Supplementary file to

Prognostic impact of expression of CD2, CD25, and/or CD30 in/on mast cells in systemic mastocytosis: a registry study of the ECNM

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BACKGROUND EUROPEAN COMPETENCE NETWORK ON MASTOCYTOSIS (ECNM)

The ECNM was established in 2002 as a non-profit network of an international group of scientists and clinicians working in the field of mastocytosis. As a collaborative initiative and scientific network of participating centers in Europe, it includes Reference Centers and Centers of Excellence [1,2]. There is a close collaboration of the members of the ECNM in order to discuss basic research as well as clinical concepts on diagnosis, prognostication and treatment of various forms of mast cell (MC) diseases with the aim to advance the field in this rare disease, involving specialists from different medical disciplines. With such an interdisciplinary approach connecting various medical fields and their specialists, including basic researchers, dermatologists, allergologists, hematologists or pathologists, all aspects of MC diseases are covered in the ECNM. Within the ECNM network, researchers and physicians take care of patients with various MC disorders, such as cutaneous and systemic mastocytosis (SM) as well as mast cell activation syndromes (MCAS). Moreover, genetic anomalies, such as hereditary alpha tryptasemia (HαT), are subject of research in ECNM projects. The members of the ECNM are organizing annual meetings with the aim of providing new information about MC diseases, including diagnostics and management strategies involving new therapeutic options for treatment of mastocytosis. These annual meetings are not only providing a platform for discussion and exchange of ideas and new developments in the field but are also offering new information for patient advocacy groups. Within the ECNM, active centers also promote education and the development of standards and optimal patient care and facilitate referral to medical experts and specialized centers in the field. A valuable source of all relevant information is the ECNM homepage at the Medical University of Vienna, Austria.

THE ECNM REGISTRY

Introduction

The establishment of the ECNM registry has been an initiative of participating ECNM centers in 2012, aimed at creating a large set of data obtained from patients with various MC diseases. Patients with cutaneous mastocytosis as well as patients with SM have been enrolled since 2012. SM patients include cases with non-advanced forms, such as bone marrow mastocytosis (BMM), indolent SM (ISM), or smoldering SM (SSM), and advanced forms, such as aggressive SM (ASM), SM with an associated hematologic neoplasm (SM-AHN), and mast cell leukemia (MCL). Further aims of the ECNM registry are to obtain data on the prevalence, etiology, diagnosis, prognosis and the clinical course of all forms of MC diseases as well as additional potentially relevant disorders (co-morbidities) [3].

To achieve these goals and evaluate data, several ECNM registry projects have been launched, each of these projects focusing on a certain research topic.

Collecting Data in the ECNM Registry

The ECNM registry is a web-based data base incorporating laboratory and clinical data of patients with various MC diseases [3]. The majority of patients included in the ECNM registry are seen and managed in the various Centers of Excellence of the ECNM. Data are collected both retro- and prospectively and are entered in the ECNM registry either by the clinical research coordinator (CRC) or the local principle investigator (PI) of such a center. Patient data are pseudonymized using a unique code for each patient ensuring that identification of a patient is only possible for the CRC or the PI of the local center. To ensure high quality data a regular data check is performed, there is a data clearing once yearly, and technical adjustments are implemented as needed. Queries are sent to the participating centers in case of inconsistencies or ambiguities. The Medical University of Vienna, Austria, is the sponsor of the ECNM registry.

Participating Centers and ECNM Registry Projects

There is an ECNM registry board consisting of the coordinators of the ECNM registry at the Medical University of Vienna, Austria, and the local PI of the actively participating center. The ECNM registry projects are based on a registry study and a related contract between the sponsor and the participating centers where rights and obligations are defined. In fact, this consortium contract defines the distribution, conduct, and development of ECNM registry projects in various participating centers. Each actively participating center is invited to conduct one or more ECNM registry projects. The contract also defines the role of the participating centers, collaborations within the ECNM registry projects as well as details concerning publications. Participating centers are encouraged to conduct one or more ECNM registry project and to publish the results.

Inclusion and Exclusion Criteria in the ECNM Registry

All patients with confirmed mastocytosis, based on the classification of the World Health Organization (WHO), are potential candidates for inclusion in the ECNM registry [4,5]. This includes patients with cutaneous mastocytosis, SM, and mast cell sarcoma. Patients in whom mastocytosis is not confirmed or patients with mast cell activation syndromes (MCAS) without mastocytosis are excluded. There are no age restrictions to include patients in the ECNM registry. In other words, pediatric and adult patients with confirmed mastocytosis at any age can be included. Patients with mast cell infiltrates in the skin but no bone marrow examination, who have a provisional diagnosis of mastocytosis in the skin (MIS), can also be included in the ECNM registry [6]. Patients with or without allergies and patients with or without anaphylaxis can be included in the ECNM registry, provided that they are suffering from confirmed mastocytosis.

Approval of Ethics Committee

A written informed consent provided by the patient is a prerequisite for inclusion in the ECNM registry. That means that all patients in the ECNM registry provided their written informed consent to be included in the ECNM registry. The ECNM registry study was approved by the responsible local ethics committee of the participating center.

SUPPLEMENTARY METHODS

Determining associations between surface marker expression and disease-related parameters

Both, clinical and laboratory parameters, were analyzed with regard to a possible association with the expression of CD2, CD25, and/or CD30 in clonal MC in our SM patients. Laboratory parameters had to be comparable to analyze them in a statistical valuable approach. Laboratory values included basal serum tryptase, *KIT* D816V mutation, other activating *KIT* mutations, as well as chromosomal aberrations distinguishing between normal (46,XX and 46,XY) and abnormal (all other) karyotype.

Statistical analysis

Kaplan-Meier curves for overall survival, progression-free survival and event-free survival were constructed and stratified by expression patterns of CD2, CD25, and CD30 [7]. In the log-log transformed survival curves of patients with CD2-positive MC und CD2-negative MC with indolent systemic mastocytosis (ISM), smoldering systemic mastocytosis (SSM) and advanced systemic mastocytosis (advSM) both curves do not cross, therefore the risk of having an event does not significantly change over time.

A log-rank test was employed to assess differences in survival time among ISM, SSM and advSM.

P-values below 0.05 were considered to be statistically significant.

The assumption of proportional hazards was tested by visual inspection of the log-log plots.

Multivariate analyses were conducted using CD2 expression of MC, CD25 expression of MC, age at inclusion and sex.

Supplemental Table S1

Variance inflation factors (VIF) in the variables CD2 expression of MC, CD25 expression of MC, age and sex.

Variable	Variance Inflation Factor (VIF)
CD2 expression of MC	1.08
CD25 expression of MC	1.09
Age at inclusion	1.01
Sex	1.0

Multicollinearity was checked visually and by calculating the VIF listed in the table. There was no evidence of multicollinearity (all VIF < 5).

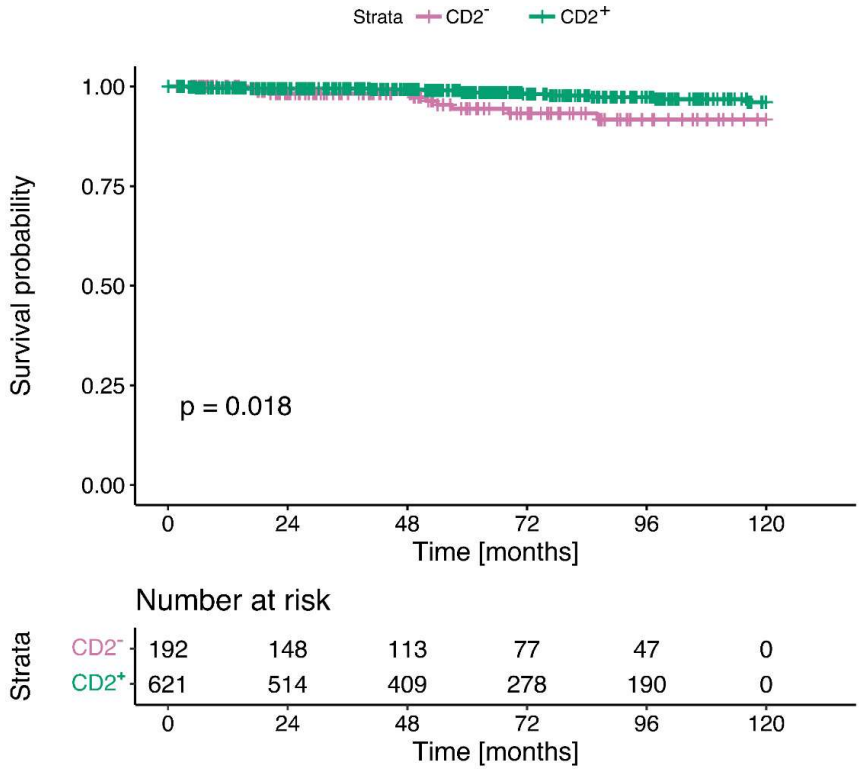
SUPPLEMENTARY RESULTS

***Overall survival* (OS) in patients with SM stratified by CD2 expression pattern in three groups of MC disorders, indolent systemic mastocytosis (ISM), smoldering systemic mastocytosis (SSM) and advanced systemic mastocytosis (advSM), is depicted in the Supplementary Figures S1 a, b, c.**

Supplemental Figures S1 a, b, c.

Figure S1 a.

OS in patients with ISM stratified by CD2 expression pattern in MC



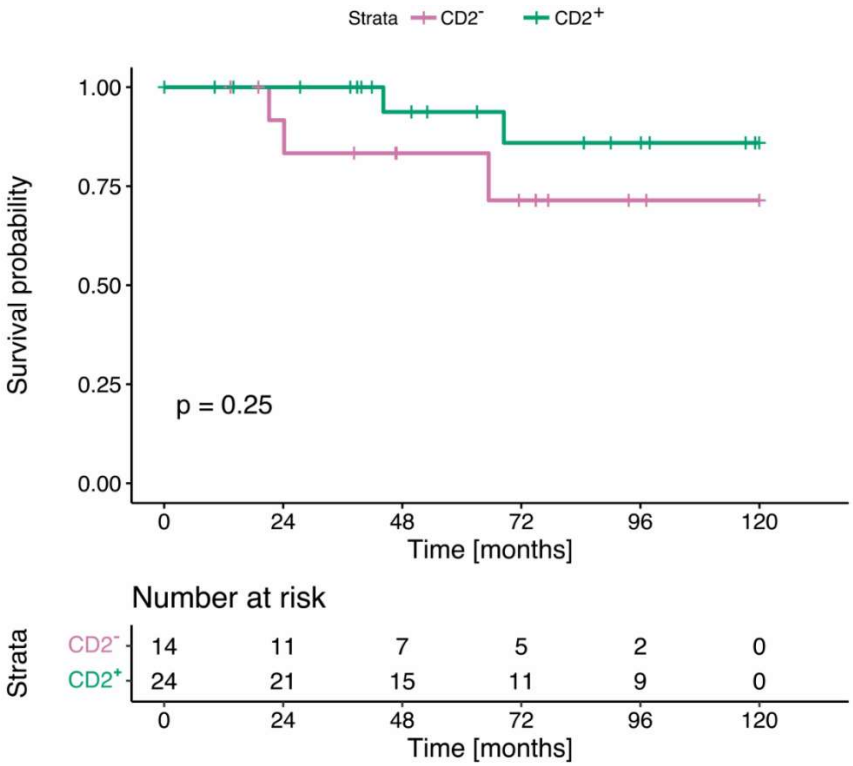
OS was analyzed in a univariate analysis in patients with *indolent systemic mastocytosis* in whom FU and survival data were reported and results by flow cytometry and/or immunohistochemistry for CD2 on/in MC were available. Two groups of patients were examined based on the pattern of CD2 expression on/in MC: CD2 negativity and CD2 positivity. The probability of OS in these two groups of patients was determined according to the method of Kaplan and Meier.

In patients with ISM there was a statistically significant reduction in OS with lack of expression of CD2 (p=0.018). The p-value refers to the comparison of survival curves as assessed by log-rank test.

Abbreviations: OS, overall survival; ISM, indolent systemic mastocytosis, MC, mast cell; FU, follow-up.

Figure S1 b.

OS in patients with SSM stratified by CD2 expression pattern in MC

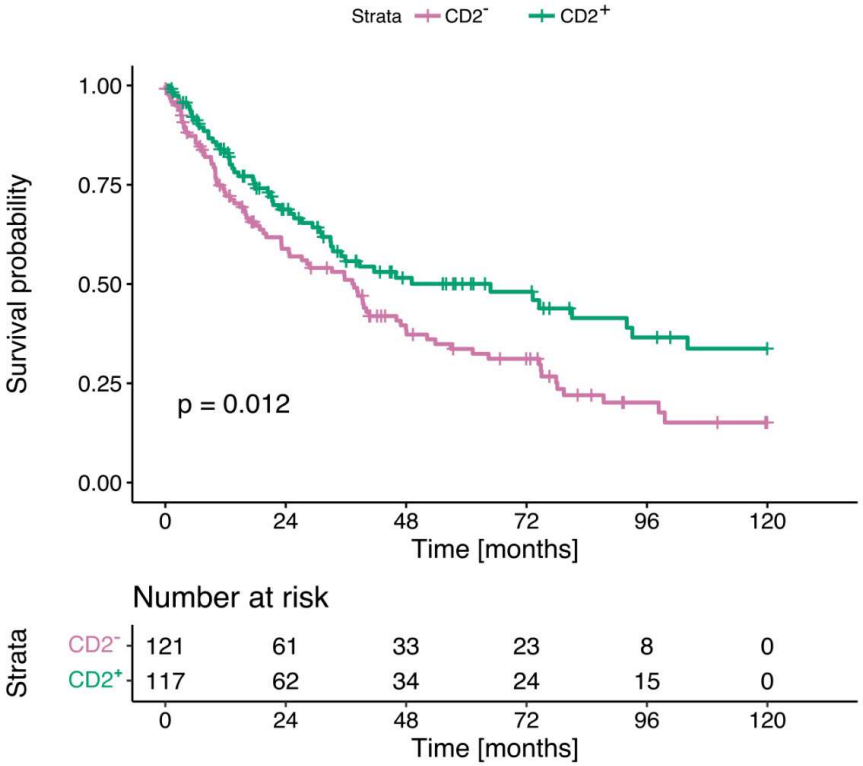


OS was analyzed in a univariate analysis in patients with *smoldering systemic mastocytosis* in whom FU and survival data were reported and results by flow cytometry and/or immunohistochemistry for CD2 on/in MC were available. Two groups of patients were examined based on the pattern of CD2 expression on/in MC: CD2 negativity and CD2 positivity. The probability of OS in these two groups of patients was determined according to the method of Kaplan and Meier.

In patients with SSM there was a trend towards a reduced OS in patients with lack of expression of CD2 (p=0.25). The p-value refers to the comparison of survival curves as assessed by log-rank test. Abbreviations: OS, overall survival; SSM, smoldering systemic mastocytosis, MC, mast cell; FU, follow-up.

Figure S1 c.

OS in patients with advSM stratified by CD2 expression pattern in MC



OS was analyzed in a univariate analysis in patients with *advanced systemic mastocytosis* in whom FU and survival data were reported and results by flow cytometry and/or immunohistochemistry for CD2 on/in MC were available. Two groups of patients were examined based on the pattern of CD2 expression on/in MC: CD2 negativity and CD2 positivity. The probability of OS in these two groups of patients was determined according to the method of Kaplan and Meier.

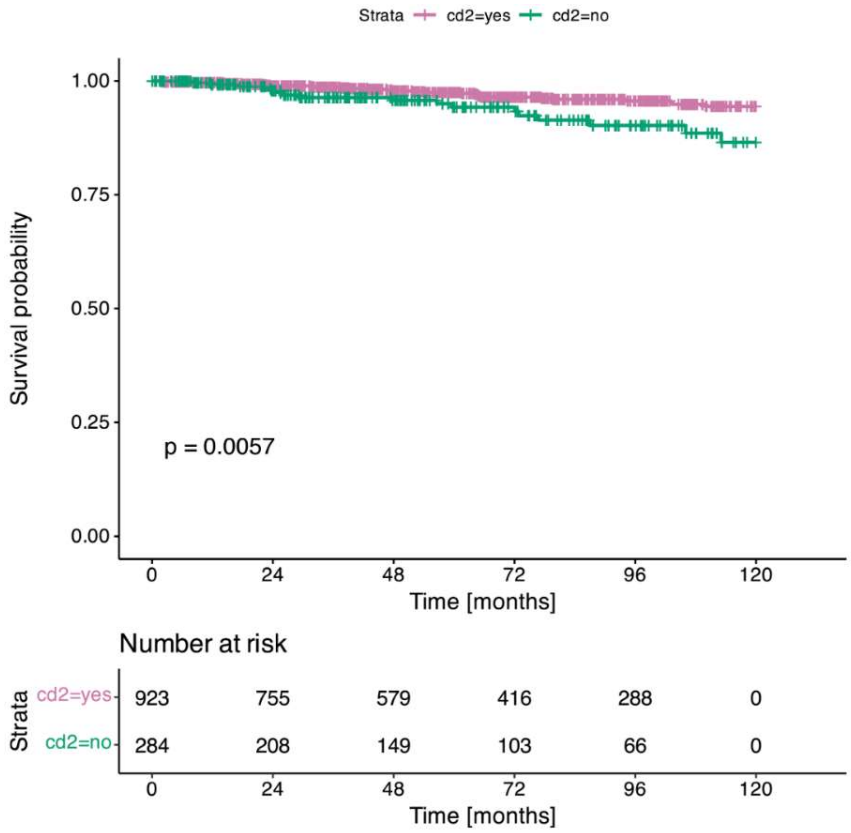
In patients with advSM there was a statistically significant reduction in OS with lack of expression of CD2 (p=0.012). The p-value refers to the comparison of survival curves as assessed by log-rank test.

Abbreviations: OS, overall survival; advSM, advanced systemic mastocytosis, MC, mast cell; FU, follow-up.

***Progression-free survival* (PFS) in patients with ISM and with advSM stratified by CD2 expression pattern in MC, and in patients with SM stratified by CD2 and CD25 expression patterns in MC, is depicted in the Supplementary Figures S2 to S4.**

Supplemental Figure S2

PFS in patients with ISM stratified by CD2 expression pattern in MC



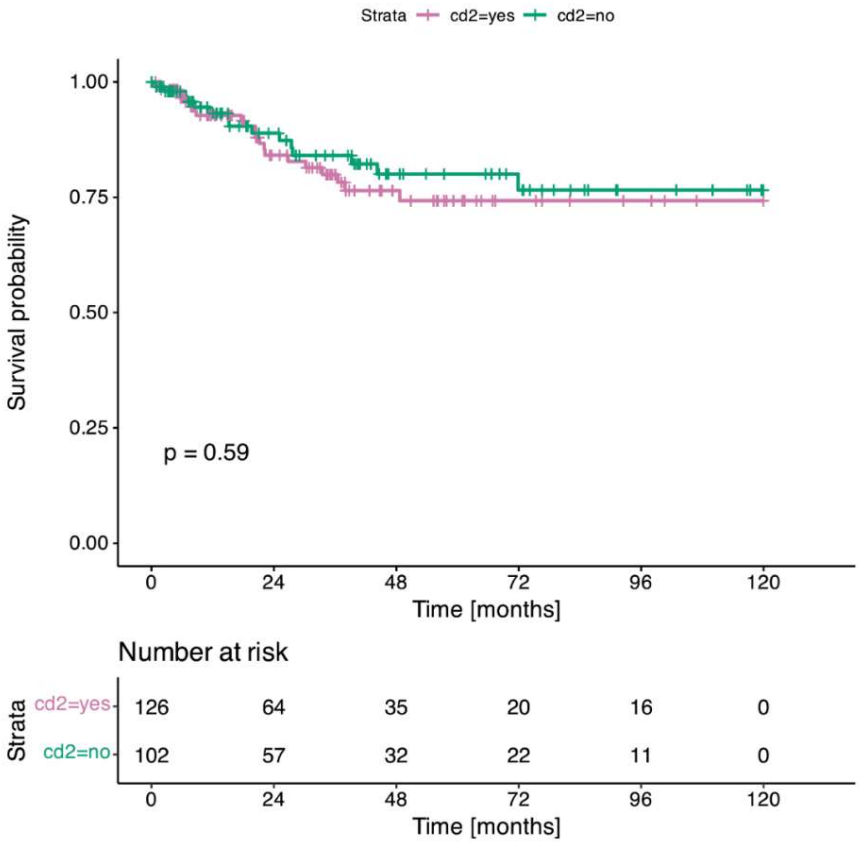
PFS was analyzed in a univariate analysis in patients with *indolent systemic mastocytosis* in whom FU and survival data were reported and results by flow cytometry and/or immunohistochemistry for CD2 on/in MC were available. Two groups of patients were examined based on the pattern of CD2 expression on/in MC: CD2 negativity and CD2 positivity. The probability of PFS in these two groups of patients was determined according to the method of Kaplan and Meier.

PFS in patients with ISM is influenced by CD2 expression. There is a significantly reduced PFS in patients with ISM and lack of CD2 expression (p=0.0057). The p-value refers to the comparison of survival curves as assessed by log-rank test.

Abbreviations: PFS, progression-free survival; ISM, indolent systemic mastocytosis; MC, mast cell; FU, follow-up.

Supplemental Figure S3

PFS in patients with advSM stratified by CD2 expression pattern in MC



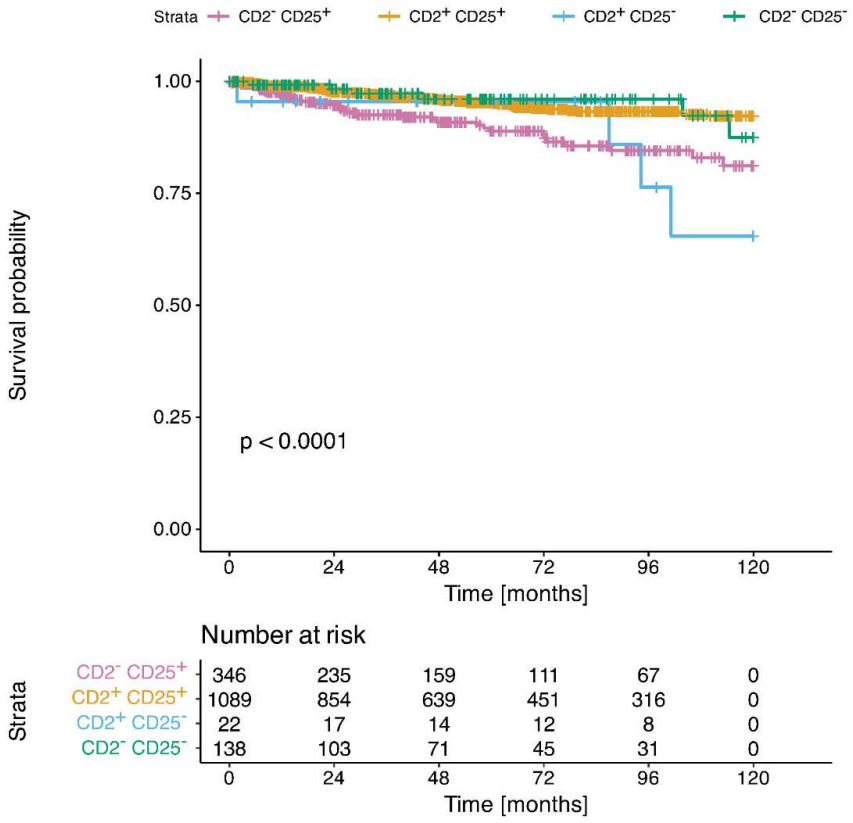
PFS was analyzed in a univariate analysis in patients with *advanced systemic mastocytosis* in whom FU and survival data were reported and results by flow cytometry and/or immunohistochemistry for CD2 on/in MC were available. Two groups of patients were examined based on the pattern of CD2 expression on/in MC: CD2 negativity and CD2 positivity. The probability of PFS in these two groups of patients was determined according to the method of Kaplan and Meier.

PFS in patients with advSM is not influenced by CD2 expression (p=0.59). The p-value refers to the comparison of survival curves as assessed by log-rank test.

Abbreviations: PFS, progression-free survival; advSM, advanced systemic mastocytosis; MC, mast cell; FU, follow-up.

Supplemental Figure S4

PFS in patients with SM stratified by CD2 and CD25 expression patterns in MC



PFS was analyzed in a univariate analysis in 1183 patients with *systemic mastocytosis* in whom FU and survival data were reported and results by flow cytometry and/or immunohistochemistry for CD2 expression and CD25 expression on/in MC were available. Four groups of patients were examined based on the pattern of CD2 expression and CD25 expression on/in MC: $CD2^-/CD25^+$ MC, $CD2^+/CD25^+$ MC, $CD2^+/CD25^-$ MC; and $CD2^-/CD25^-$ MC. The probability of PFS in these 4 groups of patients was determined according to the method of Kaplan and Meier.

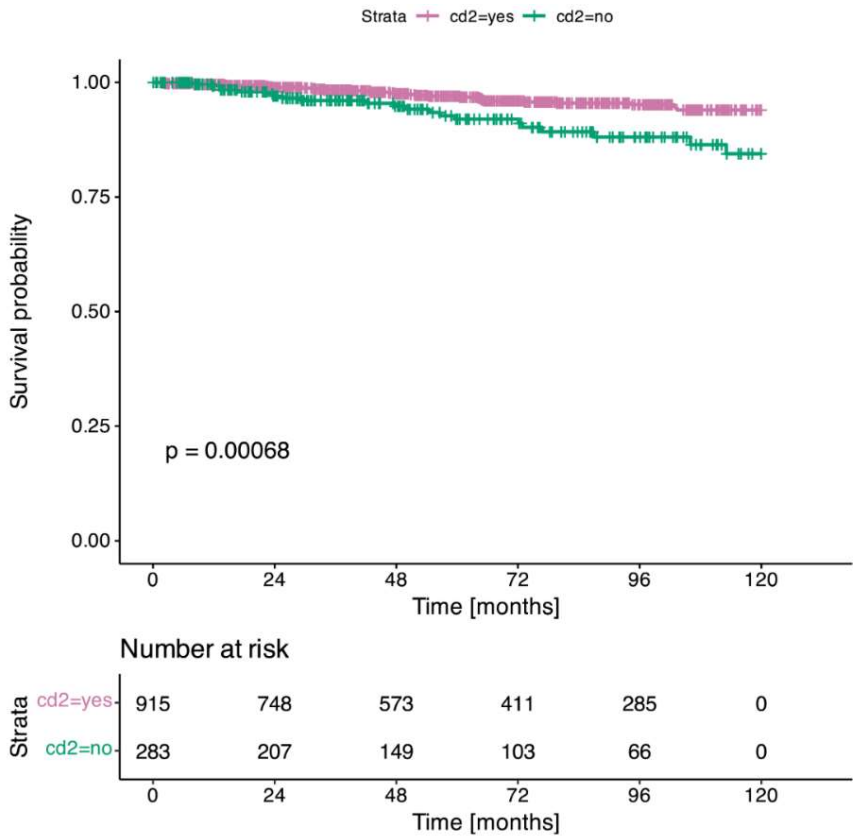
Patients with CD2-negative MC expressing CD25 had a significantly reduced PFS compared to patients with MC expressing both, CD2 and CD25 ($p<0.0001$). The p-value refers to the comparison of all survival curves as assessed by log-rank test.

Abbreviations: PFS, progression-free survival; SM, systemic mastocytosis; MC, mast cell; FU, follow-up.

***Event-free survival* (EFS) in patients with ISM and with advSM stratified by CD2 expression pattern in MC, and in patients with SM stratified by CD2 and CD25 expression patterns in MC, is depicted in the Supplementary Figures S5 to S7.**

Supplemental Figure S5

EFS in patients with ISM stratified by CD2 expression pattern in MC



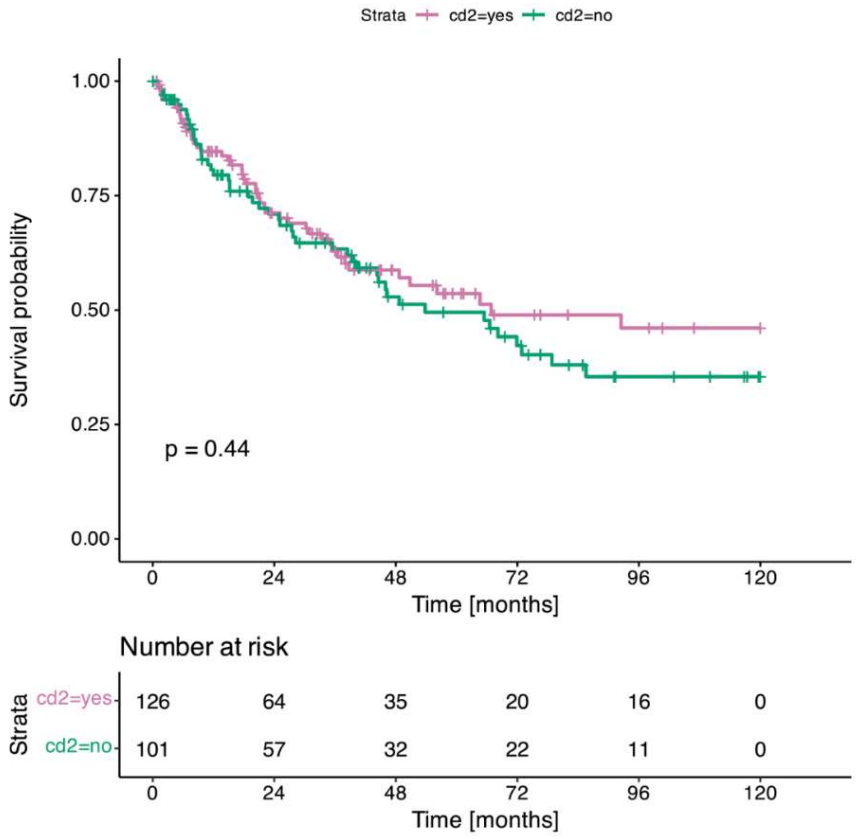
EFS was analyzed in a univariate analysis in patients with *indolent systemic mastocytosis* in whom FU and survival data were reported and results by flow cytometry and/or immunohistochemistry for CD2 on/in MC were available. Two groups of patients were examined based on the pattern of CD2 expression on/in MC: CD2 negativity and CD2 positivity. The probability of EFS in these two groups of patients was determined according to the method of Kaplan and Meier.

EFS in patients with ISM is influenced by CD2 expression. There is a significantly reduced EFS in patients with ISM and lack of CD2 expression (p=0.00068). The p-value refers to the comparison of survival curves as assessed by log-rank test.

Abbreviations: EFS, event-free survival; ISM, indolent systemic mastocytosis; MC, mast cell; FU, follow-up.

Supplemental Figure S6

EFS in patients with advSM stratified by CD2 expression pattern in MC



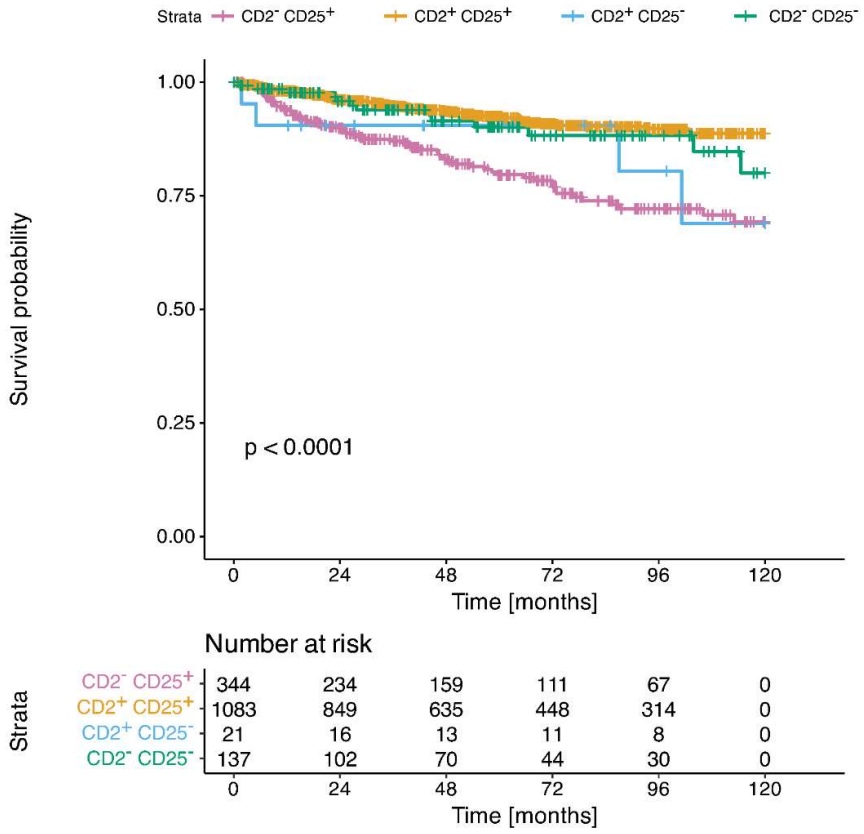
EFS was analyzed in a univariate analysis in patients with *advanced systemic mastocytosis* in whom FU and survival data were reported and results by flow cytometry and/or immunohistochemistry for CD2 on/in MC were available. Two groups of patients were examined based on the pattern of CD2 expression on/in MC: CD2 negativity and CD2 positivity. The probability of EFS in these two groups of patients was determined according to the method of Kaplan and Meier.

EFS in patients with advSM is not influenced by CD2 expression (p=0.44). The p-value refers to the comparison of survival curves as assessed by log-rank test.

Abbreviations: EFS, event-free survival; advSM, advanced systemic mastocytosis; MC, mast cell; FU, follow-up.

Supplemental Figure S7

EFS in patients with SM stratified by CD2 and CD25 expression patterns in MC



EFS was analyzed in a univariate analysis in 1183 patients with *systemic mastocytosis* in whom FU and survival data were reported and results by flow cytometry and/or immunohistochemistry for CD2 expression and CD25 expression on/in MC were available. Four groups of patients were examined based on the pattern of CD2 and CD25 expression on/in MC: $CD2^-/CD25^+$ MC, $CD2^+/CD25^+$ MC, $CD2^+/CD25^-$ MC; and $CD2^-/CD25^-$ MC. The probability of EFS in these 4 groups of patients was determined according to the method of Kaplan and Meier.

Patients with CD2-negative MC expressing CD25 had a significantly reduced EFS compared to patients with MC expressing both, CD2 and CD25 ($p<0.0001$). The p-value refers to the comparison of all survival curves as assessed by log-rank test.

Abbreviations: EFS, event-free survival; SM, systemic mastocytosis; MC, mast cell; FU, follow-up.

Due to the small number of patients with SSM, the majority having CD25 positive MC, establishment of contrast for CD25 was not done.

Survival analysis with multivariate analysis in patients with SM, taking into account CD2 expression and CD25 expression, age and sex, are summarized in the Supplementary Tables S2 to S4.

Supplementary Table S2

Multivariate analysis of *overall survival* in patients with SM

Variable	Overall			ISM			SSM			AdvSM		
	HR[1]	95% CI[2]	p-value	HR[1]	95% CI[2]	p-value	HR[1]	95% CI[2]	p-value	HR[1]	95% CI[2]	p-value
$CD2^+$ MC[3]	-	-		-	-		-	-		-	-	
$CD2^-$ MC[3]	2.73	1.97, 3.78	< 0.001	2.73	1.12, 6.67	0.027	0.83	0.10, 6.61	0.9	1.41	1.0, 2.01	0.053
$CD25^-$ MC[3]	-	-		-	-		-	-		-	-	
$CD25^+$ MC[3]	1.53	0.85, 2.75	0.2	1.63	0.2, 13.0	0.6				0.77	0.39, 1.53	0.5
Age	1.09	1.08, 1.11	< 0.001	1.14	1.09, 1.20	< 0.001	1.30	1.04, 1.63	0.023	1.04	1.02, 1.05	< 0.001
Female	-	-		-	-		-	-		-	-	
Male	1.95	1.4, 2.71	< 0.001	1.13	0.47, 2.69	0.8	1.8	0.23, 14.3	0.6	1.36	0.94, 1.99	0.11

[1] HR, Hazard Ratio; [2] CI, Confidence Interval; [3] MC, mast cell

OS calculation with multivariate analysis in indolent systemic mastocytosis (ISM), smoldering systemic mastocytosis (SSM) and advanced systemic mastocytosis (advSM), taking into account CD2 expression and CD25 expression in MC, age and sex.

There is a significantly reduced OS in patients with SM with CD2-negative MC in the whole cohort and in patients with ISM.

Supplementary Table S3

Multivariate analysis of *progression-free survival* in patients with SM

Variable	Overall			ISM			SSM			AdvSM		
	HR[1]	95% CI[2]	p-value	HR[1]	95% CI[2]	p-value	HR[1]	95% CI[2]	p-value	HR[1]	95% CI[2]	p-value
$CD2^+$ MC[3]	-	-		-	-		-	-		-	-	
$CD2^-$ MC[3]	2.18	1.40, 3.40	< 0.001	2.49	1.34, 4.60	0.004	0.83	0.10, 6.61	0.9	0.7	0.34, 1.42	0.3
$CD25^-$ MC[3]	-	-		-	-		-	-		-	-	
$CD25^+$ MC[3]	1.2	0.60, 2.44	0.6	1.85	0.43, 7.96	0.4				0.89	0.21, 3.83	0.9
Age	1.02	1.00, 1.04	0.011	1.00	0.98, 1.02	> 0.9	1.3	1.04, 1.63	0.023	1.02	0.99, 1.04	0.2
Female	-	-		-	-		-	-		-	-	
Male	1.53	1.01, 2.32	0.043	1.25	0.7, 2.23	0.5	1.8	0.23, 14.3	0.6	1.97	0.91, 4.26	0.084

[1] HR, Hazard Ratio; [2] CI, Confidence Interval; [3] MC, mast cell

PFS calculation with multivariate analysis in indolent systemic mastocytosis (ISM), smoldering systemic mastocytosis (SSM) and advanced systemic mastocytosis (advSM), taking into account CD2 expression und CD25 expression in MC, age and sex.

There is a significantly reduced PFS in patients with SM with CD2-negative MC in the whole cohort and in patients with ISM.

Supplementary Table S4

Multivariate analysis of *event-free survival* in patients with SM

Variable	Overall			ISM			SSM			AdvSM		
	HR[1]	95% CI[2]	p-value	HR[1]	95% CI[2]	p-value	HR[1]	95% CI[2]	p-value	HR[1]	95% CI[2]	p-value
$CD2^+$ MC[3]	-	-		-	-		-	-		-	-	
$CD2^-$ MC[3]	2.40	1.73, 3.33	< 0.001	2.81	1.60, 4.93	< 0.001	0.83	0.10, 6.61	0.9	1.00	0.66, 1.53	> 0.9
$CD25^-$ MC[3]	-	-		-	-		-	-		-	-	
$CD25^+$ MC[3]	1.23	0.72, 2.09	0.4	2.09	0.49, 8.89	0.3				0.7	0.33, 1.47	0.3
Age	1.05	1.04, 1.06	< 0.001	1.03	1.01, 1.05	0.009	1.3	1.04, 1.63	0.023	1.03	1.01, 1.05	< 0.001
Female	-	-		-	-		-	-		-	-	
Male	1.63	1.19, 2.22	0.002	1.09	0.63, 1.87	0.8	1.8	0.23, 14.3	0.6	1.68	1.07, 2.64	0.023

[1] HR, Hazard Ratio; [2] CI, Confidence Interval; [3] MC, mast cell

EFS calculation with multivariate analysis in indolent systemic mastocytosis (ISM), smoldering systemic mastocytosis (SSM) and advanced systemic mastocytosis (advSM), taking into account CD2 expression und CD25 expression in MC, age and sex.

There is a significantly reduced EFS in patients with SM with CD2-negative MC in the whole cohort and in patients with ISM.

Reasons of death in patients with SM are depicted in the Supplemental Table S5.

Supplementary Table S5

Reasons of death in patients with SM

Variable	ISM	SSM	AdvSM	p-value	Missing values (n %)
Death	32 (2.3)	6 (10.7)	212 (53.5)	<0.001	735 (25.9)
Reasons of death (%)				<0.001	5 (2.0)
Disease related	7 (25.0)	2 (33.3)	140 (66.0)	<0.01	
Other	17 (53.1)	4 (66.7)	38 (18.4)	<0.01	
Treatment related	1 (3.1)	0 (0.0)	13 (6.1)	0.65	
Unknown	5 (15.6)	0 (0.0)	18 (8.9)	0.35	

Analyzing reasons of death and comparing indolent systemic mastocytosis (ISM), smoldering systemic mastocytosis (SSM) and advanced systemic mastocytosis (advSM), death was significantly less disease related in ISM compared to SSM and advSM, although power of statistical analysis is limited due to small number of cases. In two patients with ISM and in three patients with advSM there were missing values.

Associations between expression of aberrant CD2 and CD25 in MC with other clinical and laboratory parameters in multivariate analysis are summarized in the Supplementary Tables S6 to S8.

Supplemental Table S6

Multivariate analysis of allergies, constitutional / cardiovascular symptoms and SM-related osteopathy in patients with SM

Variable	Allergy			Constitutional / Cardiovascular symptoms			SM-related osteopathy (osteopenia and osteoporosis)		
	OR[1]	95% CI[2]	p-value	OR[1]	95% CI[2]	p-value	OR[1]	95% CI[2]	p-value
$CD2^+$ MC[3]	-	-		-	-		-	-	
$CD2^-$ MC[3]	0.53	0.42, 0.67	< 0.001	0.65	0.50, 0.84	< 0.001	0.62	0.49, 0.79	<0.001
$CD25^-$ MC[3]	-	-		-	-		-	-	
$CD25^+$ MC[3]	1.37	0.88, 2.17	0.200	1.44	0.88, 2.45	0.3	1.21	0.78, 1.93	0.4
Age	1.00	0.99, 1.00	0.120	0.99, 1.00	1.01, 1.05	0.009	1.03	1.02, 1.03	<0.001
Female	-	-		-	-		-	-	
Male	1.54	1.27, 1.87	< 0.001	0.96	0.78, 1.17	0.8	0.91	0.75, 1.12	0.4

[1] OR, Odds Ratio; [2] CI, Confidence Interval; [3] MC, mast cell

Multivariate analysis of allergies, constitutional / cardiovascular symptoms and SM-related osteopathy (osteopenia and osteoporosis), adjusted for sex and age.

CD2-negativity was associated with lower rates of allergies, constitutional / cardiovascular symptoms and SM-related osteopathy.

Supplemental Table S7

Multivariate analysis of pruritus, blistering/bullae and gastrointestinal (GI) symptoms in patients with SM

Variable	Pruritus (≥ mild or moderate frequent)			Blistering/Bullae			GI symptoms		
	OR[1]	95% CI[2]	p-value	OR[1]	95% CI[2]	p-value	OR[1]	95% CI[2]	p-value
$CD2^+$ MC[3]	-	-		-	-		-	-	
$CD2^-$ MC[3]	0.76	0.56, 1.03	0.082	1.06	0.54, 1.95	0.9	1.09	0.87, 1.36	0.5
$CD25^-$ MC[3]	-	-		-	-		-	-	
$CD25^+$ MC[3]	0.99	0.59, 1.71	>0.9	1.08	0.39, 3.86	0.9	1.17	0.77, 1.77	0.5
Age	1.01	1.00, 1.02	0.064	1.00	0.98, 1.02	>0.9	0.99	0.99, 1.00	0.040
Female	-	-		-	-		-	-	
Male	0.87	0.68, 1.12	0.3	1.48	0.87, 2.50	0.14	0.49	0.41, 0.59	<0.001

[1] OR, Odds Ratio; [2] CI, Confidence Interval; [3] MC, mast cell

Multivariate analysis of pruritus, blistering/bullae and GI symptoms, adjusted for sex and age.

CD2-negativity was not associated with the presence of pruritus, blistering/bullae or GI symptoms.

Supplemental Table S8

Multivariate analysis of karyotype and *KIT* mutations in patients with SM

	CD2 $^{-}$		CD25 $^{+}$		CD2^{-} CD25^{+}	
	OR[1] [95% CI[2]]	p-value	OR[1] [95% CI[2]]	p-value	OR[1] [95% CI[2]]	p-value
Abnormal karyotype	1.32 [0.69, 2.5]	0.4	0.69 [0.28, 2.11]	0.5	1.11 [0.57, 2.13]	0.8
KIT mutations						
c-*KIT* D816V	0.36 [0.27, 0,47]	< 0.001	5.76 [3.85, 8.55]	< 0.001	0.63 [0.48, 0.88]	0.005
Other *KIT*-mutations	0.38 [0.10, 1.14]	0.1	3.86 [0.77, 70,3]	0.2	0.57 [0.12, 1.92]	0.4
Mutations in genes other than *KIT*	7.54 [1.90, 50,6]	0.011	0.35 [0.01, 3,94]	0.5	5.43 [1.36, 36.6]	0.065

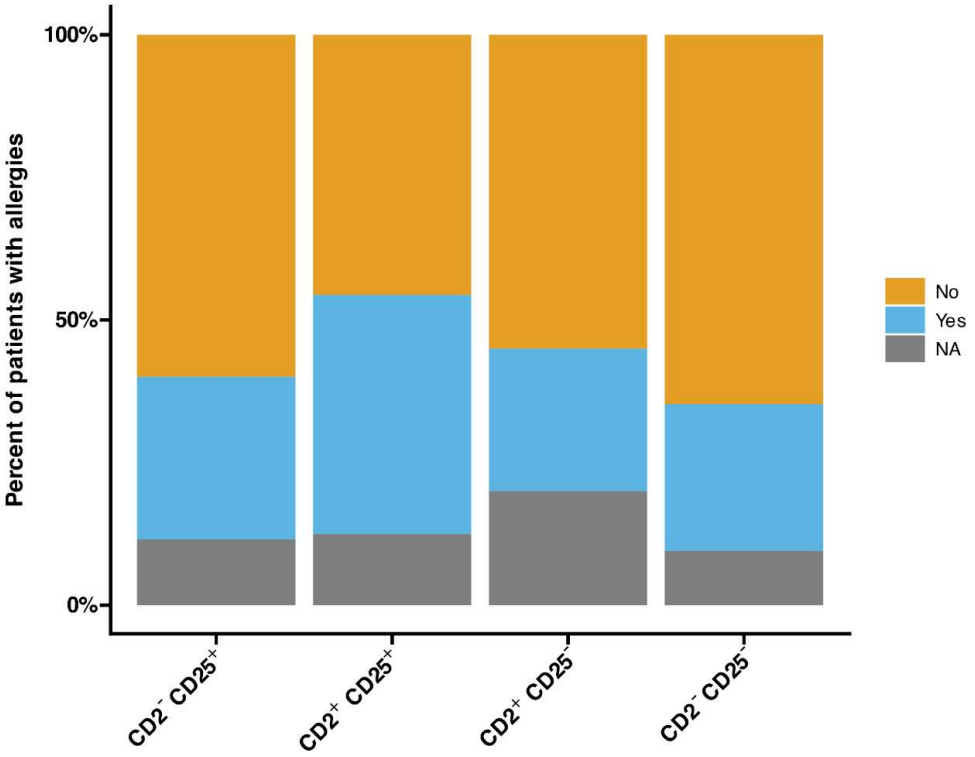
[1] OR, Odds Ratio; [2] CI, Confidence Interval

Multivariate analysis of karyotype and *KIT* mutations, adjusted for sex and age, in patients with SM. There was a positive association between CD2-negativity and an abnormal karyotype with conventional cytogenetic analysis, which did not reach statistical significance. However, CD2-negativity was associated with mutations in genes other than *KIT* with an odds ratio of 7.54.

Associations between expression of CD2 and CD25 in MC and selected *clinical* and *laboratory parameters* are depicted in Figures S8 to S13.

Supplemental Figure S8

Percentage of patients with SM and *allergies* stratified by CD2 and CD25 expression patterns in MC

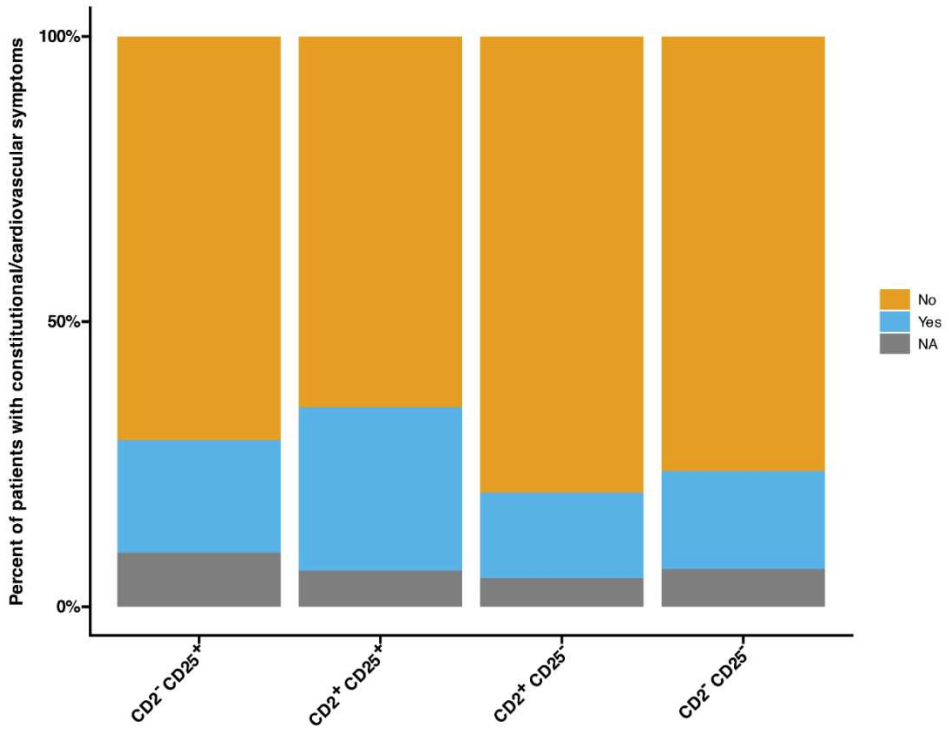


Four groups of patients with SM and allergies were examined based on the pattern of CD2 and CD25 expression on/in MC as determined by flow cytometry or immunohistochemistry: $CD2^-/CD25^+$ MC, $CD2^+/CD25^+$ MC, $CD2^+/CD25^-$ MC; and $CD2^-/CD25^-$ MC. CD2-negativity was associated with lower rates of allergies (OR: 0.53; 95% CI 0.42, 0.67; $p < 0.001$).

Abbreviations: SM, systemic mastocytosis; MC, mast cell; NA, not applicable (no data available).

Supplemental Figure S9

Percentage of patients with SM and *constitutional/cardiovascular symptoms* stratified by CD2 and CD25 expression patterns in MC

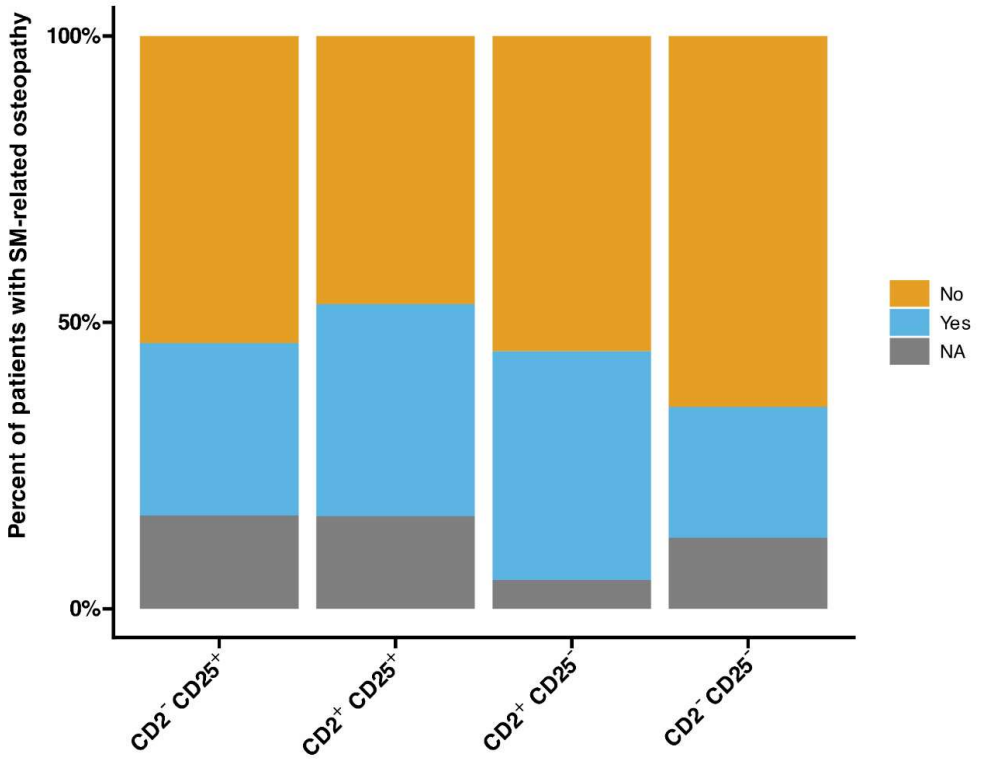


Four groups of patients with SM and constitutional/cardiovascular symptoms were examined based on the pattern of CD2 and CD25 expression on/in MC as determined by flow cytometry or immunohistochemistry: $CD2^-/CD25^+$ MC, $CD2^+/CD25^+$ MC, $CD2^+/CD25^-$ MC; and $CD2^-/CD25^-$ MC. CD2-negativity was associated with lower rates of constitutional/cardiovascular symptoms (OR: 0.65; 95% CI: 0.50, 0.84; p=0.001).

Abbreviations: SM, systemic mastocytosis; MC, mast cell; NA, not applicable (no data available).

Supplemental Figure S10

Percentage of patients with SM and *SM-related osteopathy* (osteopenia or osteoporosis) stratified by CD2 and CD25 expression patterns in MC

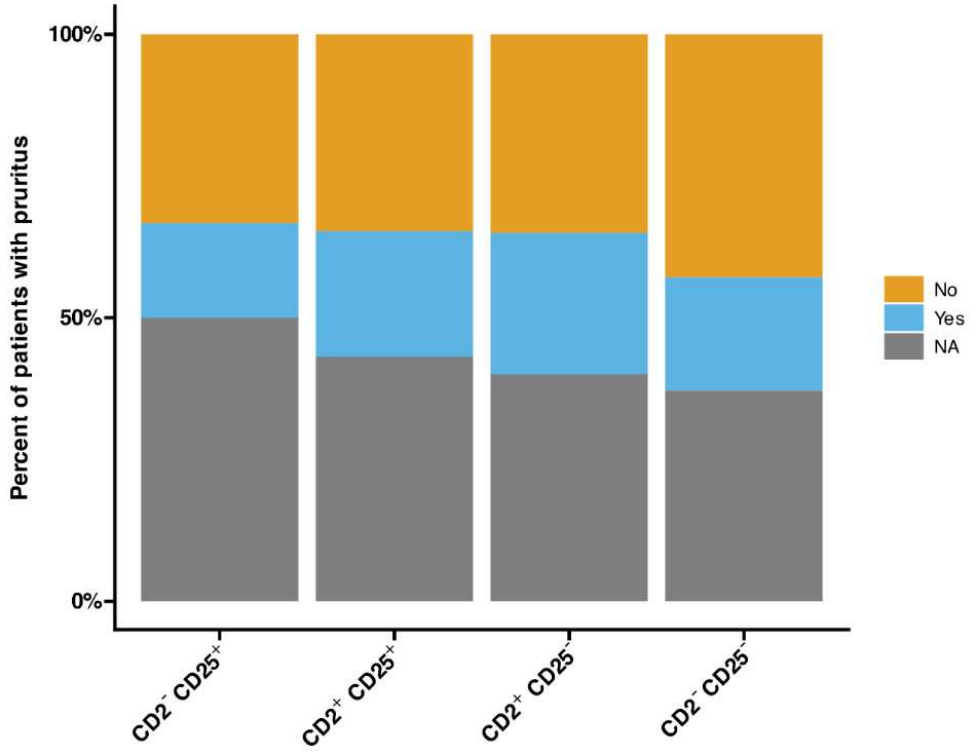


Four groups of patients with SM and SM-related osteopathy (osteopenia or osteoporosis) were examined based on the pattern of CD2 and CD25 expression on/in MC as determined by flow cytometry or immunohistochemistry: $CD2^-/CD25^+$ MC, $CD2^+/CD25^+$ MC, $CD2^+/CD25^-$ MC; and $CD2^-/CD25^-$ MC. CD2-negativity was associated with lower rates of SM-related osteopathy with osteopenia or osteoporosis (OR: 0.62; 95% CI: 0.49, 0.79; $p < 0.001$).

Abbreviations: SM, systemic mastocytosis; MC, mast cell; NA, not applicable (no data available).

Supplemental Figure S11

Percentage of patients with SM and *pruritus* stratified by CD2 and CD25 expression patterns in MC

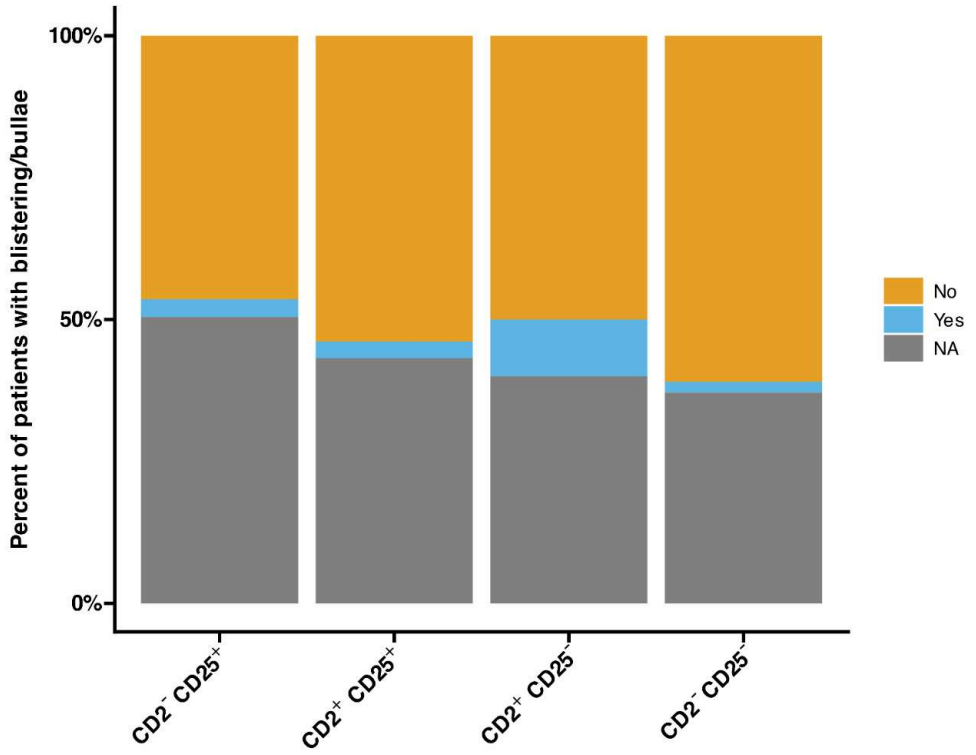


Four groups of patients with SM and pruritus were examined based on the pattern of CD2 and CD25 expression on/in MC as determined by flow cytometry or immunohistochemistry: $CD2^-/CD25^+$ MC, $CD2^+/CD25^+$ MC, $CD2^+/CD25^-$ MC; and $CD2^-/CD25^-$ MC. No difference was detected between the severity of pruritus and different antigen marker expression.

Abbreviations: SM, systemic mastocytosis; MC, mast cell; NA, not applicable (no data available).

Supplemental Figure S12

Percentage of patients with SM and *blistering or bullae* stratified by CD2 and CD25 expression patterns in MC

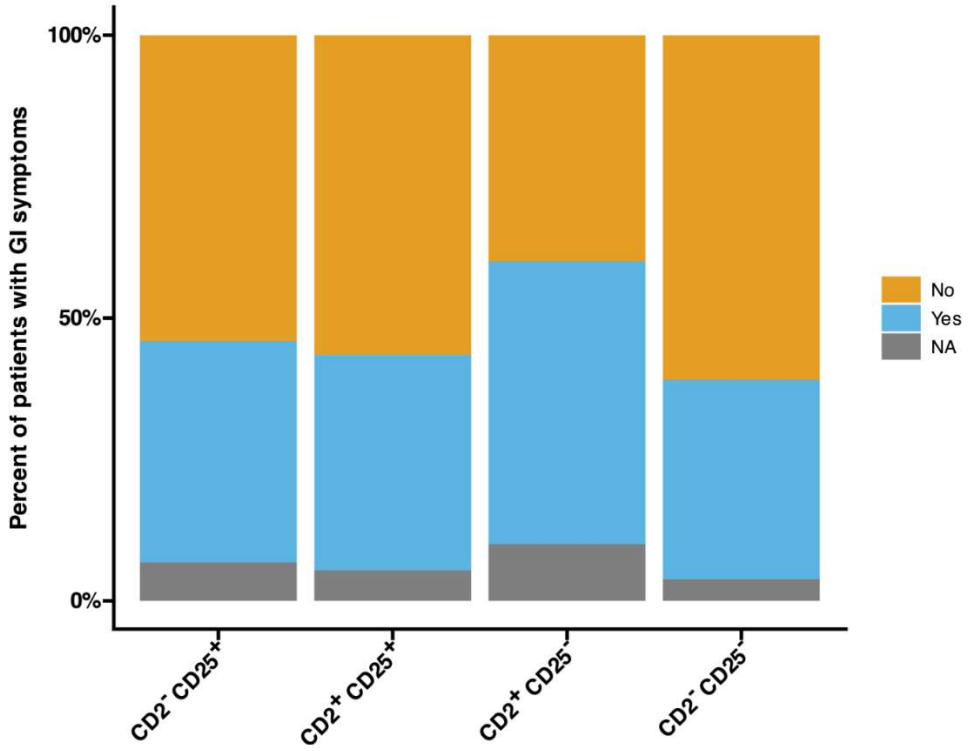


Four groups of patients with SM and blistering or bullae were examined based on the pattern of CD2 and CD25 expression on/in MC as determined by flow cytometry or immunohistochemistry: $CD2^-/CD25^+$ MC, $CD2^+/CD25^+$ MC, $CD2^+/CD25^-$ MC; and $CD2^-/CD25^-$ MC. No difference was detected between the severity of blistering of bullae and different antigen marker expression.

Abbreviations: SM, systemic mastocytosis; MC, mast cell; NA, not applicable (no data available).

Supplemental Figure S13

Percentage of patients with SM and *gastrointestinal symptoms* stratified by CD2 and CD25 expression patterns in MC



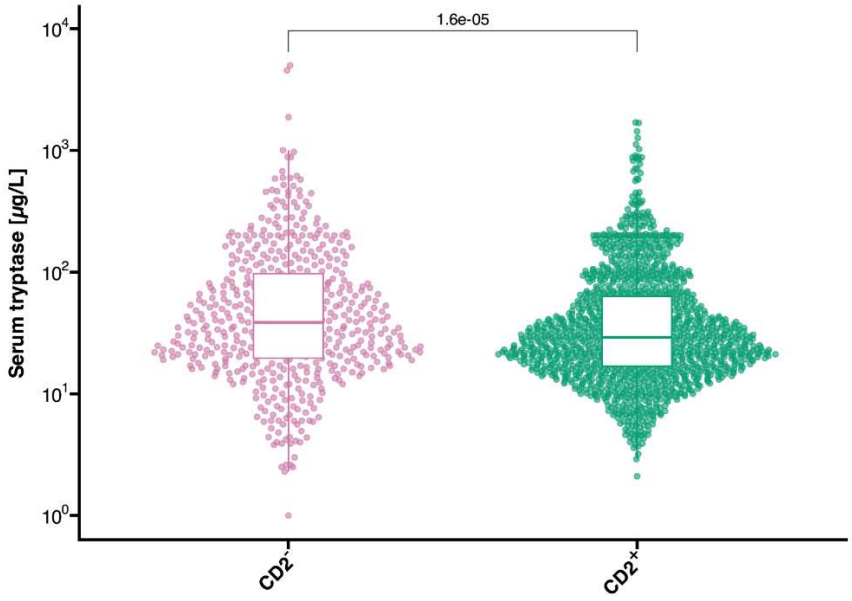
Four groups of patients with SM and GI symptoms were examined based on the pattern of CD2 and CD25 expression on/in MC as determined by flow cytometry or immunohistochemistry: $CD2^-/CD25^+$ MC, $CD2^+/CD25^+$ MC, $CD2^+/CD25^-$ MC; and $CD2^-/CD25^-$ MC. No difference was detected between the absence of presence of GI symptoms and different antigen marker expression.

Abbreviations: SM, systemic mastocytosis; MC, mast cell; GI, gastrointestinal; NA, not applicable (no data available).

Associations between *serum tryptase level* and expression of CD2 and CD25 in MC are depicted in Figures S14 to S15.

Supplemental Figure S14

Serum tryptase level in patients with SM stratified by CD2 expression pattern in MC



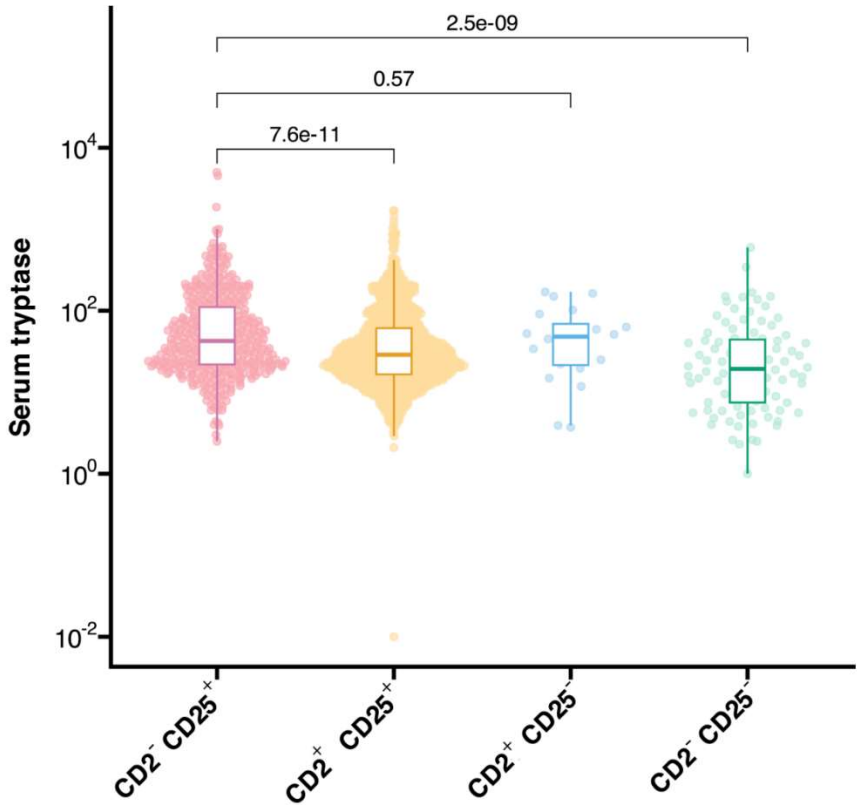
Basal serum tryptase (BST) level was analyzed in 531 patients with SM with CD2 negative MC and in 1441 patients with SM with CD2 positive MC.

The median BST was 40.55 µg/l (IQR: 21.00, 102.00) and 29.00 µg/l (IQR: 17.00, 63.40), in CD2-negative MC and in CD2-positive MC, respectively ($p<0.001$).

Abbreviations: SM, systemic mastocytosis; MC, mast cell.

Supplemental Figure S15

Serum tryptase level in patients with SM stratified by CD2 and CD25 expression patterns in MC



Four groups of patients with SM and serum tryptase level at diagnosis were examined based on the pattern of CD2 and CD25 expression on/in MC as determined by flow cytometry or immunohistochemistry: $CD2^-/CD25^+$ MC, $CD2^+/CD25^+$ MC, $CD2^+/CD25^-$ MC; and $CD2^-/CD25^-$ MC. Patients with lack of CD2 expression in MC had a significantly higher median serum tryptase level compared to patients in whom MC were found to express CD2.

Abbreviations: SM, systemic mastocytosis; MC, mast cell.

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