Supplemental Tables

Supplemental Table 1

IPSS-R* risk score	- Very low, low or intermediate risk
Anemic status	- Symptomatic anemia (patient-reported symptoms) - Transfusion-dependent anemia, irrespective of symptoms - Preferably, patients should be treated before the onset of permanent transfusion need
Criteria for non-recommendation	- S-Epo >500 U/L + transfusion intensity ≥ 2 units / 4 weeks

*Revised International Prognostic Scoring System

Supplemental Table 1. MDS-RIGHT indications for treatment with erythropoiesis-stimulating agents (ESAs).[1]

Supplemental Table 2

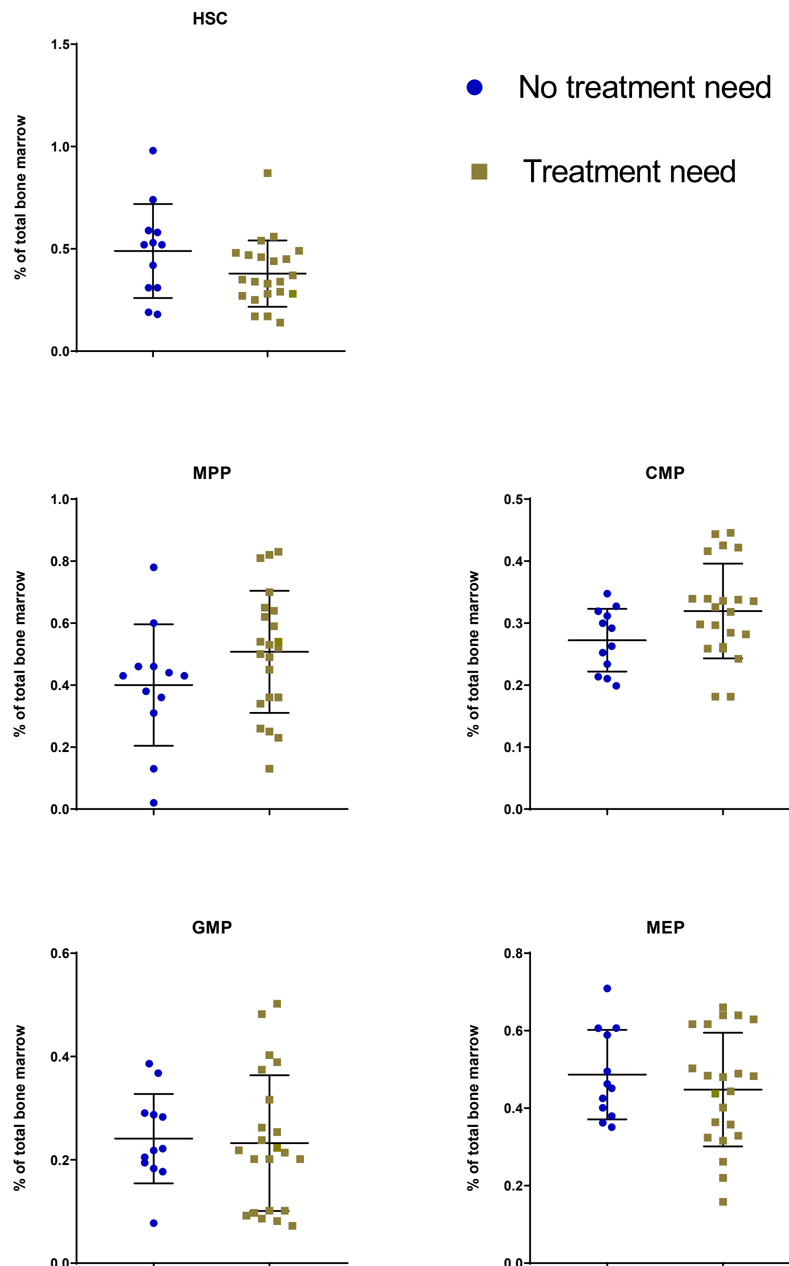
		Mutations, n (%)			
Response category	**n (%)**	**SF3B1 only**	**SF3B1 + 1**	**SF3B1 + ≥2**	**SF3B1 VAF, median (interquartile range)**
Complete response*	16 (53)	12 (71)	3 (38)	1 (20)	35 (28-41)
Partial response*	13 (43)	5 (29)	5 (62)	3 (60)	38 (29-41)
No response	1 (3)	0 (0)	0 (0)	1 (20)	38 (NA)
P†	NA	0.18			0.56

*Complete response = Hb ≥15.0 g/dL, partial response = Hb increase of ≥1.5 g/dL or achieving transfusion independency

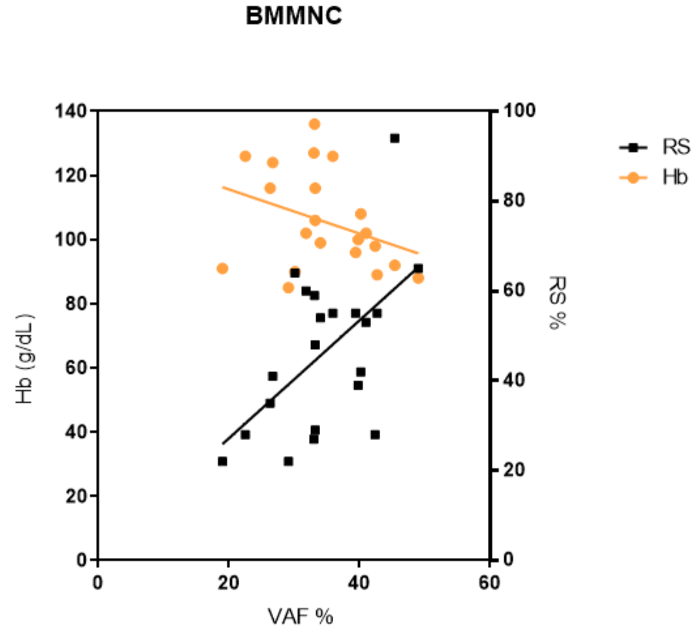
† Complete vs. partial responders; Fisher's exact test for categorical variables, Wilcoxon rank sum test for continuous variables

Supplemental Table 2. *SF3B1* mutational load and number of co-mutations are not associated with type of response to ESA in MDS-RS.

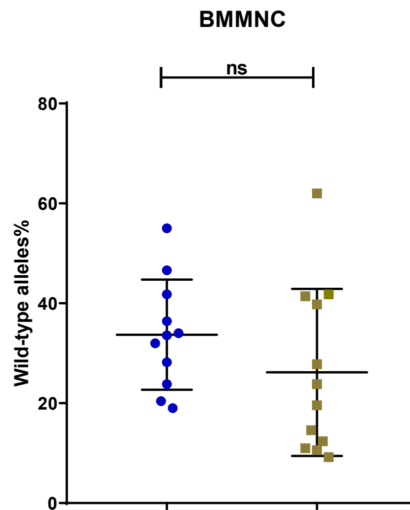
Supplemental Figure



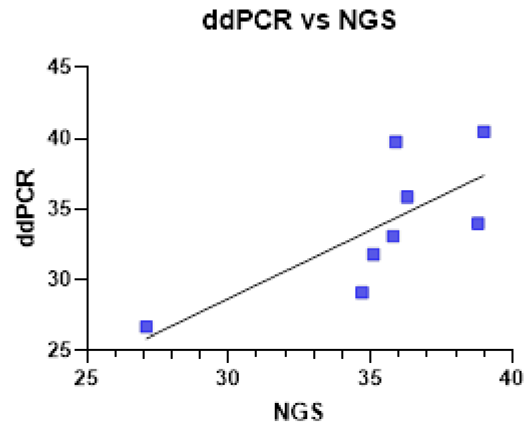
Supplemental Figure 1. Treatment need status does not impact HSPCs subset frequencies. Total bone marrow frequencies of the different HSPCs populations: hematopoietic stem cells (HSC), multipotent progenitor cells (MPP), common myeloid progenitor cells (CMP), granulocyte-macrophage progenitor cells (GMP) and megakaryocyte-erythroid progenitor cells (MEP). Comparison patients with asymptomatic anemia (no treatment need (blue)) and patients with symptomatic anemia (treatment need (yellow)). Number of patients included: no treatment need n= 12; treatment need= 22. Mann-Whitney U test was used to calculate p-values and no statistically significant differences were identified.



Supplemental Figure 2. Correlation between *SF3B1* variant allele frequency (VAF%) and ring sideroblasts (RS) and hemoglobin levels (Hb g/dl). BM ring sideroblast % increases as VAF grows (*p*= 0.008), while the relation to Hb levels is not significant (p=0.127). Numbers of investigated patients: 21. Linear regression test was used to fit the straight line and calculate statistical significance.



Supplemental Figure 3. Comparison of the proportions of the remaining wild-type allele % between patients with asymptomatic anemia (no treatment need (blue)) and patients with symptomatic anemia (treatment need (yellow)). Number of patients included: no treatment need n= 12; treatment need= 22. Whitney U test was used to calculate p-values and no statistically significant difference was identified.



Supplemental Figure 4. Correlation between ddPCR and Next-Generation Sequencing (NGS). Numbers of investigated patients: 8. Linear regression test was used to fit the straight line and calculate statistical significance.

Supplemental methods

Patient samples, flow cytometry and fluorescent-activated cell sorting (FACS)

Cryopreserved BM mononuclear cells (MNCs) or CD34-enriched cells were thawed in thawing medium (IMDM + 20% FBS + 0.1 mg/ml DNase I (Merck, Darmstadt, Germany)) and washed with Phosphate Buffered Saline (PBS, Thermo Fisher Scientific, Waltham, MA, USA) with 5% FBS before staining. Cells were sorted on a FACS ARIA II Fusion (Becton Dickinson, Franklin Lakes, NJ, USA) at the WIRM FACS facility at the Karolinska Institute, Flemingsberg. All experiments included fluorescent-minus-one (FMO) and single-stained controls.[2] A previously described strategy was used to define the HSPC populations.[3,4]

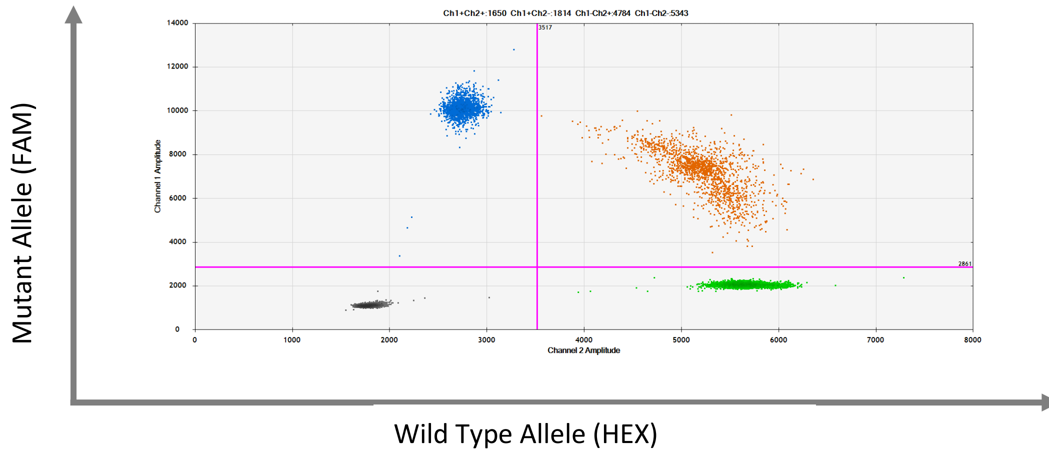
Antibody panel for hematopoietic stem and progenitor cells

Antibody	Conjugate	Clone	Supplier
CD38	TxRED	MHCD3817	Life Technologies
CD45RA	BV421	HI100	Biolegend
CD71	FITC	CY1G4	Biolegend
CD123	PECy7	6H6	Biolegend
CD90	PE	5E10	Biolegend
CD34	APC	581	Biolegend
CD2	PE-Cy5	RPA-2.10	Biolegend
CD3	PE-Cy5	HIT3a	Biolegend
CD4	PE-Cy5	RPA-T4	Biolegend
CD7	PE-Cy5	CD7-6B7	Biolegend
CD8	PE-Cy5	RPA-T8	Biolegend
CD10	PE-Cy5	HI10a	Biolegend
CD11b	PE-Cy5	ICRF44	Biolegend
CD14	PE-Cy5	RMO52	Beckman Coulter
CD19	PE-Cy5	HIB19	Biolegend
CD20	PE-Cy5	2H7	Biolegend
CD56	PE-Cy5	B159	BD Biosciences
CD235a	PE-Cy5	HIR2	Biolegend
7AAD	PE-Cy5		eBiosciences

Droplet digital PCR (ddPCR)

PCR reaction mixtures were prepared according to the manufacturer's protocols using the Supermix for Probes without dUTP (Bio-Rad, Berkeley, CA, USA), respective assay, 20 ng of DNA and nuclease-free water (Life Technologies, Carlsbad, CA, USA). 20 µl of PCR reaction mixture was transferred to a sample well in a disposable droplet generator cassette (Bio-Rad); 70 µl of droplet generation oil (Bio-Rad) was then loaded into the oil well for each channel and the cassette loaded into a QX200 Droplet Digital PCR System (Bio-Rad). The droplets were then transferred to a 96-well PCR semi-skirted plate (Eppendorf, Hamburg, Germany). Emulsified PCR reactions were run on a C1000 Touch thermal cycler (Bio-Rad) incubating the plates at 95°C for 10 min followed by 40 cycles of 94°C for 30 seconds and 55°C for 60 seconds, 10 minutes incubation at 98°C, and 4°C for 10 minutes. Each well was then read using the QX200 Droplet Reader (Bio-Rad). Two-dimensional plots displaying measured HEX (wild type) and FAM (mutant) fluorescent signal for each droplet show four main populations; droplets containing no amplified DNA and no fluorescent signal (lower left quadrant; HEX-FAM-), droplets with only mutant DNA and high FAM fluorescent signal (upper left quadrant), droplets with only wild-type DNA and high

HEX fluorescent signal (lower right quadrant) and droplets with both wild-type and mutant amplified DNA and high FAM and HEX fluorescent signal (upper right quadrant). QuantaSoft software (Bio-Rad) was used to calculate the VAF of the mutated allele based on Poisson distribution. We included at least one known mutated sample, one wildtype sample and one H_2O sample as controls in every run. All the primers and probes were designed and ordered from Bio-Rad using available online tools.



Representative ddPCR 2D plot for quantification of the presence of *SF3B1* E622D bulk BM MNC isolated from peripheral blood from a selected patient. Numbers in graph quadrants show threshold value, events in top left and top right quadrants are included to support detection of mutant allele. FAM: Mutant allele, HEX: Wild type allele. In this study we aimed to analyzed using ddPCR a maximum of 1000 cells per RepliG-amplified sample, with a minimum number of (70) cells used equaling a LoD (level of detection) of (1/70) %.

References

1. Therapeutic options for MDS, approved by EMA or part of standard care. (Accessed Oct 1, 2020, at https://mds-europe.org/management/treatment/mds-right/therapeutic_options/approved.)
2. Maecker HT, Trotter J. Flow cytometry controls, instrument setup, and the determination of positivity. Cytometry Part A 2006;69A:1037-42.
3. Woll PS, Kjallquist U, Chowdhury O, et al. Myelodysplastic syndromes are propagated by rare and distinct human cancer stem cells in vivo. Cancer Cell 2014;25:794-808.
4. Tehranchi R, Woll PS, Anderson K, et al. Persistent malignant stem cells in del(5q) myelodysplasia in remission. N Engl J Med 2010;363:1025-37.