Supplementary Information for

The overexpression of *WT1* and *PRAME* predicts poor outcomes of myelodysplastic syndromes patients with thrombocytopenia

Table of Contents

Table S1. International working group response criteria of MDS

Category	Response criteria (response must last 4 weeks)
Complete remission (CR)	Bone marrow: ≤5% myeloblasts with normal maturation of all cell lines
	Persistent dysplasia will be noted
	Peripheral blood
	Hemoglobin ≥11g/dL
	Platelets ≥ 100 x10^9/L
	Neutrophils ≥ 1.0 x10^9/L
	Blasts 0%
Progression	For patients with:
	Less than 5% blasts: ≥ 50% increase in blasts to > 5% blasts
	5%-10% blasts: ≥ 50% increase to >10% blasts
	10%-20% blasts: ≥50% increase to > 20% blasts
	20%-30% blasts: ≥50% increase to > 30% blasts
	Any of the following:
	At least 50% decrement from maximum remission/response in granulocytes or platelets
	Reduction in hemoglobin by ≥ 2 g/dL
	Transfusion dependence
Survival	Endpoints
	Overall survival: death from any cause
	PFS: disease progression or death from MDS

Table S2. *WT1* and *PRAME* transcript levels among three groups

	WT1 transcript levels	*PRAME* transcript levels
MDS with isolated thrombocytopenia	1.80 (0.29-5.50)	1.10 (0.14-19.70)
MDS with bicytopenia	1.70 (0.30-7.90)	1.05 (0.18-20.75)
MDS with pancytopenia	2.80 (0.64-10.50)	0.97 (0.24-19.08)

Data are median (IQR). MDS: myelodysplastic syndromes. *WT1*: Wilms tumor 1. *PRAME*: Preferentially expressed antigen of melanoma.

Table S3. Clinical profile of MDS patients with thrombocytopenia in low risk

Feature	Low-favorable group N=53	Low-adverse group N=50	*P*
Age at diagnosis (years)	50.0(30.5-65.0)	52.5(36.7-64.2)	0.502
Sex			0.896
Female	29(54.7%)	28(56.0%)	
Male	24(45.3%)	22(44.0%)	
Cytopenias			
HGB (g/dL)	103(88-115）	107(95.75-120）	0.443
ANC ($\times 10^9$/L)	2.14(1.20-3.09)	1.99(1.21-2.53)	0.476
Platelet ($\times 10^9$/L)	39.0(24.0-50.5)	56.0(33.3-76.0)	0.855
Severe thrombocytopenia	15(28.3%)	9(18.0%)	0.216
Bone marrow blasts (%)	1.0(0-2.0)	2.0(1.0-2.6)	0.099
WHO classification			0.175
MDS-SLD	15(28.3%)	18(36.0%)	
MDS-MLD	2(3.8%)	7(14.0%)	
MDS-RS	0	0	
5q-	0	0	
MDS-EB-1	4(7.5%)	3(6.0%)	

MDS-EB-2	0	0	
MDS-U	32(60.4%)	22(44.0%)	
Cytogenetic risk			0.527
Very good	1(1.9%)	0	
Good	42(79.2%)	39(78.0%)	
Intermediate	9(17.0%)	11(22.0%)	
Poor	1(1.9%)	0	
Very poor	0	0	
AML evolution	2(3.8%)	3(6.0%)	0.601

Data are n (%) or median (IQR). HGB: hemoglobin. ANC: absolute neutrophil count. WBC: white blood cell. MDS-SLD: MDS with single-lineage dysplasia. MDS-MLD: MDS with multilineage dysplasia. MDS-RS: MDS with ring sideroblasts. MDS-EB: MDS with excess blasts. MDS-U: MDS-unclassifiable. 5q-: MDS with isolated del(5q).

Table S4. Characteristics of allo-HSCT patients

Feature	Patients N=124
Age (years)	39(28, 50)
Sex (M/F)	
Male	78(62.9%)
Female	46(37.1%)
Interval from diagnosis to HSCT mo,	
Median (IQR)	
S-AML, n(%)	64(51.6%)
WHO subtype, n(%)	
MDS-SLD	10(8.1%)
MDS-MLD	4(3.2%)
MDS-RS	0
5q-	0
MDS-EB-1	34(27.4%)
MDS-EB-2	61(49.2%)
MDS-U	15(12.1%)
Cytogenetics, n(%)	
Very good	0
Good	65(52.4%)
Intermediate	37(29.8%)
Poor	12(9.7%)
Very poor	10(8.1%)
IPSS-R	
Intermediate	24(19.3%)
High	47(37.9%)
Very high	53(42.7%)
Patient-donor sex matching, n(%)	

M-M	40(32.3%)
M-F	37(29.8%)
F-F	20(16.1%)
F-M	27(21.8%)
HLA match, n(%)	36(29.0%)
Donor relation, n(%)	
MUD	1(0.8%)
MMURD	0
MRD	34(27.4%)
MMRD	89(71.8%)

Data are n (%) or median(IQR). M: male. F: female. S-AML: secondary acute myeloid leukemia. WHO: World Health Organization. MDS-SLD: MDS with single lineage dysplasia. MDS-MLD: MDS with multilineage dysplasia. MDS-RS: MDS with ring sideroblasts. MDS-EB: MDS with excess blasts. MDS-U: MDS-unclassifiable. 5q-: MDS with isolated del(5q). IPSS-R: Revised International Prognostic Scoring System. MRD: matched related donor. MURD: matched unrelated donor. MMRD: mismatched related donor. MMURD: mismatched unrelated donor.

Table S5. Characteristics of MDS patients undergoing chemotherapy and HMA

Feature	Patients N=50
Age (years)	59(49.8, 64.0)
Sex	
Male	32(64.0%)
Female	18(36.0%)
S-AML, n(%)	37(74.0%)
WHO subtype, n(%)	
MDS-SLD	1(2.0%)
MDS-MLD	2(4.0%)
MDS-RS	0
5q-	0
MDS-EB-1	11(22.0%)
MDS-EB-2	34(68.0%)
MDS-U	2(4.0%)
Cytogenetics, n(%)	
Very good	0
Good	26(52.0%)
Intermediate	11(22.0%)
Poor	3(6.0%)
Very poor	10(20.0%)
IPSS-R	
Intermediate	8(16.0%)
High	17(34.0%)
Very high	25(50.0%)
Chemotherapy and HMA	
HMA	33(66.0%)
Chemotherapy	17(34.0%)

Data are n (%) or median(IQR). M: male. F: female. S-AML: secondary acute myeloid leukemia. WHO: World Health Organization. MDS-SLD: MDS with single lineage dysplasia. MDS-MLD: MDS with multilineage dysplasia. MDS-RS: MDS with ring sideroblasts. MDS-EB: MDS with excess blasts. MDS-U: MDS-unclassifiable. 5q-: MDS with isolated del(5q). IPSS-R: Revised International Prognostic Scoring System.

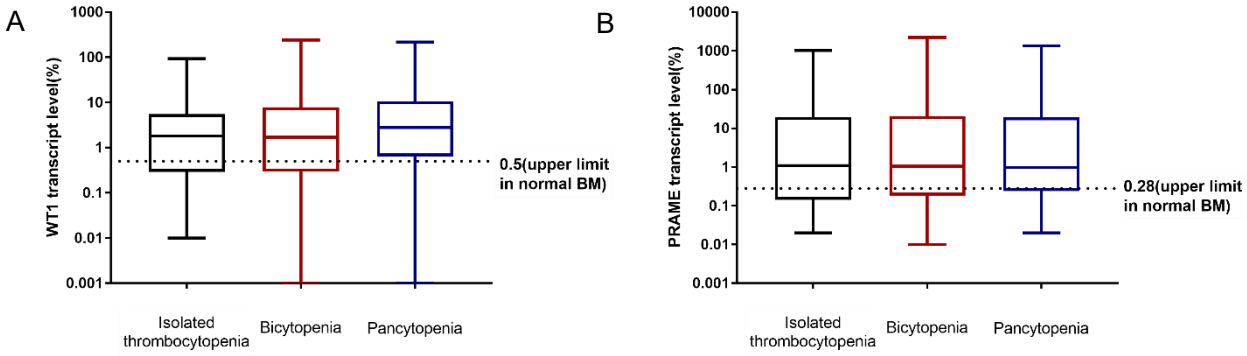

Figure S1. Majority patients in all three groups had a higher *WT1* (A) and *PRAME* (B) transcript level than the normal range. Additional lines (at 0.5 for *WT1* and 0.28 for *PRAME*) were the upper limit of *WT1* and *PRAME* transcript levels from normal bone marrow.

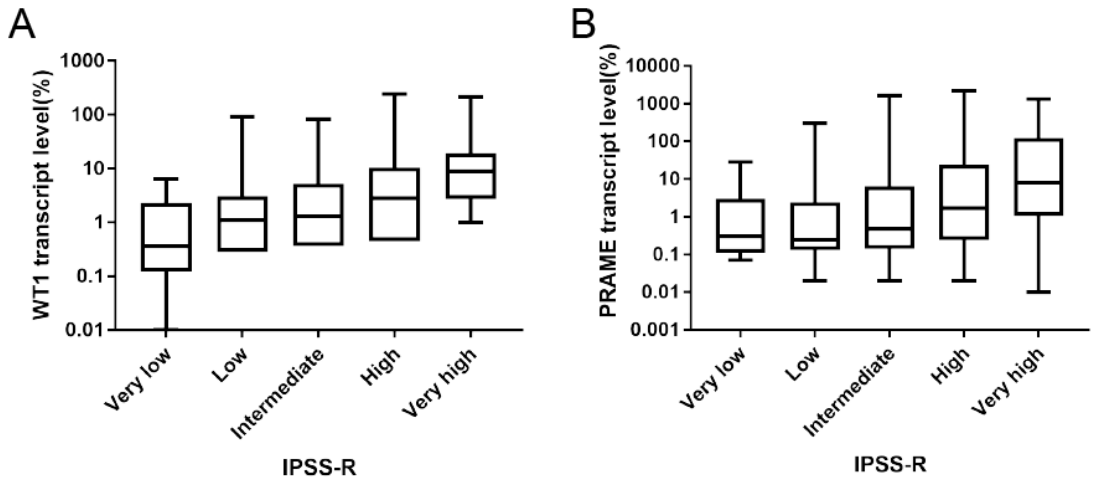

Figure S2. *WT1* (A) and *PRAME* (B) transcript levels according to IPSS-R. The transcript levels of *WT1* and *PRAME* were higher in the higher-risk group compared with those in the lower-risk group (p=0.000 for *WT1*, p=0.001 for *PRAME*).

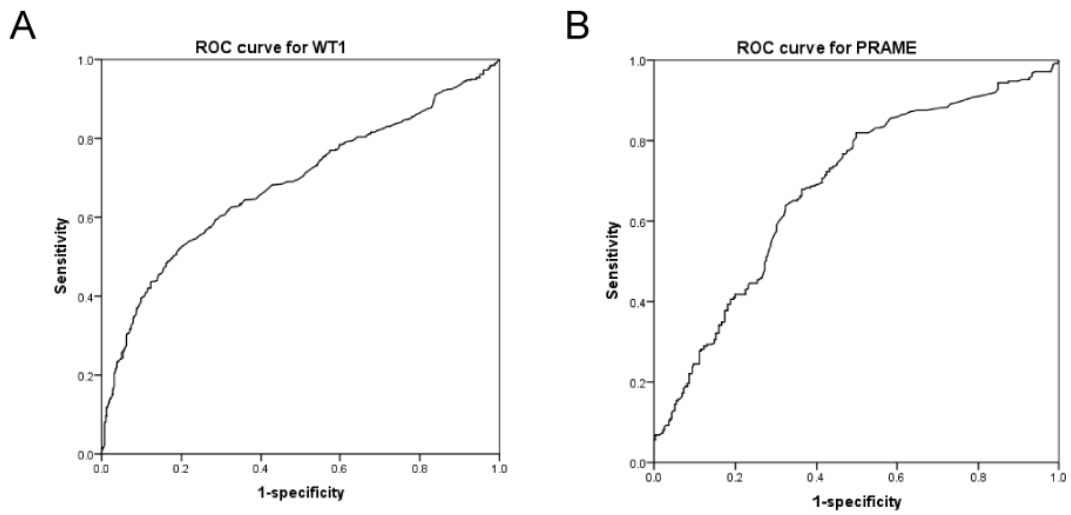

Figure S3. ROC curve analysis of *WT1* (A) and *PRAME* (B) gene transcript levels and the IPSS-R higher risk rate.

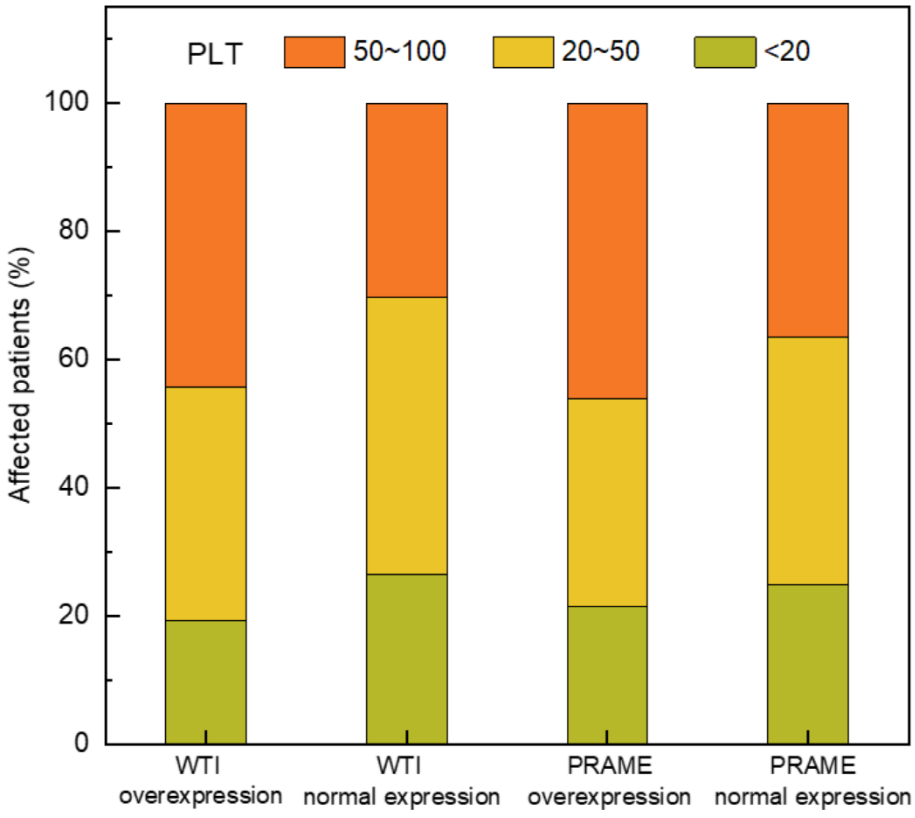

Figure S4. Proportions of patients with *WT1* and *PRAME* transcript levels according to platelet count.

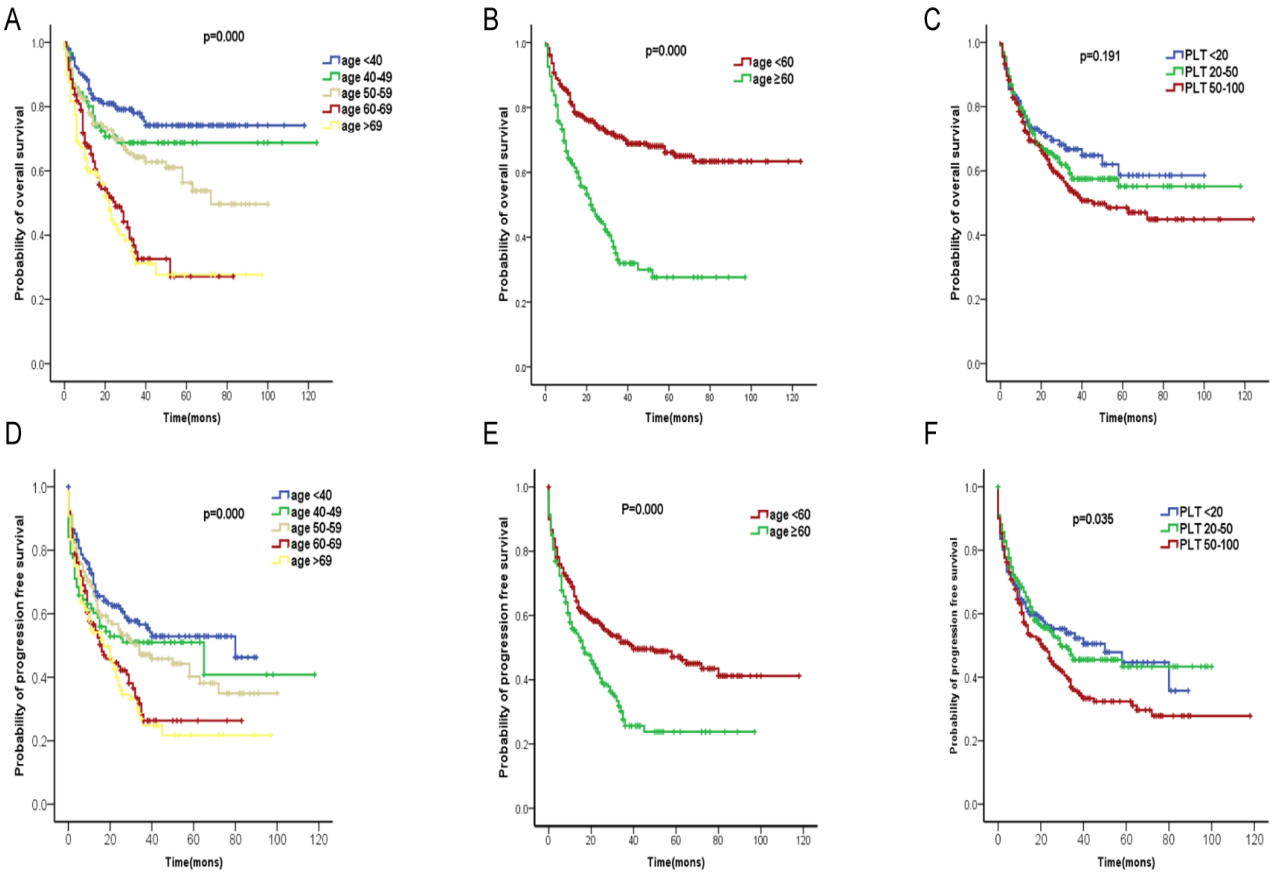

Figure S5. OS and PFS of MDS patients according to age group and degree of thrombocytopenia. (A) OS of MDS patients based on age group. (B) OS of MDS patients with age <60 years old compared with older patients. (C) OS of MDS patients based on degree of thrombocytopenia. (D) PFS of MDS patients based on age group. (E) PFS of MDS patients with age <60 years old compared with older patients. (F) PFS of MDS patients based on degree of thrombocytopenia.

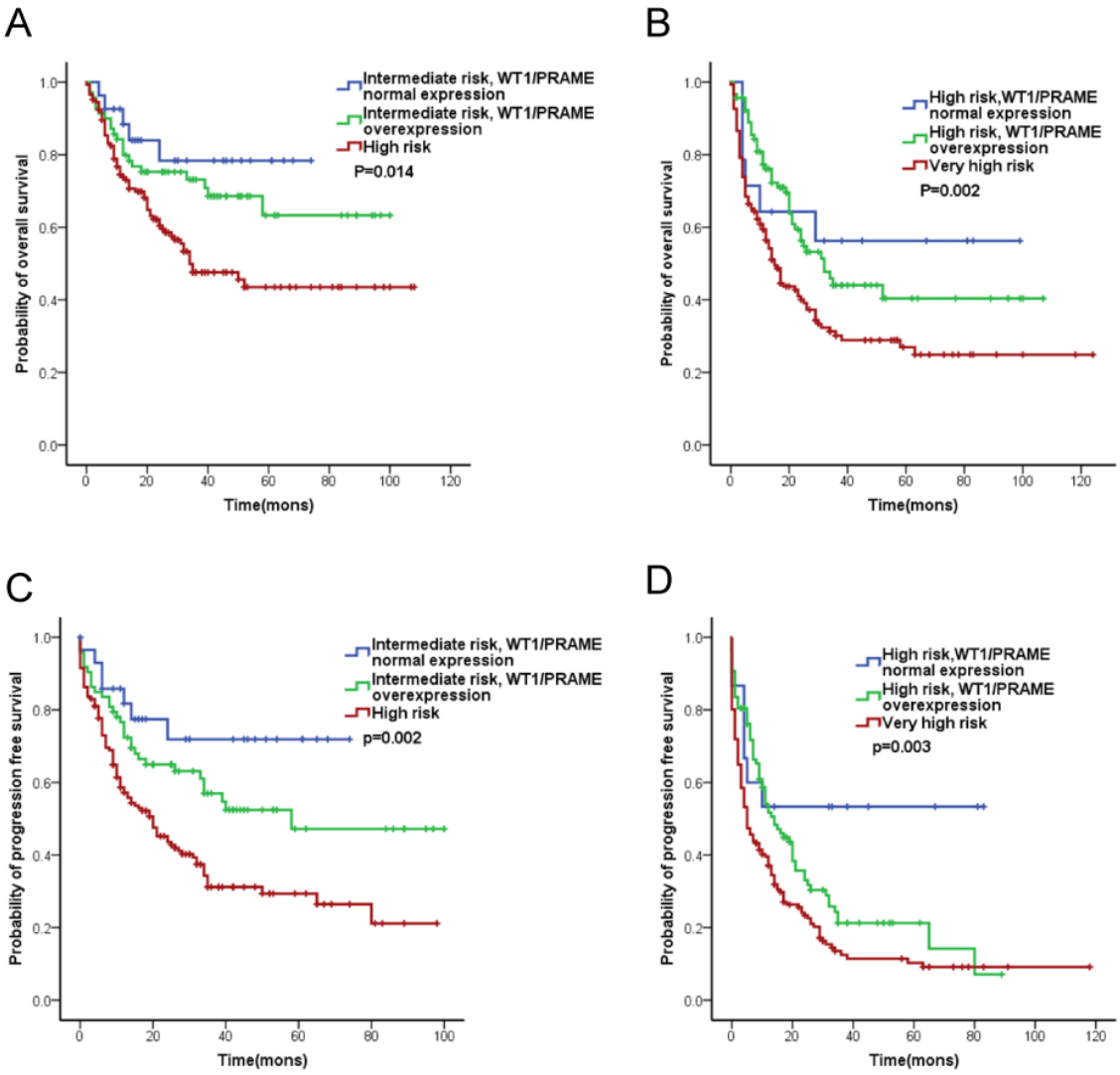

Figure S6. OS and PFS of MDS patients according to IPSS-R and *WT1* and *PRAME* transcript levels. (A-D) OS of MDS patients according to the presence and absence of *WT1* or *PRAME* overexpression and according to IPSS risk group. OS (A-B) and PFS (C-D) of MDS patients in the next-highest IPSS risk group are included for the purpose of comparison. P values were calculated between MDS patients with *WT1*/*PRAME* overexpression for the given IPSS-R risk group and those in the next-highest IPSS risk group. The patients were not enough to calculate p value in very low risk and very high risk group.