categories (high/very high) is higher in the training cohort than in the validation cohort (23.3% vs. 17.5%), but not statistically significant (P-value = 0.079) (Table 1).

Flow cytometry data

In the training cohort ($n = 399$ MDS patients), 73 distinct FCM-parameters (Table 2) based on the recent ELN recommendations[32] were investigated, considering aberrancies in their expression in progenitor cells, granulopoiesis, monocytopoiesis, erythropoiesis, and lymphopoiesis. The quality check, as described in detail in the methods section, resulted in the inclusion of 55 non-correlated FCM-parameters (Table 2) in the study defining various cell populations. These 55 parameters were considered as potential prognostic parameters for OS in MDS patients and included in the subsequent analyses.

Computational component

In *Phase 1*, following a data-driven approach as outlined in the methods section (Figure 1B), 24 parameters were significantly associated with OS after univariate analysis (Table S2). Following multivariate testing, a total of 9 different FCM-parameters that significantly impacted OS across the four models (named A–D) were identified. The significant FCM-parameters were related to progenitor cells (increased CD45 mean fluorescence intensity (MFI)-ratio of lymphocytes and myeloid progenitor cells, decreased % of lymphatic progenitor cells), granulopoiesis (increased CD33 MFI, decreased CD15 MFI, decreased SSC-ratio of granulopoiesis and lymphocytes, increased ratio of % lymphocytes and % granulopoiesis), monocytopoiesis (decreased ratio % lymphocytes and monocytes), lymphocytes (decreased/increased % of $CD19^+$ B-lymphocytes), and pDC (increased percentage). Some of the parameters are shared among the four models and therefore among the identified prognostic FCM-scores. Further details on the FCM-parameters with the lab-specific reference ranges included in each model are provided in Table 3. Detailed information on their biological significance can be found in Table S3.

In *Phase 2*, to quantify the identified models, four composite FCM-scores (FCM-score A, FCM-score B, FCM-score C, and FCM-score D) were constructed, one for each model, comprising six parameters in FCM-scores A and B and four parameters in FCM-scores C and D (Table 3).

Next, to calculate the FCM-scores per patient, we applied the *non-weighted* and *weighted* scoring methods as detailed in the methods section. FCM-scores A to D using the *non-weighted* calculation outperformed the *weighted* scores in the multivariate Cox regression analysis for OS. Therefore, in the following section (*Phase 3*), the results using the *non-weighted* scoring method are presented only.

In *Phase 3*, the performance of the FCM-scores A to D was assessed. In the multivariate proportional Cox regression analysis for OS, FCM-score B (vs. FCM-scores A, C and D), comprising six

TABLE 3 Flow cytometry (FCM)-parameters with prognostic significance after multivariable analysis identified in each of the four models tested.

FCM-parameters	Reference ranges[a]	FCM-scores			
		A	B	C	D
ProgC: CD45 (MFI-ratio: Lympho/myProgC)	≤7.0 (≤7.5)	x	x		
ProgC: lyProgC (%)	≥5.0 (≥5.0)	x	x		
Granulo: CD33 MFI (MFI-ratio)[b]	≤6600 (≤29.75)	x	x	x	x
Granulo: CD15 MFI (MFI-ratio)[b]	≥1500 (≥61.85)	x			
Granulo: SSC-ratio (Granulo/Lympho)	≥6.0 (≥6.0)		x	x	x
Gran: Lympho/Granulo-ratio (%)	≤1.0 (≤1.0)	x			
Mono: Lympho/Mono-ratio (%)	≥2.66 (≥3.22)			x	x
Lympho: CD19 (%)	A: 3.0–15.0; B: ≤15.0; C: ≤17.1; D: 6.3–17.1 (A–D: 1.47–19.40)	x	x	x	x
Plasmacytoid dendritic cells (%)	≤1.0 (≤0.36)		x		

Note: Details on the multivariate models can be found in Table S2. FCM-score B is later on termed FCM-PS. The "x" shows the FCM-parameters having a significant prognostic impact on the respective FCM-scores.

Abbreviations: FCM, flow cytometry; Granulo, granulopoiesis; Lympho, lymphocytes; lyProgC, lymphatic progenitor cells; MFI, mean fluorescence intensity; Mono, monopoiesis; myProgC, myeloid progenitor cells; ProgC, progenitor cells; SSC, sideward scatter.

[a]Lab-specific reference ranges of the training cohort (TU Dresden) and the external validation cohort (Amsterdam UMC-VUMC; reference ranges in parentheses).

[b]In the Amsterdam UMC-VUMC lab, an MFI-ratio of the Granulo/Lympho was calculated.

TABLE 4 Stratified Cox model and concordance C-index for overall survival (OS) of flow cytometry-prognostic score (FCM-PS) (high ≥ 2 points vs. low score 0–1 points), International Prognostic Scoring System-Revised (IPSS-R), and International Prognostic Scoring System-Molecular (IPSS-M) in the training and validation cohort.

Training cohort	HR (CI 95%)	P-value	C-index
FCM-PS (continuous)	1.905 (1.598–2.271)	<0.0001	0.6810
FCM-PS (high vs. low)	**4.079 (2.540–6.548)**	**<0.0001**	**0.6380**
IPSS-R (continuous)	2.368 (1.226–1.514)	<0.0001	0.6520
IPSS-R (high vs. low)[a]	2.368 (1.607–3.490)	<0.0001	**0.6130**
IPSS-M (continuous)	0.985 (0.617–1.573)	0.9509	0.5200
IPSS-M (high vs. low)[b]	0.816 (0.303–2.196)	0.6876	0.5549
Validation cohort	**HR (CI 95%)**	**P-value**	**C-index**
FCM-PS (continuous)	1.525 (1.231–1.889)	0.0001	0.6469
FCM-PS (high vs. low)	**2.672 (1.416–5.041)**	**0.0024**	**0.5894**
IPSS-R (continuous)	1.380 (1.202–1.583)	<0.0001[b]	0.6817
IPSS-R (high vs. low)[a]	2.640 (1.581–4.407)	0.0002	0.6333

Note: Statistically significant results are highlighted in bold.

Abbreviations: CI, confidence interval; FCM, flow cytometry; FCM-PS, flow cytometry prognostic score; HR, hazard ratio; IPSS-M, International Prognostic Scoring System-Molecular; IPSS-R, International Prognostic Scoring System-Revised.

[a]Higher IPSS-R includes the intermediate-, high-, and very high-risk groups, and lower IPSS-R includes the very low- and low-risk groups.

[b]Higher IPSS-M includes moderate high-, high-, and very high-risk groups, and lower IPSS-M includes very low-, moderate low-, and low-risk groups. IPSS-M sample size $n = 84$.

parameters (**ProgC: CD45** [MFI-ratio: Lympho/myProgC]; **ProgC: lyProgC** [%]; **Granulo: CD33** MFI [MFI-ratio]; **Granulo: SSC-ratio** [Granulo/Lympho]; **Lympho: CD19** [%]; **Plasmacytoid dendritic cells** [%]; for further information, please refer to Table 3), exhibited the most significant impact on OS after its categorization into low (0–1) versus high (≥2) score via non-weighted calculation (HR [95% CI]: 4.079 [2.540–6.548]), outperforming well-known FCM diagnostic scores, for example, Ogata-score (HR [95% CI]: 2.441 [1.611–3.699]), iFS (HR [95% CI]: 1.941 [1.161–3.247]), and FCSS (HR [95% CI]: 1.891 [1.115–3.205]). The new FCM-score B (HR [95% CI]: 4.079 [2.540–6.548]) provided superior prognostic grading compared to IPSS-R (HR [95% CI]: 2.368 [1.607–3.490]) as well as to IPSS-M (HR [95% CI]: 0.816 [0.303–2.196]) ($n = 84$), as detailed in Tables 4 and S4. A similar trend was observed in the C-index with stratified FCM-score B obtaining the best prediction potential (C-Index: FCM-B 0.6380 and IPSS-R 0.6130) (Table 4).

In the following, we focused on the FCM-score with the strongest prognostic impact, FCM-score B, renamed FCM-PS for the rest of this paper. The FCM-PS included the final panel: CD45/CD34/CD117/CD33/CD19/CD123/HLA-DR. Two examples of FCM measurements, one displaying a high and the other a low FCM-PS, are shown in Figure S2.

The distribution of FCM-PS score was visualized against the HRs for OS (Figure 2). It was calculated with respect to the average patient (median FCM-PS) in the training population and showed a clear gradient from low to very high risk according to FCM-PS. The median score for a patient was 2, which was taken as the reference for the survival analysis. Patients with FCM-PS equal to zero and patients with FCM-PS equal to one have better survival probabilities compared to patients with a FCM-PS score equal to 2. Patients presenting a FCM-PS score of 0 are 66% less likely to die than patients with a score of 2, and patients withan FCM-PS equal to 1 are 63% less likely to die than patients with a score of 2. Furthermore, patients with scores from 3 onwards present a much higher risk of dying (range of HR: 2–7) than reference patients (Figure 2).

Subsequent ROC-analysis, incorporating known prognostic factors, showed that FCM-PS outperformed even IPSS-R and IPSS-M in accurately predicting progression (Figure S3A/B).

Notably, patients with low FCM-PS exhibited better OS probability in the Kaplan–Meier curve (Figure 3A) ($P < 0.0001$). Remarkably, FCM-PS added substantial prognostic information to IPSS-R by distinguishing both IPSS-R lower and higher-risk patients considering OS (Figure 4A/B). For IPSS-M lower-risk patients, we observed a trend in the same direction ($n = 84$) (Figure 4C/D).

Furthermore, FCM-PS also presented prognostic capabilities to predict transformation to AML in the training cohort (patients transformed to AML = 58; FCM-PS (HR [95% CI]: 3.304 [1.747–6.249]) (Table S5).

Moreover, Kaplan–Meier curves were performed for different risk categories of the FCM-PS (very low risk [0–1 points in the score], low risk [2 points in the score], intermediate risk [3 points in the score], high risk [4–5 points in the score]), presenting a clear separation of the different risk categories for OS (FCM-PS log-rank P-value < 0.001) (Figure 5).

Next, reliability testing in the training cohort ($n = 77$ healthy donors vs. $n = 399$ MDS patients) revealed a clear difference in the distribution of the FCM-PS (chi-square test $P < 0.0001$) (Figure S4A). Thus, a small proportion of HD presented with a high FCM-PS (12.9% of HD vs. 58.4% of MDS patients). The main aberrancies included increased % of $CD19^+$ lymphocytes (8/10 HD)

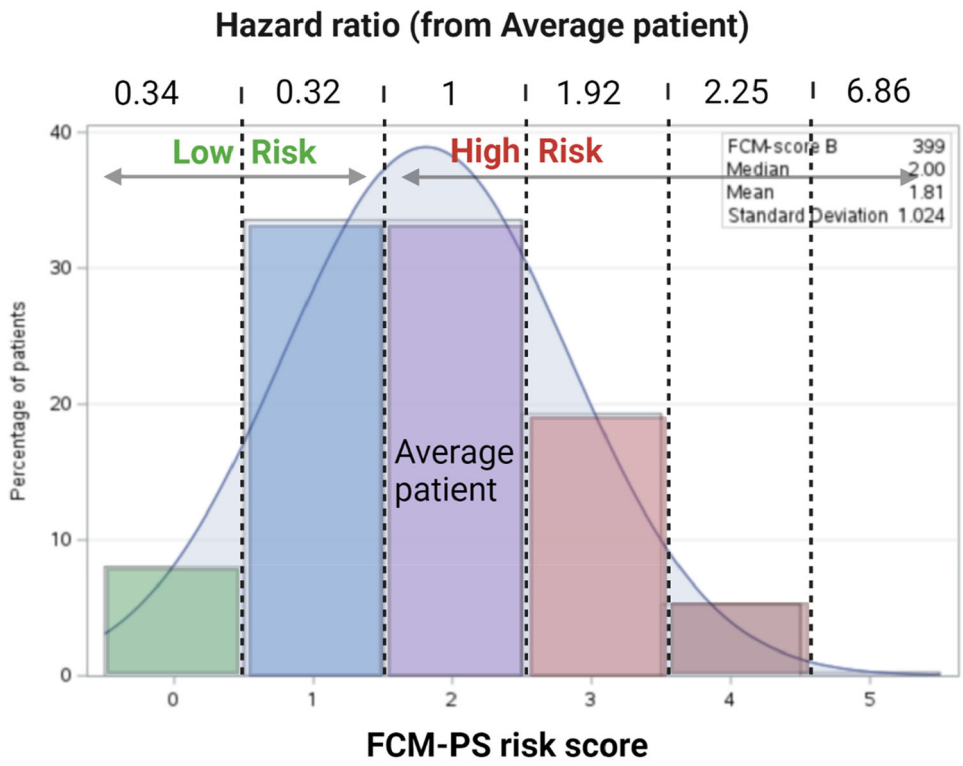


FIGURE 2 **Distribution of flow cytometry-prognostic score (FCM-PS) in the training population.** Hazard ratios for overall survival (OS) calculated with respect to average patient for each score category (399 patients; median FCM-PS = 2.00 (mean = 1.81; standard deviation = 1.024). Abbreviations: FCM-PS, flow cytometry prognostic score; OS, overall survival.

and decreased % of lyProgC (5/10 HD). However, the maximum FCM-PS score never exceeded a score of 2 in HD. Moreover, there was no significant difference regarding the presence of a *TP53* mutation between low- and high-scored MDS patients ($n = 107$ wildtype [wt*TP53*] vs. $n = 15$ mutated [m*TP53*]) in the training cohort (chi-square test $P < 0.2317$; Fisher's exact test $P < 0.2067$) (Figure S4B). Even in the group of patients presenting either a high variant allele fraction (VAF) or biallelic *TP53* mutation plus patients with a complex karyotype ($n = 17$), an equal distribution of low- and high-scored MDS patients was present (chi-square test $P < 0.7511$; Fisher's exact test $P < 0.4695$) (Figure S4C). Four of the five patients who presented with the above-mentioned high-risk features, but a low FCM-PS are alive with a follow-up of 35 to 83 months. This suggests that the prognostic value of the FCM-PS in MDS patients is independent of the presence of the here considered high-risk molecular and cytogenetic abnormalities.

The new FCM-scores, FCM-PS as well as FCM-scores A, C, and D, were validated in an independent, predominantly lower-risk MDS-cohort, demonstrating robust OS discrimination performance. Specifically, FCM-PS (HR [95% CI]: 2.672 [1.416–5.041]) outperformed the well-known FCM diagnostic scores (Tables 4 and S4) and proved superior prognostic grading to IPSS-R (HR [95% CI]: 2.640 [1.581–4.407]). Subsequent ROC-analysis, incorporating known prognostic factors, showed that FCM-PS (area under the curve [AUC]: 0.70) outperformed IPSS-R (AUC: 0.57) in OS prediction accuracy (Figure S3C). Kaplan–Meier curve (Figure 3B) exhibited a significantly better OS for patients with low FCM-PS ($P < 0.0001$). Therefore, we conclude that FCM-PS was able to separate OS in an independent cohort.

DISCUSSION

FCM is recommended as one part of the integrated diagnostic approach for MDS, according to the WHO classification.[5-12] However, the predictive value of FCM in MDS has been reported primarily through studies focusing on the expression of a low number of antigens in selected cell compartments.[9,13-23] Our study aimed to extend the scope of these investigations by examining a large cohort of MDS patients, utilizing a comprehensive panel of FCM-parameters for OS prognostication. This approach allowed for the analysis of progenitor cells and the maturation of granulo-, monocyto-, erythro-, and lymphopoiesis. An extensive manual expert gating strategy, complemented by the application of a computational algorithm, facilitated this analysis.

Nine independent FCM-parameters indicating abnormal maturation of the progenitor cell compartment as crucial features of myeloid dysplasia (abnormal expression of CD45 on myPCs, reduced percentage of lymphatic progenitors), hypogranularity (reduced SSC) and impaired maturation within the granulopoietic cell compartment (reduced percentage, decreased CD15 and CD33 expression), and abnormal accumulation of monocytic cells underscore FCM's prognostic importance beyond its established diagnostic utility (Table S3).[8-11,32] Especially abnormal CD45 expression on myPC and reduced SSC on mature granulocytes proved to be independently associated with MDS diagnosis in the multicenter study of the ELN iMDS flow working group.[12] Remarkably, the two additional parameters, abnormal percentage of mature B-lymphocytes and increased percentage of pDC, might reflect the disturbed immune system in MDS.[38-40] Controversial results are reported in the literature,

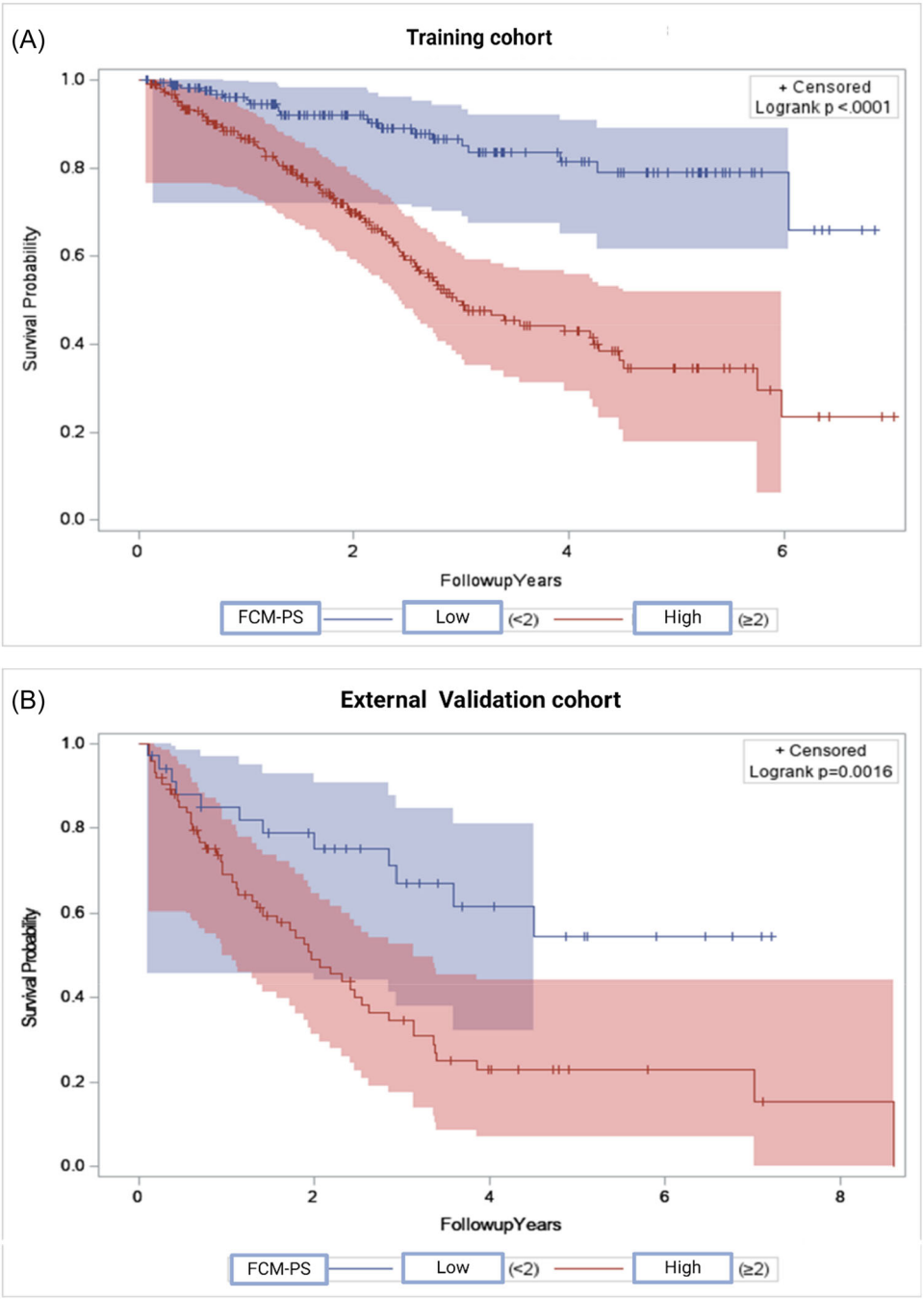


FIGURE 3 **Prognostic value of the flow cytometry-prognostic score (FCM-PS) for overall survival (OS) prediction.** Kaplan–Meier curves of high versus low FCM-PS in the training cohort **(A)** and the external validation cohort **(B)**. Abbreviation: FCM-PS, flow cytometry prognostic score.

especially with regard to the proportion of pDC. Alhan et al.[23] and van Leeuwen-Kerkhoff et al.[38] also described an increased proportion of pDC in the BM. Van Leeuwen-Kerkhoff reports this specifically for MDS with low blasts (MDS-LB), whereas in MDS with increased blasts (MDS-IB), the proportion is reduced, as are the other DC subpopulations and SLAN$^+$ non-classical monocytes. In contrast to our results, Saft et al.[41] showed a decreased percentage of pDC in BM. Furthermore, Jachiet et al.[42] analyzed pDC in the peripheral blood of MDS without and with systemic inflammatory or dysimmune diseases and found only in the latter patient group reduced numbers of pDC. The MDS patients with increased numbers of pDC in our study presented with increased blast counts and higher-risk IPSS-R categories. An increased number of pDC might reflect the presence of viral infections in these patients.

In this context, we developed and validated the easy-to-use, one tube FCM-based prognostic score (panel: CD45/CD34/CD117/CD33/CD19/CD123/HLA-DR; costs around $200 per examination), termed FCM-PS, effectively identifying MDS patients with better OS. Remarkably, we observed that this new FCM-PS surpassed the established IPSS-R clinical prognostic score and diagnostic FCM-scores

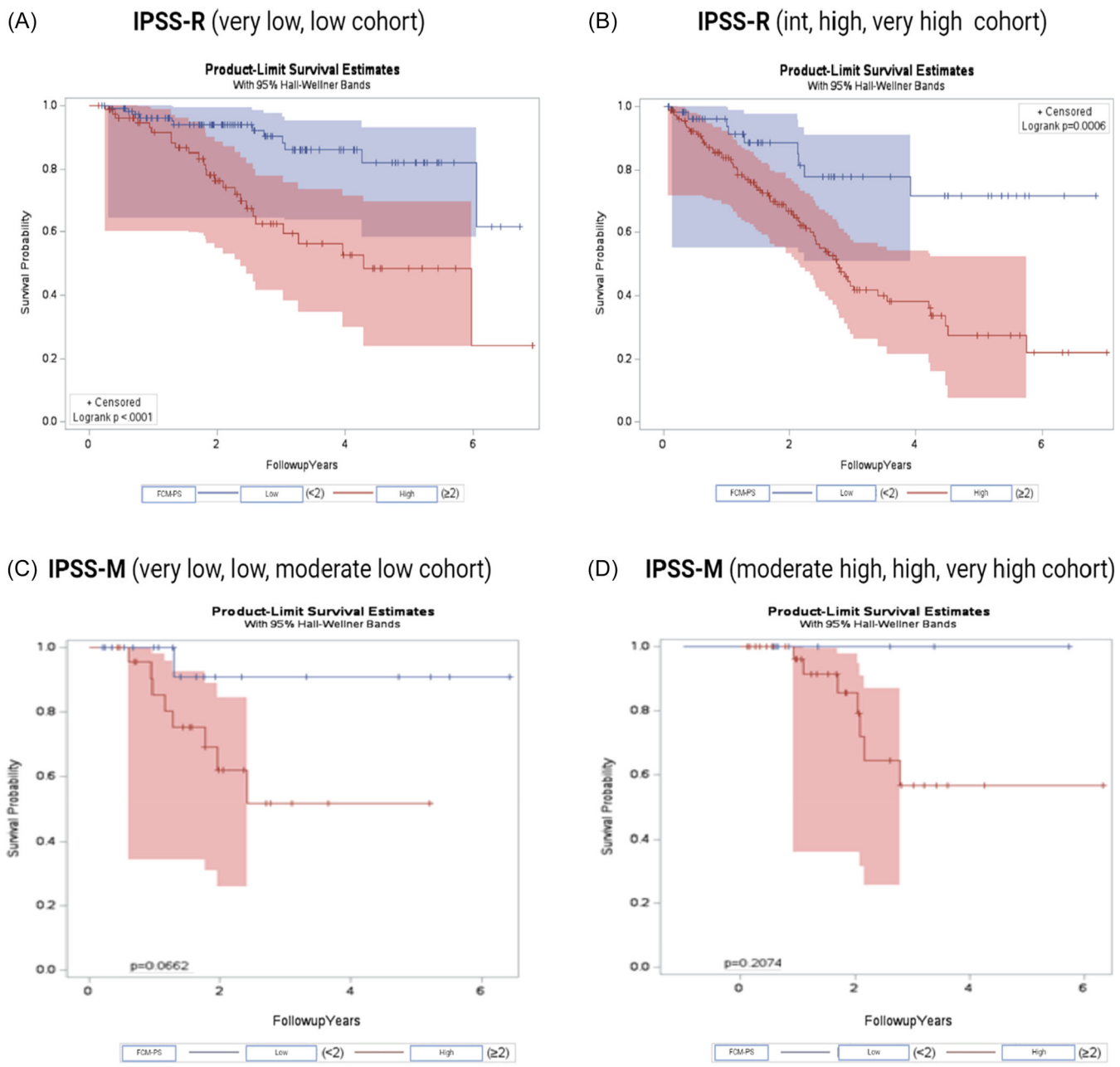


FIGURE 4 **Flow cytometry-prognostic score (FCM-PS) refines International Prognostic Scoring System-Revised (IPSS-R) subgroups for overall survival (OS) prediction (training cohort).** A clear separation of two distinct groups with different prognosis could be identified within the IPSS-R lower risk **(A)** as well as in the higher risk **(B)** categories. Considering the International Prognostic Scoring System-Molecular (IPSS-M), a trend in the same direction was observed **(C/D)**. Abbreviations: FCM-PS, flow cytometry prognostic score; int, intermediate; IPSS-M, International Prognostic Scoring System–Molecular; IPSS-R, International Prognostic Scoring System-Revised; OS, overall survival.

like the Ogata-score, FCSS, and iFS. A clear trend in the same direction was also observed with regard to the prognostic IPSS-M score. Our new FCM-PS comprised six FCM-parameters, including three parameters already known from the Ogata-score. However, the addition of CD33, a granulopoiesis parameter, the percentage of $CD19^{+}$ cells (mature B-cells), and of pDC as additional decision parameters revealed a more powerful prognostic impact than the Ogata-score. The reliability of the score was confirmed by parallel testing of BM samples from MDS patients and healthy donors, with a low presence of a FCM-PS score of 2 in the latter group (13% of the healthy donors vs. 58% of the MDS patients). One of 18 healthy donors with available molecular data (data not shown) presented with a CHIP mutation (m*TET2*; VAF 2.6%) combined with a high FCM-PS. This might be an explanation for the other donors with higher FCM-scores. The distribution of mutations in the *TP53* gene, even among high-risk MDS patients with a high VAF or biallelic *TP53* mutations or the presence of a complex aberrant karyotype, further validated the scores' reliability. Additionally, consistent results obtained for an external validation cohort confirmed the robustness of the new FCM-PSs in this predominantly lower-IPSS-R-risk MDS cohort.

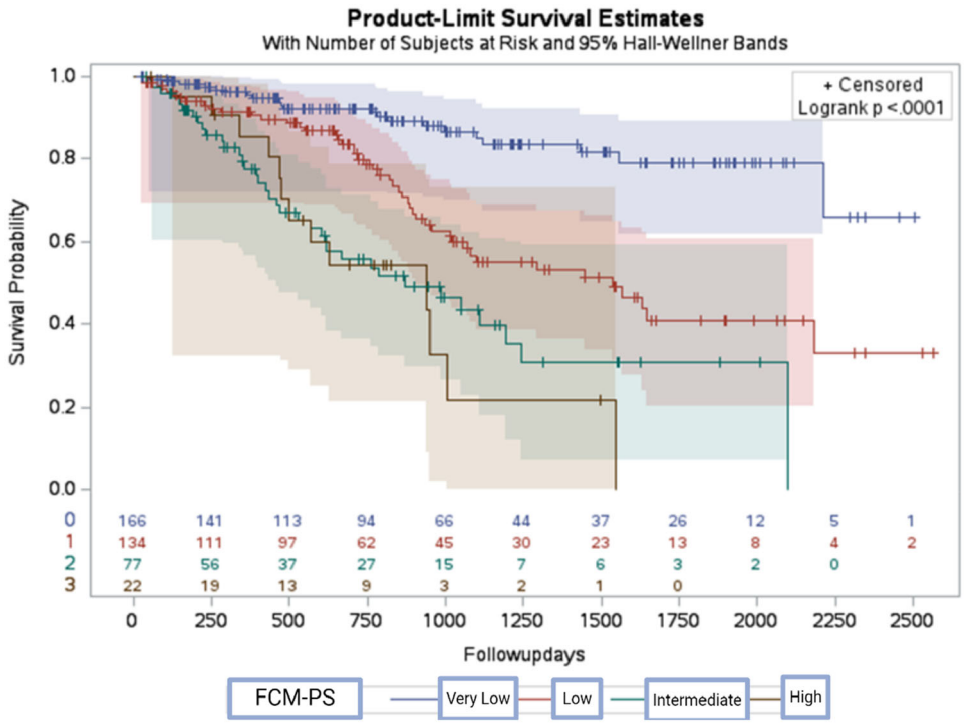


FIGURE 5 **Overall survival in different risk categories for the flow cytometry-prognostic score (FCM-PS).** The Kaplan–Meier curves for overall survival (OS) were performed for different risk categories of the FCM-PS (very low risk [0–1 points in the score], low risk [2 points in the score], intermediate risk [3 points in the score], and high risk [4–5 points in the score]). Abbreviation: FCM-PS, flow cytometry prognostic score; OS, overall survival.

The main limitation of the study is the limited molecular characterization of both cohorts, restricting the interpretation of the IPSS-M risk stratification based on our FCM-PS score. Although the IPSS-M has increased the ability to predict patient outcomes, the molecular tests on which the IPSS-M is based take time to return results and may not be available in all centers and feasible for all patients. Circumventing this, we propose to use the widely accessible and relatively cost-effective MDS FCM-panel as an add-on to the IPSS-R as a predictive tool for disease outcomes. This strategy aims to bridge the gap in centers with limited advanced molecular diagnostics.

Additionally, the role of dendritic cells (DCs) in MDS has been addressed in several studies, most of which consistently report reduced frequencies and impaired function. For example, Jachiet et al. demonstrated significantly decreased DC and monocyte subsets in MDS patients with associated systemic inflammatory and autoimmune diseases, particularly in those with VEXAS syndrome.[42] In our own previous work, we showed that BM DC subsets and slan+ nonclassical monocytes are reduced in frequency, display downregulated pro-inflammatory transcripts, and exhibit impaired T-cell stimulatory capacity in MDS, highlighting an immunosuppressive phenotype that may favor disease progression.[39] These data, together with other published studies, firmly support the view that DC numbers are generally diminished in MDS compared with healthy controls. In contrast, our current analysis identified relatively higher plasmacytoid DC frequencies as a parameter within the prognostic model. This apparently paradoxical observation should not be interpreted as a contradiction of previous biological findings, but rather as an indication that variation within the low DC compartment of MDS patients may still carry prognostic relevance. Importantly, our sample size for this parameter was small, and differences in cohort cutoffs or center-specific methodology may also have contributed. Thus, while the finding is intriguing, it requires confirmation in larger independent cohorts before firm conclusions can be drawn.

In summary, the new FCM-PS offers predictive insight into OS in MDS patients by assessing myeloid cell maturation and BM immune cell composition. It provides nuanced insights into IPSS-R and IPSS-M subcategories and could potentially aid in tracking treatment responses, although this was not the aim of the current study. The FCM-PS is particularly valuable in settings with limited access to mutational analysis, offering a feasible and complementary approach for disease outcome prediction.

ACKNOWLEDGMENTS

We would like to thank all the technicians in the flow cytometry laboratories for processing the samples and acquiring the raw data. Moreover, we would like to thank all the patients and families who have contributed to this study.

AUTHOR CONTRIBUTIONS

Aida Santaolalla: Conceptualization; methodology; formal analysis; visualization; writing—original draft; writing—review and editing; validation; investigation; data curation. **Uta Oelschlaegel**: Conceptualization; methodology; data curation; visualization; writing—original draft; writing—review and editing; supervision; investigation; resources; formal analysis. **Susann Winter**: Methodology; data curation; conceptualization; writing—review and editing; writing—original draft; visualization; resources; formal analysis. **Shirin Jamshidi**: Formal analysis; software. **Theresia M. Westers**: Conceptualization; methodology; data curation; investigation; writing—original draft; writing—review and editing; validation; resources. **Katja Sockel**: Writing—original draft; writing—review and editing. **Martin Bornhäuser**:

Writing—original draft; writing—review and editing. **Rosa Andres Ejarque**: Formal analysis; software. **Farzin Farzaneh**: Writing—original draft; writing—review and editing. **Antonella Poloni**: Writing—original draft; writing—review and editing. **Anne-Sophie Kubasch**: Writing—original draft; writing—review and editing. **Mieke Van Hemelrijck**: Writing—review and editing; supervision. **Arjan A. van de Loosdrecht**: Conceptualization; methodology; investigation; data curation; supervision; project administration; writing—original draft; writing—review and editing; resources. **Uwe Platzbecker**: Conceptualization; methodology; writing—original draft; writing—review and editing; supervision; resources. **Shahram Kordasti**: Conceptualization; methodology; investigation; supervision; project administration; funding acquisition; resources; writing—original draft; writing—review and editing.

CONFLICT OF INTEREST STATEMENT

Aida Santaolalla: Employment: Kite, a Gilead company. Stocks: Gilead Sciences. None of these are relevant to the current work. Uwe Platzbecker: Research support and honoraria: BMS, Geron, Janssen, Jazz, Curis. None of these are relevant to the current work. Shahram Kordasti: Research support and honoraria: Novartis, Alexion, Beckman Coulter, MorphoSys, Pfizer. None of these are relevant to the current work. The remaining authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Original data is overseen by the Institutional Review Boards of the Technical University of Dresden and the Amsterdam UMC. Access to the original data will be via request to the relevant access committee.

FUNDING

This work was supported by the CRUK City of London Centre Award (CTRQQR-2021/100004) at KCL.

SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article.

ORCID

Aida Santaolalla https://orcid.org/0000-0003-2748-3252
Uta Oelschlaegel https://orcid.org/0000-0002-2287-437X
Katja Sockel https://orcid.org/0000-0003-1732-7299

REFERENCES

1. Garcia-Manero G. Myelodysplastic syndromes: 2023 update on diagnosis, risk-stratification, and management. *Am J Hematol*. 2023;98(8):1307-1325.
2. Adès L, Itzykson R, Fenaux P. Myelodysplastic syndromes. *Lancet*. 2014;383(9936):2239-2252.
3. Bernard E, Tuechler H, Greenberg PL, et al. Molecular International Prognostic Scoring System for myelodysplastic syndromes. *NEJM Evid*. 2022;1(7):EVIDoa2200008. doi:10.1056/EVIDoa2200008
4. Sauta E, Robin M, Bersanelli M, et al. Real-world validation of molecular International Prognostic Scoring System for myelodysplastic syndromes. *J Clin Oncol*. 2023;41(15):2827-2842.
5. Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization classification of haematolymphoid tumours: myeloid and histiocytic/dendritic neoplasms. *Leukemia*. 2022;36(7):1703-1719.
6. Mufti GJ, McLornan DP, van de Loosdrecht AA, Germing U, Hasserjian RP. Diagnostic algorithm for lower-risk myelodysplastic syndromes. *Leukemia*. 2018;32(8):1679-1696.
7. Van de Loosdrecht AA, Kern W, Porwit A, et al. Clinical application of flow cytometry in patients with unexplained cytopenia and suspected myelodysplastic syndrome. A report of the Europoean LeukemiaNet International MDS-Flow Cytometry Working Group. *Cytometry B Clin Cytom*. 2023;104(1):77-86.
8. Ogata K, Della Porta MG, Malcovati L, et al. Diagnostic utility of flow cytometry in low-grade myelodysplastic syndromes: a prospective validation study. *Haematologica*. 2009;94(8):1066-1074.
9. Wells DA, Benesch M, Loken MR, et al. Myeloid and monocytic dyspoiesis as determined by flow cytometric scoring in myelodysplastic syndrome correlates with the IPSS and with outcome after hematopoietic stem cell transplantation. *Blood*. 2003;102(1):394-403.
10. Cremers EMP, Westers TM, Alhan C, et al. Implementation of erythroid lineage analysis by flow cytometry in diagnostic models for myelodysplastic syndromes. *Haematologica*. 2017;102(2):320-326.
11. Oelschlaegel U, Oelschlaeger L, von Bonin M, et al. Comparison of five diagnostic flow cytometry scores in patients with myelodysplastic syndromes: diagnostic power and prognostic impact. *Cytometry B Clin Cytom*. 2023;104(2):141-150.
12. Kern W, Westers TM, Bellos F, et al. Multicenter prospective evaluation of diagnostic potential of flow cytometric aberrancies in myelodysplastic syndromes by the ELN iMDS flow working group. *Cytometry B Clin Cytom*. 2023;104(1):51-65.
13. Molteni A, Riva M, Cesana C, et al. Prognostic relevance of the flow cytometric count of medullar blasts in myelodysplastic syndromes. *Eur J Haematol*. 2015;94(6):519-525.
14. Della Porta MG, Picone C, Tenore A, et al. Prognostic significance of reproducible immunophenotypic markers of marrow dysplasia. *Haematologica*. 2014;99(1):e8-e10.
15. Kárai B, Bedekovics J, Miltényi Z, et al. A single-tube flow cytometric procedure for enhancing the diagnosis and prognostic classification of patients with myelodysplastic syndromes. *Int J Lab Hematol*. 2017;39(6):577-584.
16. Barreau S, Green AS, Dussiau C, et al. Phenotypic landscape of granulocytes and monocytes by multiparametric flow cytometry: a prospective study of a 1-tube panel strategy for diagnosis and prognosis of patients with MDS. *Cytometry B Clin Cytom*. 2020;98(3):226-237.
17. Chu S-C, Wang T-F, Li C-C, et al. Flow cytometric scoring system as a diagnostic and prognostic tool in myelodysplastic syndrome. *Leuk Res*. 2011;35(7):868-873.
18. Falco P, Levis A, Stacchini A, et al. Prognostic relevance of cytometric quantitative assessment in patients with myelodysplastic syndromes. *Eur J Haematol*. 2011;87(5):409-418.
19. Scott BL, Wells DA, Loken MR, Myerson D, Leisenring WM, Deeg HJ. Validation of a flow cytometric scoring system as a prognostic indicator for posttransplantation outcome in patients with myelodysplastic syndrome. *Blood*. 2008;112(7):2681-2686.
20. Kern W, Bacher U, Haferlach C, Alpermann T, Schnittger S, Haferlach T. Multiparameter flow cytometry provides independent prognostic information in patients with suspected myelodysplastic syndromes: a study on 804 patients. *Cytometry B Clin Cytom*. 2015;88(3):154-164.
21. Vido-Marques JR, Reis-Alves SC, Saad STO, Metze K, Lorand-Metze I. A simple score derived from bone marrow immunophenotyping is important for prognostic evaluation in myelodysplastic syndromes. *Sci Rep*. 2020;10(1):20281.
22. Matarraz S, López A, Barrena S, et al. Bone marrow cells from myelodysplastic syndromes show altered immunophenotypic profiles that may contribute to the diagnosis and prognostic stratification of the disease: a pilot study on a series of 56 patients. *Cytometry B Clin Cytom*. 2010;78B(3):154-168.
23. Alhan C, Westers TM, Cremers EMP, et al. The myelodysplastic syndromes flow cytometric score: a three-parameter prognostic flow cytometric scoring system. *Leukemia*. 2016;30(3):658-665.

24. Park S, Kosmider O, Maloisel F, et al. Dyserythropoiesis evaluated by the RED score and hepcidin:ferritin ratio predicts response to erythropoietin in lower-risk myelodysplastic syndromes. *Haematologica*. 2019;104(3):497-504.
25. Guarnera L, Fabiani E, Attrotto C, et al. Reliability of flow-cytometry in diagnosis and prognostic stratification of myelodysplastic syndromes: correlations with morphology and mutational profile. *Ann Hematol*. 2023;102(11):3015-3023.
26. Li L, Yu S, Hu X, et al. Immunophenotypic changes of monocytes in myelodysplastic syndrome and clinical significance. *Clin Exp Med*. 2023;23(3):787-801.
27. Johansson U, McIver-Brown N, Cullen M, et al. The flow cytometry myeloid progenitor count: a reproducible parameter for diagnosis and prognosis of myelodysplastic syndromes. *Cytometry B Clin Cytom*. 2023;104(2):115-127.
28. Majcherek M, Kiernicka-Parulska J, Mierzwa A, et al. The diagnostic and prognostic significance of flow cytometric bone marrow assessment in myelodysplastic syndromes according to the European LeukemiaNet recommendations in single-centre real-life experience. *Scand J Immunol*. 2021;94(2):e13028.
29. Verigou E, Lampropoulou P, Smyrni N, et al. Evaluation of a bone marrow dysmyelopoiesis immunophenotypic index for the diagnosis and prognosis of myelodysplastic syndromes. *Cardiovasc Hematol Disord Drug Targets*. 2015;15(2):148-161.
30. Van de Loosdrecht AA, Westers TM, Westra AH, Dräger AM, van der Velden VHJ, Ossenkoppele GJ. Identification of distinct prognostic subgroups in low- and intermediate-1-risk myelodysplastic syndromes by flow cytometry. *Blood*. 2008;111(3):1067-1077.
31. Aanei CM, Picot T, Tavernier E, Guyotat D, Campos Catafal L. Diagnostic utility of flow cytometry in myelodysplastic syndromes. *Front Oncol*. 2016;6:161.
32. Porwit A, Béné MC, Duetz C, et al. Multiparameter flow cytometry in the evaluation of myelodysplasia: analytical issues. *Cytometry B Clin Cytom*. 2023;104(1):27-50.
33. Cherlin S, Bigirumurame T, Grayling MJ, et al. Utilising high-dimensional data in randomised clinical trials: a review of methods and practice. *arXiv*. Preprint posted online May 17, 2023. arXiv:2305.10174.
34. Bhattacharyya A, Rai SN. Adaptive signature design-review of the biomarker guided adaptive phase-iii controlled design. *Contemp Clin Trials Commun*. 2019;15:100378.
35. Van de Loosdrecht AA, Cremers EMP, Alhan C, et al. Determinants of lenalidomide response with or without erythropoiesis-stimulating agents in myelodysplastic syndromes: the HOVON89 trial. *Leukemia*. 2024;38(4):840-850.
36. Van der Velden VHJ, Preijers F, Johansson U, et al. Flow cytometric analysis of myelodysplasia: pre-analytical and technical issues-Recommendations from the European LeukemiaNet. *Cytometry B Clin Cytom*. 2023;104(1):15-26.
37. Greenberg PL, Tuechler H, Schanz J, et al. Revised international prognostic scoring system for myelodysplastic syndromes. *Blood*. 2012;120:2454-2465.
38. Van Leeuwen-Kerkhoff N, Westers TM, Poddighe PJ, et al. Reduced frequencies and functional impairment of dendritic cell subsets and non-classical monocytes in mtelodysplastic symdromes. *Haematologica*. 2022;107(3):655-667.
39. Winter S, Shoaie S, Kordasti S, Platzbecker U. Integrating the "Immunome" in the stratification of myelodysplastic syndromes and future clinical trial design. *J Clin Oncol*. 2020;38(15):1723-1735.
40. Kahn JD, Chamuleau MED, Westers TM, et al. Regulatory T cells and progenitor B cells are independent prognostic predictors in lower risk myelodysplastic syndromes. *Haematologica*. 2015;100(6):e220-e222.
41. Saft L, Björklund E, Berg E, Hellström-Lindberg E, Porwit A. Bone marrow dendritic cells are reduced in patients with high-risk myelodysplastic syndromes. *Leuk Res*. 2013;37:266-273.
42. Jachiet V, Ricard L, Hirsch P, et al. Reduced peripheral blood dendritic cell and monocyte subsets in MDS patients with systemic inflammatory or dysimmune diseases. *Clin Exp Med*. 2023;23(3):803-813.