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Pretreatment CD34$^+$/CD38$^-$ Cell Burden as Prognostic Factor in Myelodysplastic Syndrome Patients Receiving Allogeneic Stem Cell Transplantation

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Myelodysplastic syndrome (MDS) is a highly heterogeneous clonal hematopoietic disorder. Allogeneic hematopoietic stem cell transplantation (HSCT) remains the only curative treatment and is of particular interest in patients at high risk for progression to acute myeloid leukemia (AML). In MDS, CD34$^+$/CD38$^-$ cells possess MDS stem cell potential, and secondary AML (sAML) clones originate from the MDS disease stage. However, the prognostic impact of the pretreatment stem cell population burden in MDS remains unknown. We retrospectively analyzed the prognostic impact of the pretreatment CD34$^+$/CD38$^-$ cell burden in 124 MDS patients who received allogeneic HSCT at our institution. A high pretreatment bone marrow CD34$^+$/CD38$^-$ cell burden ($\geq$1%) was associated with worse genetic risk and a higher incidence of blast excess. Patients with a high CD34$^+$/CD38$^-$ cell burden had a significantly higher cumulative incidence of MDS relapse, a higher cumulative incidence of secondary AML, and a trend for shorter overall survival after allogeneic HSCT. In multivariable analyses this prognostic impact was shown to be independent of other clinical and cytogenetic risk factors in MDS. Patients suffering MDS relapse or progression to AML also had a higher pre-treatment CD34$^+$/CD38$^-$ cell burden as a continuous variable. The observed prognostic impact is likely mediated by MDS stem cells within the CD34$^+$/CD38$^-$ cell population initiating MDS relapse or progression to AML. New therapeutic strategies targeting MDS stem cells might improve outcomes.

INTRODUCTION

Myelodysplastic syndromes (MDS) and myelodysplastic/myeloproliferative neoplasm (MDS/MPN) are heterogeneous clonal hematopoietic disorders resulting in inefficient hematopoiesis and a variable progression risk to secondary acute myeloid leukemia (AML) [1-3]. At diagnosis, the International Prognostic Scoring System (IPSS) [1] and the revised international prognostic scoring system (IPSS-R) [2] are commonly used to estimate disease aggressiveness for subsequent therapeutic decision-making. Allogeneic hematopoietic stem cell transplantation (HSCT) remains the only curative treatment option for MDS patients and is of particular interest in high-risk disease [4]. Retrospective analyses suggested survival advantages for high-risk MDS patients receiving HSCT compared with alternative treatment options such as hypomethylating agents [5,6]. With the introduction of conditioning regimens with reduced intensity, allogeneic HSCT became available for older or comorbid individuals who represent the majority of MDS patients [4,7]. In AML patients leukemic stem cells are believed to be responsible for disease initiation and relapse [8,9]. Similar to healthy HSCs, most AML stem cells reside within the immature bone marrow CD34$^+$/CD38$^-$ cell population [8-10].

A high diagnostic burden of CD34$^+$/CD38$^-$ cells is known to provide prognostic information in AML patients because it independently associated with worse outcome [11-13]. HSCs were also reported as the origin of the disease in low-risk MDS [14,16-19], whereas in MDS progressing to secondary AML MDS-initiating clones were shown to persist in secondary AML [14,15]. Furthermore, the bone marrow CD34$^+$/CD38$^-$ cell compartment was described to harbor most MDS-initiating cells [14,16,20,21]. Some studies showed a correlation between a high expression of the immature surface antigens CD34 and CD117 and worse outcomes in MDS patients in bone marrow [22,23] or peripheral blood [24]. Especially high CD34 expression was linked to a high proportion of immature MDS bone marrow cells, high-risk MDS, poor IPSS risk groups, and worse genetic risk [22,23,25-27]. However, data on the

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prognostic utility of the highly immature $CD34^+/CD38^-$ cell burden in MDS patients remain sparse. Only one study described an association of a high fraction of $CD34^+$/intermediate forward-scatter/sideward-scatter/$CD38^{dim}$ cells with poor IPSS risk and suggested a correlation with disease progression in a small cohort of eleven MDS patients [28]. Thus, the main objective of our study is to assess the prognostic impact of the bone marrow $CD34^+/CD38^-$ cell burden in a larger set of MDS patients before initiation of therapy followed by an allogeneic HSCT as curative treatment.

METHODS

Patient Population

We retrospectively analyzed 124 consecutive adult patients diagnosed with MDS (n=107, 86%) or MDS/MPN (n=17, 14%) who received an allogeneic HSCT at our institution between January 2003 and September 2017. Median age at HSCT was 61.3 years (range, 22.2 to 74.4). For all patients bone marrow aspirates for flow cytometric analyses were collected 0 to 110 months after MDS diagnosis. Patients received no cytoreductive treatment for their MDS before sample collection. Patients were grouped according to the IPSS and IPSS-R risk groups before initiation of treatment [1,2]. Further patient characteristics are shown in Table 1 and Supplementary Table S1. Median follow-up of surviving patients after HSCT was 4.3 years (range, .2 to 13.6) for patients alive. Written informed consent for all individuals was obtained in accordance with the Declaration of Helsinki.

Treatments

Median time from assessment of the pretreatment $CD34^+/CD38^-$ cell burden to allogeneic HSCT was 128 days (range, 4 to 1218). Fifty-four patients (44%) received a reduced-intensity (RIC) conditioning before HSCT that consisted of fludarabine with busulfan (n = 49) or treosulfan (n = 5) [29,30]. Seventy patients (56%) received non-myeloablative (NMA) conditioning that consisted of fludarabine with 2 Gy (n = 68) or 3 Gy (n = 2) total body irradiation [7]. All patients received granulocyte colony-stimulating factor–mobilized peripheral blood stem cells on day 0. Donors were either HLA-matched related (n = 17, 14%), HLA-matched unrelated (n = 69, 56%), or had at least one HLA allelic mismatch (n = 38, 31%). Seventy-three patients (59%) received additional cytoreductive treatment before HSCT. Additional treatment before HSCT was administered to patients with a bone marrow blast count exceeding 10% or in patients with intermediate-2 or high-risk IPSS as bridging to HSCT or according to patient choice. Treatments consisted of either hypomethylating agents (25%), intensive chemotherapy (24%), or both (10%). For further details regarding the applied therapy protocols, see Supplemental Material. Median time from MDS diagnosis to allogeneic HSCT was 7 months (range, 1 to 114).

Healthy Control Subjects

Bone marrow aspirates of 51 healthy individuals were analyzed as healthy age-matched control subjects. Median age of the healthy control subjects was 57.1 years (range, 50.1 to 75.3). Written informed consent for all healthy individuals was obtained in accordance with the Declaration of Helsinki.

Flow Cytometry

For all samples mononuclear bone marrow cells were assessed for surface expression of CD34, CD38, and CD34/CD38 as previously described [13]. For all patients (n=124), the $CD34^+/CD38^-$ cells were assessed before initiation of treatment. Additionally, we evaluated the $CD34^+/CD38^-$ cell burden in 73 patients up to 30 days before HSCT (median, 20 days; range, 4 to 30) and in 85 patients 28 days after HSCT (median, 28 days; range, 24 to 32).

Cut-Off Point Definition for Pre-treatment $CD34^+/CD38^-$ Cell Burden

Using R's "Optimal Cutpoints" package [31], an optimal cut-off of 1% pretreatment bone marrow $CD34^+/CD38^-$ cells was assessed to differentiate patients according to their relapse probability (Supplementary Figure S1).

Definition of Clinical Endpoints and Statistical Analysis

All statistical analyses were performed using the R statistical software platform (version 3.4.3) [32]. Cumulative incidence of relapse (CIR) was calculated from HSCT to morphologic relapse. Cumulative incidence of secondary AML (CIsAML) was calculated from HSCT to development of secondary AML. CIR and CIsAML were calculated considering their competing risks (nonrelapse mortality and non-AML mortality, respectively) using the Fine and Gray model [33]. Overall survival (OS) was calculated from HSCT to death from any cause. Survival estimates were calculated using the Kaplan-Meier method, and groups were compared with a log-rank test. Associations were compared using the Kruskal-Wallis test and Fisher's exact test for continuous and categorical variables, respectively.

RESULTS

$CD34^+/CD38^-$ Cells in MDS Patients and Healthy Control Subjects

MDS or MDS/MPN patients had a median bone marrow pretreatment $CD34^+/CD38^-$ cell burden of .4% (range, 0% to 16%). This was significantly higher than the bone marrow $CD34^+/CD38^-$ cell count of the healthy control subjects (median, .2%; range, 0% to 0.8%; $P = .003$) (Supplementary Figure S2).

Prognostic Impact of a High Pretreatment $CD34^+/CD38^-$ Cell Burden

Regarding the pretreatment $CD34^+/CD38^-$ cell burden as a continuous variable, a significantly higher pretreatment $CD34^+/CD38^-$ cell burden was observed in patients suffering relapse or progression after HSCT ($P = .005$) as well as in patients developing a secondary AML ($P = .002$) compared with patients remaining in remission. For further analyses the defined optimal 1% cut-off (Supplementary Figure S1) was used to divide the cohort into patients with a high (n = 42, 34%) or low (n = 82, 66%) pretreatment $CD34^+/CD38^-$ cell burden. A high pretreatment $CD34^+/CD38^-$ cell burden was associated with a significantly higher CIR ($P < .001$; Figure 1A) and a significantly higher CIsAML ($P = .004$; Figure 1B). Despite a separation of the curves, OS did not significantly differ between patients with high or low pretreatment $CD34^+/CD38^-$ cell burden ($P = .12$; Figure 1C).

Three years after HSCT, CIR was 48% (95% confidence interval [CI], 30% to 63%) versus 18% (95% CI, 10% to 27%), CIsAML was 32% (95% CI, 17% to 47%) versus 9% (95% CI, 4% to 17%), and OS was 49% (95% CI, 35% to 69%) versus 64% (95% CI, 54% to 76%) in patients with a high or low pretreatment $CD34^+/CD38^-$ cell burden, respectively. In multivariable analyses a high pretreatment $CD34^+/CD38^-$ cell burden remained an independent factor for higher CIR after adjustment for IPSS-R genetic risk; for higher CIsAML after adjustment for IPSS-R genetic risk, age at HSCT, and HLA match; and for shorter OS after adjustment for pre-HSCT bone marrow blast count, HLA match, and donor type (Table 2). A high pretreatment $CD34^+/CD38^-$ cell burden was also associated with worse outcome when we excluded patients with MDS/MPN from our analysis (CIR, $P = .004$; CIsAML, $P = .003$; OS, $P = .08$) (Supplementary Figure S3).

Associations of a High Pretreatment $CD34^+/CD38^-$ Cell Burden

A high pretreatment $CD34^+/CD38^-$ cell burden was associated with a higher bone marrow expression of surface antigens indicating myeloid differentiation (CD13, $P < .001$, and CD33, $P < .001$) and a higher bone marrow expression of the immature surface antigens CD34 ($P < .001$) and CD117 ($P < .001$). All patients with a high pretreatment $CD34^+/CD38^-$ cell burden (n = 42, 100%) had an excess of blasts in bone marrow (≥5%) and/or peripheral blood (≥1%) compared with 59 patients (72%) with a low $CD34^+/CD38^-$ cell burden ($P < .001$). Patients with a high pretreatment $CD34^+/CD38^-$ cell burden were significantly more likely to have received cytoreductive treatment before HSCT (76% versus 51%, $P = .007$) than patients with a low pretreatment $CD34^+/CD38^-$ cell burden. A high pretreatment $CD34^+/CD38^-$ cell burden was significantly associated with an abnormal karyotype ($P = .04$), complex karyotype ($P = .002$), and monosomal karyotype ($P = .004$). Furthermore, a high pretreatment $CD34^+/CD38^-$ cell burden was associated with worse IPSS and IPSS-R genetic risk ($P < .001$ and $P = .02$, respectively) and with worse risk groups according to IPSS and IPSS-R ($P < .001$ and $P = .03$, respectively; Table 1 and Supplementary Table S1).

Table 1
Clinical, Genetic, and HSCT-Related Characteristics of MDS Patients Treated with HSCT according to the retreatment $CD34^+/CD38^-$ Cell Burden (High versus Low, 1% Cut, n=124)

	All Patients (n=124)	Low pretreatment BM $CD34^+/CD38^-$ Cell Burden (n = 82)	High Pre-treatment BM $CD34^+/CD38^-$ Cell Burden (n = 42)	*P*
Pre-treatment characteristics				
Pretreatment BM $CD34^+/CD38^-$ cell burden, %				<.001
Median	.4	.2	2.65	
Range	0-16	0-.9	1-16	
Sex, n (%)				.13
Male	71	51 (62)	20 (48)	
Female	53	31 (38)	22 (52)	
WHO 2008 classification, n (%)				NA
MDS				
RA	15	15 (18)	0 (0)	
RCMD	8	8 (10)	0 (0)	
del(5q)	1	1 (1)	0 (0)	
RAEB 1	29	19 (23)	10 (24)	
RAEB 2	54	28 (34)	26 (62)	
MDS/MPN				
CMML1	6	8 (7)	0 (0)	
CMML2	8	4 (5)	4 (10)	
MDS/MPN-U	3	1 (1)	2 (5)	
Disease, n (%)				1
MDS	107	71 (87)	36 (86)	
MDS/MPN	17	11 (13)	6 (14)	
Blast excess, n (%)				<.001
No	23	23 (28)	0 (0)	
Yes	101	59 (72)	42 (100)	
Disease origin, n (%)				.51
Secondary	30	18 (22)	12 (29)	
De novo	94	64 (78)	30 (71)	
IPSS-R score, n (%)				.03
Very low	0	0 (0)	0 (0)	
Low	9	7 (9)	2 (5)	
Intermediate	35	29 (38)	6 (15)	
High	36	16 (26)	10 (26)	
Very high	45	24 (32)	21 (54)	
IPSS-R genetic risk, n (%)				.02
Very good	4	3 (4)	1 (3)	
Good	58	43 (52)	15 (38)	
Intermediate	16	14 (17)	2 (5)	
Poor	17	11 (13)	6 (15)	
Very poor	26	11 (13)	15 (38)	
Normal karyotype, n (%)				.04
Absent	77	47 (57)	30 (77)	
Present	44	35 (43)	9 (23)	
Complex karyotype, n (%)				.002
Absent	83	70 (85)	23 (59)	
Present	28	12 (15)	16 (41)	
Monosomal karyotype, n (%)				.004
Absent	87	65 (81)	22 (55)	
Present	33	15 (19)	18 (45)	
HSCT related characteristics				
Age at HSCT, yr				.82
Median	61.3	61.3	61.3	
Range	22.2-74.4	22.2-74.4	47.1-69.1	
Therapy before HSCT, n (%)				.007
MDS untreated	50	40 (49)	10 (24)	
MDS treated	73	41 (51)	32 (76)	
Hypomethylating agents alone	31	21 (26)	10 (24)	.13

(*continued*)

Table 1 (*Continued*)

	All Patients (n=124)	Low pretreatment BM $CD34^+/CD38^-$ Cell Burden (n = 82)	High Pre-treatment BM $CD34^+/CD38^-$ Cell Burden (n = 42)	*P*
AML chemotherapy alone	30	16 (20)	14 (33)	
Both	12	4 (5)	8 (19)	
HCT-CI score, n (%)				.66
0	28	20 (25)	8 (20)	
1/2	34	23 (29)	11 (27)	
≥3	58	36 (46)	22 (53)	
BM blasts before HSCT, n (%)				.66
≤10%	119	79 (96)	37 (95)	
≥10%	5	3 (4)	2(5)	
Pre-HSCT $CD34^{\pm}/CD38^-$ cell burden, n (%)				≤.001
Low (≤1%)	56	46 (96)	10 (40)	
High (≥1%)	17	2 (4)	15 (60)	
Conditioning regimens, n (%)				.85
Reduced intensity	54	35 (43)	19 (45)	
Non-myeloablative	70	47 (57)	23 (55)	
HLA allelic match, n (%)				.15
Matched	86	53 (65)	33 (79)	
Mismatched	38	29 (35)	9 (21)	
Donor type, n (%)				.79
Related	17	12 (15)	5 (12)	
Unrelated	107	70 (85)	37 (88)	
Donor sex, n (%)				.75
Female into male	106	70 (91)	36 (88)	
All others	12	7 (9)	5 (12)	
CMV risk, n (%)				.54
No high risk	77	49 (64)	28 (70)	
High risk	40	28 (36)	12 (30)	

BM indicates bone marrow; CMML, chronic myelomonocytic leukemia; CMV, cytomegalovirus; HCT-CI, hematopoietic cell transplant–specific comorbidity index; HSCT hematopoietic stem cell transplantation; RAEB, refractory anemia with excess blasts; RCMD, refractory cytopenia with multilineage dysplasia; WHO, World Health Organization.

Subgroup Analyses for Patients with Blast Excess Before Therapy

Because of the strong association of a high $CD34^+/CD38^-$ cell burden and an excess of blasts, we performed subgroup analyses for patients presenting with an excess of blasts before therapy (n = 101). Here, similar associations as in the whole patient cohort were observed (Supplementary Table S2). In patients with an excess of blasts before therapy, a high pretreatment $CD34^+CD38^-$ cell burden was also associated with a significantly higher CIR (*P* = .002; Figure 2A), higher CIsAML (*P* = .004; Figure 2B), but no significantly different OS (*P* = .21; Figure 2C), which was also independent from other variables in multivariable analyses (Supplementary Table S3). The presence versus absence of an excess of blasts in patients with a low pretreatment $CD34^+/CD38^-$ cell burden (23 [19%] versus 69 [56%]) did not impact any of the analyzed endpoints (CIR, *P* = .42; CIsAML, *P* = .23; OS, *P* = .21; Figure 2A-C).

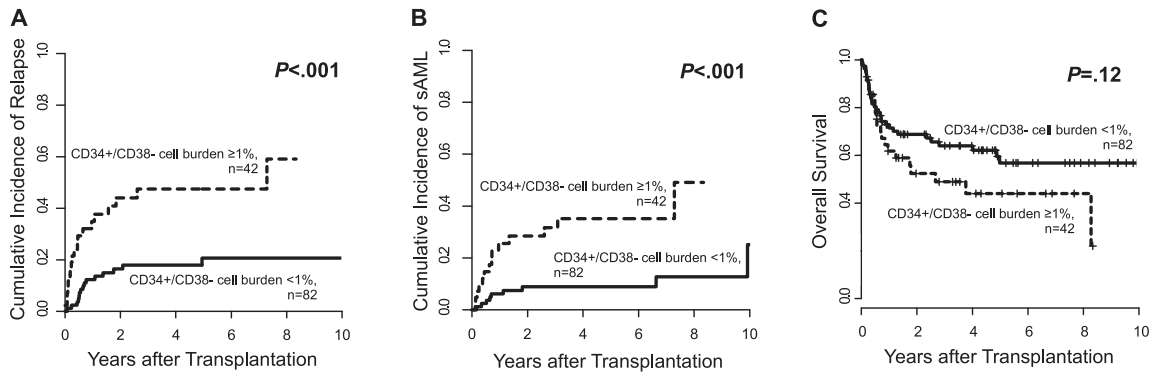

Figure 1. Outcome according to the pretreatment bone marrow $CD34^+/CD38^-$ cell burden (high versus low, 1% cut). (A) CIR, (B) CIsAML, and (C) OS of the entire patient cohort (N = 128).

Table 2
Multivariable Analyses

	CIR		CIsAML		OS	
	HR (95% CI)	*P*	HR (95% CI)	*P*	Odds Ratio* (95% CI)	*P*
Pretreatment BM $CD34^+/CD38^-$ cell burden (high vs. low, 1% cut)	2.88 (1.37-6.06)	.005	3.13 (1.23-7.99)	.02	.47 (.26-.84)	.01
IPSS-R genetic risk	1.36 (1.01-1.83)	.04	1.43 (1.02-2.02)	.04	—	—
Age at HSCT, yr	—	—	1.11 (1.02-1.18)	.01	—	—
Pre-HSCT BM blasts (<10% vs. >10%)	—	—	—	—	5.88 (1.96-16.67)	.001
HLA match (allelic MM vs. match)	—	—	.24 (.06-1.02)	.05	.34 (.18-.64)	<.001
Donor type (unrelated vs. related)	—	—			3.23 (1.45-7.14)	.004

Variables considered in the models were those significant at $\alpha = .20$ in univariable analyses. For CIR endpoint, variables considered were pretreatment $CD34^+/CD28^-$ cell burden (high vs. low, 1% cut), age at HSCT, blast excess (absent vs. present), IPSS-R genetic risk, donor sex (female into male vs. all others), and HLA match (allelic MM vs. match). For CIsAML endpoint, variables considered were $CD34^+/CD28^-$ cell burden (high vs. low, 1% cut), age at HSCT, blast excess (absent vs. present), IPSS-R genetic risk, pre-HSCT BM blast count (<10% vs. ≥10%), and HLA match (allelic MM vs. match). For OS endpoint, variables considered were $CD34^+/CD38^-$ cell burden (high vs. low, 1% cut), IPSS-R genetic risk, pre-HSCT BM blast count (<10% vs. ≥10%), HLA match (allelic MM vs. match), and donor type (unrelated vs. related). HR indicates hazard ratio; MM, mismatch.

* Less than 1 (>1) indicate lower (higher) risk for an event for the first category listed for the dichotomous variables.

Pretreatment $CD34^+/CD38^-$ Cell Burden in the Context of IPSS-R Risk Groups

Because the IPSS-R is known to provide prognostic information in MDS patients [2], we aimed to evaluate if the pretreatment $CD34^+/CD38^-$ cell burden allows additional risk stratification. In a separate analysis of patients with low or intermediate IPSS-R risk, a high $CD34^+/CD38^-$ cell burden was associated with a significantly higher CIR ($P < .001$; Figure 3A), a higher CIsAML ($P = .003$; Figure 3B), but similar OS ($P = .73$; Figure 3C). In patients with a high or very high IPSS-R risk, a higher pre-treatment $CD34^+/CD38^-$ cell burden was associated with higher CIR ($P = .05$; Figure 3A), higher CIsAML ($P = .03$; Figure 3B), and a shorter OS ($P = .02$; Figure 3C).

$CD34^+/CD38^-$ Cell Burden during Disease Course

We observed no prognostic impact of the pre-HSCT $CD34^+/CD38^-$ cell burden or the $CD34^+/CD38^-$ cell burden evaluated 28 days after HSCT. However, these analyses are limited by restricted sample sizes (n = 73 and n = 85, respectively) and heterogeneous cytoreductive treatments before HSCT. For a detailed analysis, see Supplemental Material and Supplementary Figures S4 and S5.

DISCUSSION

Similar to healthy hematopoiesis, primitive $CD34^+/CD38^-$ stem cells have been identified as the disease-initiating cells in MDS [10,14,16,21,34]. $CD34^+/CD38^-$ cells have been shown to

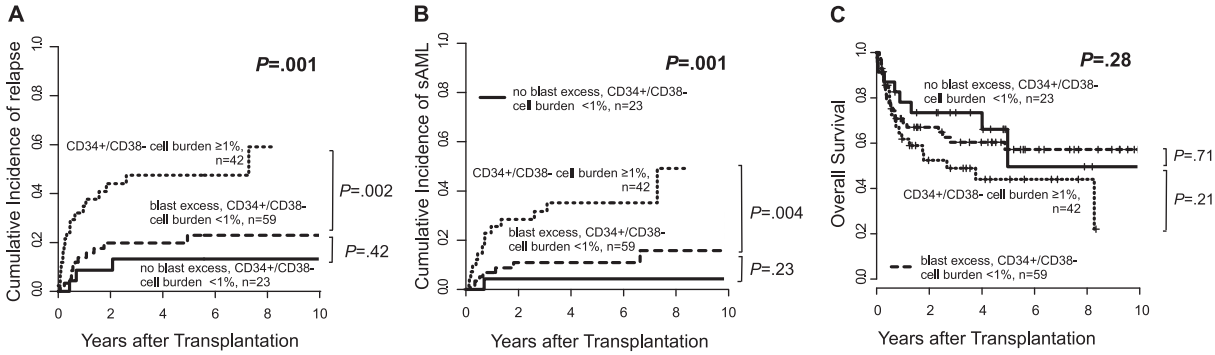

Figure 2. Outcome according to the pretreatment bone marrow $CD34^+/CD38^-$ cell burden (high versus low, 1% cut) in relation to the existence of blast excess. (A) CIR, (B) CIsAML, and (C) OS of the entire patient cohort (N = 124).

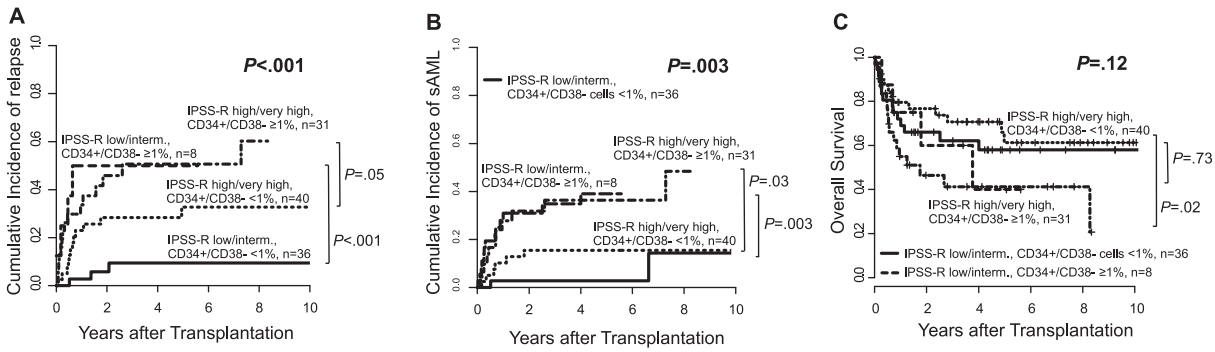

Figure 3. Outcome according to the pretreatment bone marrow $CD34^+/CD38^-$ cell burden (high versus low, 1% cut) and the IPSS-R risk group (low or intermediate versus high or very high) (n = 115). (A) CIR. (B) CIsAML. (C) OS.

contain MDS founding mutations [14,16,21], which also persisted in individuals progressing to secondary AML. Thus, MDS seems to be as clonal as AML irrespective of the blast count at MDS diagnosis [15]. MDS stem cells were also shown ineffective in reconstituting normal hematopoiesis in mice, explaining the frequently occurring cytopenias [20].

Hypomethylating agents represent the most frequently used treatment option for MDS patients but fail to eradicate the MDS founding clone [35]. In contrast, an allogeneic HSCT may have the potential to eradicate MDS stem cells [35] and remains the only curative treatment option for MDS patients [4]. In this context, identification of MDS patients with a high risk for MDS relapse or consecutive progression to secondary AML after allogeneic HSCT seems crucial to improve outcomes. In AML patients a high burden of leukemic stem cells at diagnosis is known to be associated with adverse outcomes, irrespective of chemotherapy- or HSCT-based treatment approaches [11-13]. Thus, we speculated that a high burden of MDS stem cells before initiation of therapy might also be a prognostic factor in MDS patients who receive curative treatment by applying allogeneic HSCT. Monreal et al. [28] suggested a higher incidence of disease progression in patients with a high fraction of $CD34^+$/intermediate forward-scatter/sideward-scatter/$CD38^{dim}$ cells in a cohort of 11 individuals, but no other study analyzed the prognostic impact of the $CD34^+/CD38^-$ cell burden in MDS patients. Two studies correlated the expression of immature surface antigens with MDS patient outcome. An increased risk of sAML and shorter OS was shown for a portion of more than 1% bone marrow myeloid $CD34^+$ cells alone and additionally to the IPSS-R intermediate risk group in 260 MDS patients without disease-modifying treatment [23]. A second study that excluded chronic myelomonocytic leukemia patients showed that not only a high bone marrow expression of CD34 (3% and higher) but also the immature surface antigen CD117 (5% and higher) were associated with shorter OS and leukemia-free survival [22].

Analyzing these cut-offs in our patient population, only the CD34 expression was associated with outcome (Supplementary Figure S4). However, comparison with the Bayesian information criterion identified the model including the $CD34^+/CD38^-$ cell burden as the preferred model over CD34 expression alone (Supplementary Table S4). We observed a significantly higher CIR and a strikingly higher CIsAML in patients with a high pretreatment $CD34^+/CD38^-$ cell burden but, despite a separation of the survival curve, no significantly different OS. A high bone marrow expression of $CD34^+/CD38^{dim}$ and/or high $CD34^+$ cells have been previously associated with parameters with known dismal impact on outcome as refractory anemia with excess blast MDS subtypes, higher blast counts, higher IPSS and IPSS-R risk, and worse cytogenetic risk [22,28,37]. Similar associations were observed for MDS patients with a high pretreatment $CD34^+/CD38^-$ cell burden in our study. However, in multivariable analyses the observed prognostic impact was independent from other clinic and genetic factors for all 3 analyzed endpoints. The presence of a monosomal or complex karyotype has a highly dismal prognostic impact in MDS patients [38,39] and is also associated with the presence of a high pretreatment $CD34^+/CD38^-$ cell burden in our study. In bivariable analyses of the pretreatment $CD34^+/CD38^-$ cell burden and a monosomal or complex karyotype, a high pretreatment $CD34^+/CD38$ cell burden retained its impact on CIR and CIsAML (Supplementary Tables S5 and S6).

Because of the strong association of the pretreatment $CD34^+/CD38^-$ cell burden with the presence of an excess of blasts, we also analyzed outcome according to both parameters separately. Here, a high pre-treatment $CD34^+/CD38^-$ cell burden and not the mere presence of an excess of blasts before initiation of therapy was the main prognostic factor. Finally, we evaluated whether the pre-treatment $CD34^+/CD38^-$ cell burden provided prognostic information additionally to the IPSS-R. Because of restricted patient numbers the IPSS-R low- and intermediate-risk groups and the high- and very-high-risk groups were analyzed together. Despite the strong prognostic influence of the IPSS-R in our patient group, a high pre-treatment $CD34^+/CD38^-$ cell burden identified patients with a higher CIR and CIsAML, irrespective of their IPSS-R category. Previously, our group showed in AML patients undergoing allogeneic HSCT a significantly shorter leukemia-free survival and shorter OS for individuals with a $CD34^+/CD38^-$ cell burden $\geq$ 6% at diagnosis [13]. Applying this cut-off in our MDS patients set, we observed a clear separation in all survival curves with the highest impact in CIsAML (Supplementary Figure S7). Of the seven patients with a pre-treatment $CD34^+/CD38^-$ cell burden $\geq$ 6% surviving longer than 100 days after HSCT, four developed a secondary AML after HSCT.

Because dose intensities differ between RIC and NMA-HSCT, we also performed separate analyses for both conditioning regimens. The pretreatment $CD34^+/CD38^-$ cell burden had the strongest prognostic impact in the 70 MDS patients receiving NMA-HSCT (Supplementary Figure S8D-E). Despite a separation of the CIR and CIsAML curves, outcome was not significantly different between the 54 patients with a high or a low pretreatment $CD34^+/CD38^-$ cell burden after RIC- HSCT (Supplementary Figure S8A-C). Although these analyses are restricted because of limited patient numbers, they indicate that the more intensive RIC conditioning might provide better disease control in patients with a high pretreatment $CD34^+/CD38^-$ cell burden.

Taken together, our data suggest that evaluation of the pretreatment $CD34^+/CD38^-$ cell burden provides prognostic information additionally to other prognostic factors. Our study is limited by its restriction to patients receiving allogeneic HSCT and its retrospective character, and our findings should be validated in larger prospective analyses. Also, increased expression of genes related to immature progenitors and several gene mutations have been shown to adversely influence MDS patient outcome [36,40,41]. Unfortunately, material for further molecular analyses was not available for our patient set, and further studies are needed to evaluate the pretreatment $CD34^+/CD38^-$ cell burden in the context of the mutational landscape in MDS.

In conclusion, we observed a strong association of a high pretreatment $CD34^+/CD38^-$ cell burden with high-risk disease features in MDS patients. However, evaluation of the pretreatment MDS stem cell burden by the $CD34^+/CD38^-$ cells provided independent prognostic information additionally to IPSS-R risk categories and the presence of blast excess and monosomal or complex karyotypes in patients receiving an allogeneic HSCT. Future prospective studies are needed to evaluate whether intensification of conditioning regimens in eligible patients or introducing an MDS-targeting treatment option can reduce relapse rates in patients with a high pre-treatment $CD34^+/CD38^-$ cell burden.

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SUPPLEMENTARY DATA

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