Characteristics and outcomes associated with CD2 and CD25 expression on bone marrow mast cells in patients with systemic mastocytosis

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Online supplementary

Supplemental Table 1: demographic, clinical and laboratory characteristics of mastocytosis patients in the discovery cohort.

	Total	**Non-advanced[1]**	**Advanced[2]**	**p-value**
	N = 81	**N = 54**	**N = 27**	
Age (years); median [IQR]	51 [23]	47 [22]	60 [26]	**0.003[5]**
Sex (male); n (%)	39 (48%)	21 (39%)	18 (67%)	**0.02[6]**
WHO classification; n (%)				-
ISM	54 (67%)	54 (100%)		
ASM	15 (18%)		15 (55%)	
SM-AHN[3]	8 (11%)		8 (30%)	
MCL	2 (2%)		2 (7%)	
MCS	2 (2%)		2 (7%)	
Phenotype[4]; n (%)				**0.003[6]**
Detectable	**73 (86%)**	**50 (86%)**	**23 (85%)**	
CD2+	58/73 (79%)	45/50 (90%)	13/23 (57%)	
CD2-	15/73 (21%)	5/50 (10%)	10/23 (43%)	
CD2+/CD25+	58/73 (79%)	45/50 (90%)	13/23 (57%)	
CD2+/CD25-	0/73 (0%)	0/50 (0%)	0/23 (0%)	
CD2-/CD25+	10/73 (14%)	4/50 (8%)	6/23 (26%)	
CD2-/CD25-	5/73 (7%)	1/50 (2%)	4/23 (17%)	
Undetectable	**12 (14%)**	**8 (14%)**	**4 (15%)**	
***KIT* genotype;** n (%)				0.13[6]
TKD-mutations	66 (82%)	45 (83%)	21 (77%)	
wild type	13 (16%)	9 (17%)	4 (15%)	
non-TKD mutations	2 (2%)	0 (0%)	2 (7%)	
Death; n (%)				**<0.001[7]**
Yes	11 (14%)	1 (2%)	10 (37%)	
No	70 (86%)	53 (98%)	17 (63%)	

Supplemental table 1: WHO: World Health Organization. SM: systemic mastocytosis. ISM: indolent systemic mastocytosis. ASM: aggressive systemic mastocytosis. SM-AHN: systemic mastocytosis with an associated hematological neoplasm. MCL: mast cell leukemia. MCS: mast cell sarcoma. [1]Non-advanced = ISM. [2]Advanced = ASM, MCL, MCS, SM-AHN. [3]The AHN diagnoses were chronic myelomonocytic leukemia (n=2), myelodysplasia (n=2), myeloproliferative neoplasia (n=1), acute myeloid leukemia (n=1), hairy cell leukemia (n=1), and lymphoplasmacytic lymphoma (n=1). **[4]**Including 4 patients with partial expression of CD2 and 2 patients with bimodal expression of CD2. [5]Wilcoxon's rank sum test. [6]Pearson's chi-

squared test [7]Fisher's exact test. Data were quoted as the median [interquartile range (IQR)] for continuous variables and the frequency (percentage) for categorical variables. Groups were compared in a non-parametric Wilcoxon test for continuous variables and in a Chi-squared or Fisher's exact test (as appropriate) for categorial variables. The threshold for statistical significance was set to $p<0.05$. All *KIT* sequencing in the discovery cohort was performed by sequencing from RNA extraction as reported in Polivka et al., JACI 2024. Immunophenotyping was performed using anti-CD2 (clone L303.1) and anti-CD25 (clone 2A3) antibodies.

Supplemental Table 2: Univariable and multivariable analyses of OS after midostaurin initiation, according to the WHO diagnostic classification, hemoglobin level, platelet count, CD2 expression, and number of *S/A/R* mutations.

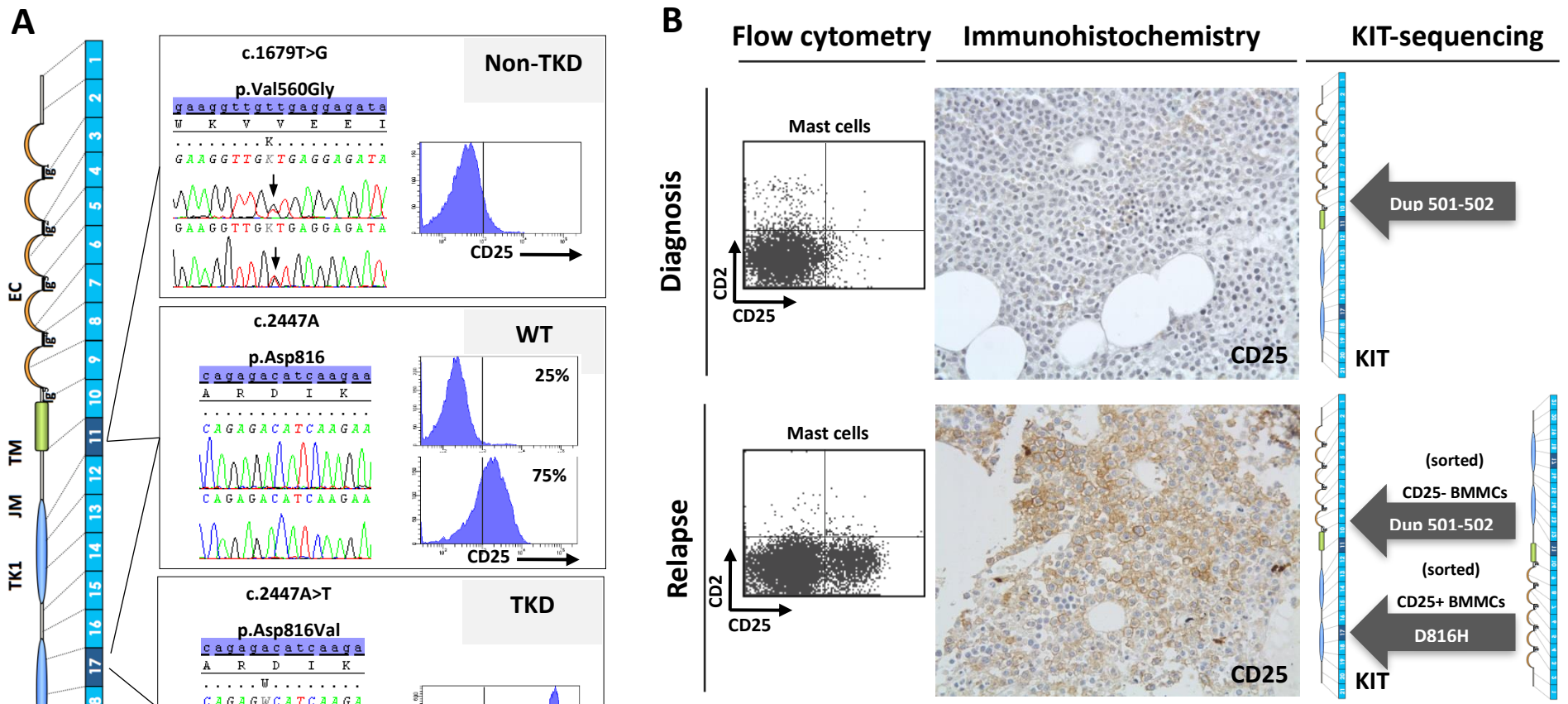
		Univariable analysis				Multivariable analysis			
Characteristic	**N**	**HR**[1]	**95%CI**[1]	**p-value**	**q-value**[2]	**N**	**HR**[1]	**95%CI**[1]	**p-value**
WHO classification				**<0.001**	<0.001				0.11
ASM	27	—	—			17	—	—	
MCL	9	8.77	2.96, 26.0			3	4.38	0.42, 45.2	
SM-AHN	75	3.43	1.61, 7.30			62	2.96	0.89, 9.87	
Hemoglobin (g/dL)	110	0.79	0.68, 0.90	**<0.001**	0.003	82	0.85	0.71, 1.02	0.069
Platelet count (x10^9/L)	111	1.0	0.99, 1.00	**<0.001**	0.002	82	0.99	0.99, 1.00	**0.005**
CD2 expression				**0.010**	0.084				0.9
No	53	—	—			36	—	—	
Yes	58	0.51	0.30, 0.86			46	0.96	0.48, 1.92	
SRSF2/ASXL1/RUNX1 mutations				**0.032**	0.3				0.068
S/A/R 0	42	—	—			42	—	—	
S/A/R 1	25	2.59	1.29, 5.22			25	2.26	1.08, 4.75	
S/A/R >= 2	15	1.60	0.65, 3.94			15	0.96	0.37, 2.46	
Alkaline phosphatase > ULN				0.4	>0.9				
No	24	—	—						
Yes	48	1.32	0.70, 2.51						
Tryptase ≥ 200 ng/mL				0.8	>0.9				
No	62	—	—						
Yes	47	0.93	0.55, 1.57						
Leukocyte count (x10^9/L)	108	1.01	0.98, 1.04	0.6	>0.9				

Supplemental table 2: WHO: World Health Organization. ASM: aggressive systemic mastocytosis. MCL: Mast cells leukemia. SM-AHN: systemic mastocytosis with an associated hematological neoplasm. *S/A/R*: *SRSF2/ASXL1/RUNX1*. ULN: upper limit of normal. [1]HR: hazard ratio; CI: confidence interval. [2]Bonferroni correction for multiple testing. We used Cox proportional hazard models to investigate prognostic factors and the strength of associations with OS. We first selected explanatory variables with p<0.2 in a univariable analysis. Given

the risk of a type I error, we also reported the Bonferroni correction as a q-value. Next, we included the variables as prognostic factors in multivariable models. Both univariable and multivariable estimates of the hazard ratio (HR) [95%CI] were reported. The multivariable models' assumptions were checked by plotting the Schoenfeld residuals.

Supplemental Figure 1: Correlation between CD25 expression and the mast cell genotype in patients with systemic mastocytosis.

(A) Distribution of the CD25 expression pattern and immunogenetic *KIT* status in a series of 73 patients. **(B)** An illustrative case of MCL with a $CD25^-$ immunophenotype and a Dup 501-502 *KIT*-genotype at diagnosis. The disease showed a clonal evolution at relapse, with the emergence of a D816H mutation (in addition to the Dup 501-502 abnormality) associated with the start of partial CD25 expression (detected in both flow cytometry and immunochemistry experiments). The D816H *KIT* mutation segregated with the $CD25^+$ BMMC compartment after electronic sorting and was absent from the $CD25^-$ BMMC population.



Antigen expression	TKD mutation	Absence of TKD mutation	
	D816	JM mutations	WT
CD25-	-	2 / 2 (100%) **	3/12 (25%) ***
CD25+	58/58 (100%)*	-	9/12 (75%)

* 1 case harboring a D816H mutation
** 1 MCL and 1 MCS
*** 1 ISM, 1 SM-AHN, 1 MCL