Supplemental Material to Mueller et al.

CD44 is a RAS/STAT5-regulated invasion receptor that triggers disease expansion in advanced mastocytosis

Methods

Patients

A total number of 161 patients with mastocytosis were examined. The median age was 54 years (range 10-90). Diagnoses were established according to criteria provided by the World Health Organization (WHO).[1-6] Patients were classified as cutaneous mastocytosis (CM, n=15), indolent systemic mastocytosis (ISM, n=79), smoldering SM (SSM, n=7), SM with an associated hematologic neoplasm (SM-AHN, n=40), aggressive SM (ASM, n=12), and mast cell leukemia (MCL, n=8). The patients´ characteristics are shown in Table 1 (overview) and Supplemental Table S1 (showing each individual patient). Control bone marrow (BM) samples (n=57) were obtained from individuals with normal/reactive BM (RBM, n=5), idiopathic cytopenia of undetermined significance (ICUS, n=3), monoclonal gammopathy of undetermined significance (MGUS, n=4), AL-amyloidosis (n=2), or patients with lympho-proliferative neoplasms, including Hodgkin´s disease (HD, n=2) and Non-Hodgkin lymphomas (NHL, n=41) (Table 1 and Supplemental Table S2). Control serum samples (n=15) were obtained from healthy donors (Table 1 and Supplemental Table S3). Mononuclear cells (MNC) were isolated from heparinized BM samples using Ficoll, washed and recovered in RPMI-1640 medium with 10% fetal calf serum (FCS). In addition, in 16 patients with SM (indolent SM, n=7; advanced SM, n=9), serum

soluble CD44 (sCD44) measurements and/or flow cytometry studies were performed in follow-up samples. All patients with SM received standard supportive therapy, including histamine receptor (HR) 1 and HR2 blocker and bisphosphonates as needed.[6] Patients with advanced SM or progressive disease received additional interventional therapies, following standard recommendations.[6] The type of interventional therapy administered in patients in whom sCD44 levels and/or surface CD44 levels on neoplastic cells were determined in follow-up samples is shown in Supplemental Table S10. The study was approved by the ethics committee of the Medical University of Vienna and conducted in accordance with the declaration of Helsinki. All primary material used in this study (BM, peripheral blood (PB), serum) was obtained after written informed consent was given.

Reagents

Iscove´s Modified Dulbecco´s medium (IMDM), RPMI-1640 medium and penicillin-streptomycin were purchased from Lonza (Basel, Switzerland), FCS from Gibco Life Technologies (Carlsbad, CA, USA), and amphotericin B from Pan-Biotech (Aidenbach, Germany). As a source for recombinant murine stem cell factor (SCF) Chinese hamster ovary cells transfected with the murine *SCF* gene (CHO-KL) were used.[7,8] 5-azacytidine, decitabine, pimozide, and dimethylsulfoxide (DMSO) were purchased from Sigma Aldrich (St. Louis, MO, USA) and PKC412 (midostaurin) from LC Laboratories (Woburn, MA, USA). Ponatinib, ibrutinib, JQ1, BEZ235, an inhibitor of phosphoinositide-3-kinase (PI3 kinase) and mechanistic target of rapamycin (mTOR), and lenalidomide were purchased from Selleck Chemicals (Houston, TX, USA). Nilotinib and the MEK1/2 inhibitor RDEA119 (refametinib) were purchased

from Chemietek (Indianapolis, IN, USA). 5-azacytidine and decitabine were dissolved in distilled water. All other drugs were dissolved in DMSO. A specification of monoclonal antibodies (mAb) used in this study is provided in Supplemental Table S5.

Human mast cell (MC) lines

The two sub-clones HMC-1.1 and HMC-1.2, derived from a patient with MCL, were kindly provided by Dr. J. H. Butterfield (Mayo Clinic, Rochester, MN, USA)[9] and cultured in IMDM, 10% FCS, and antibiotics at 37°C and 5% CO_2. HMC-1.1 cells display the *KIT* mutation V560G but not D816V, whereas HMC-1.2 cells express both *KIT* mutations.[10,11] The human MC lines MCPV-1 (sub-clones MCPV-1.1, MCPV-1.2, MCPV-1.3 and MCPV-1.4) were established from SCF-cultured cord blood-derived MC progenitors by lentiviral transduction with *HRAS* G12V, *TERT*, and SV40 large tumor antigen (TAg).[8] The human SCF-dependent $ROSA^{KIT\ WT}$ cell line was established by culturing cord blood-derived MC progenitors as reported.[7] The human SCF-independent $ROSA^{KIT\ D816V}$ cell line was then established by transfecting $ROSA^{KIT\ WT}$ cells with the human *KIT D816V* gene by lentiviral transduction.[7] MCPV-1 as well as ROSA cells were cultured in IMDM, 10% heat-inactivated FCS, and antibiotics at 37°C and 5% CO_2. In addition, $ROSA^{KIT\ WT}$ cells and MCPV-1 cells were cultured in the presence of SCF-containing supernatants (10%) derived from CHO-KL, whereas the $ROSA^{KIT\ D816V}$ cell line was maintained in the absence of SCF.[7,8] The identity of HMC-1, ROSA and MCPV-1.1 cells was confirmed by *KIT* mutation analyses and/or surface phenotyping. All human MC lines used in this study were re-thawed from an original stock every 4-8 week, passaged weekly, and

periodically tested for mycoplasma contamination by conventional PCR using the Venor GeM Classic Mycoplasma Detection Kit (Minerva Biolabs, Berlin, Germany).

Chronic myeloid leukemia (CML) cell lines

The CML cell line K562 (BCR-ABL1$^+$) was kindly provided by Dr. M. Deininger (Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, USA) and KU812 cells (BCR-ABL1$^+$) were kindly provided by Dr. K. Kishi (Niigata University, Niigata, Japan). KCL22 cells (BCR/ABL1$^+$) were purchased from the German Collection of Microorganism and Cell Culture (DSMZ, Braunschweig, Germany). All CML cell lines were cultured in RPMI-1640 medium with 10% FCS and antibiotics at 37°C.

Flow cytometry analyses on cell lines

Surface expression of CD44 was examined in all human MC lines (HMC-1, ROSA, MCPV-1) and in the CML cell lines K562, KU812 and KCL22 by flow cytometry. In a separate set of experiments, the MC lines were pre-incubated at 37°C with signal transduction inhibitors, including RDEA119 (refametinib) (0.1-5 µM), pimozide (2.5-10 µM), ibrutinib (1-5 µM), nilotinib (0.005-5 µM), ponatinib (0.001-1 µM), PKC412 (midostaurin) (0.005-1 µM), and BEZ235 (0.001-5 µM) for 48 hours, or with epigenetic drugs, including decitabine (0.1-5 µM), 5-azacytidine (0.1-5 µM), JQ1 (0.1-5 µM), and lenalidomide (0.1-5 µM) for 96 hours before flow cytometry experiments were performed. In addition, expression of CD44 was examined on green fluorescent protein positive (GFP$^+$) or m-Cherry$^+$ (lentiviral transduced) HMC-1.2 cells or ROSA$^{KIT\ WT}$ cells (see below). After FcR blocking (FcR blocking reagent human; Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), human MC lines were stained

with a phycoerythrin (PE)-conjugated mAb (515) against CD44 (Supplemental Table S5). After washing in PBS, cells were analyzed on a BD FACSCalibur (Becton Dickinson, Franklin Lakes, NJ, USA). Antibody reactivity was controlled using isotype-matched control antibodies (Supplemental Table S5). In contrast to GFP^+ lentiviral transduced human MC lines (stained with PE-labeled anti-CD44 and analyzed on BD FACSCalibur), m-$Cherry^+$ cells were stained with allophycocyanin (APC)-labeled anti-CD44 mAb (BJ18) (Supplemental Table S5) and analyzed on a BD LSRFortessa (Becton Dickinson, Franklin Lakes, NJ, USA). Flow cytometry data analysis was performed with FlowJo software Version 8.8.7 (FlowJo LLC, Ashland, OR, USA). All human MC lines analyzed in this study were initially gated for viability and, if applicable, examined for GFP or m-Cherry positivity. The median fluorescence intensity (MFI) of reactivity of MC with CD44 mAb and the isotype-matched control mAb was determined and the Staining Index (SI) (MFI of CD44 divided by MFI of the isotype-matched control) was calculated.

Flow cytometry analyses of primary patient-derived BM cells

Heparinized unseparated BM samples obtained from the iliac crest of 56 patients with mastocytosis (CM, n=2; ISM, n=20; SM-AHN, n=22; ASM, n=6; MCL, n=6; Supplemental Tables S1 and S4) and control patients (including lymphoproliferative disorders and normal/reactive BM, n=57; Table 1 and Supplemental Table S2) were analyzed by multi-color flow cytometry. Unseparated BM aspirate samples were stained with APC-Cyanine7 labeled anti-CD45 (HI30), Pacific Blue labeled anti-CD34 (581), PE-Cyanine7 labeled anti-CD117 (104D2), APC-labeled CD38 (HIT-2), and PE-labeled anti-CD44 (515) or isotype-matched control antibody (Supplemental Table

S5). After erythrocyte lysis (Lysing Solution; Becton Dickinson, Franklin Lakes, NJ, USA) and washing with PBS, CD44 surface expression on $CD45^+/CD117^{++}/CD34^-$ MC, $CD45^+/CD34^+/CD38^-$ stem cells (SC) and $CD45^+/CD34^+/CD38^+$ progenitor cells (PC) was examined on a BD FACSCanto II (Becton Dickinson, Franklin Lakes, NJ, USA). A specification of all mAb applied in this study is shown in Supplemental Table S5. Flow cytometry data analysis was performed with FlowJo software Version 8.8.7 (FlowJo LLC, Ashland, OR, USA). For detection and analysis of primary MC, BM cells were gated as $CD45^+/CD117^{++}/CD34^-$ cells. In addition, doublets were excluded and cell fractions were gated for viable cells. For detection and analysis of primary SC and PC, BM cells were gated for CD45 dim and $CD34^+$ viable cells. Then, these cells were gated against CD38 in order to separate the $CD34^+/CD38^-$ SC from the $CD34^+/CD38^+$ PC. The median fluorescence intensity (MFI) of CD44 and the isotype-matched control on MC, SC and PC was determined and the SI (MFI of CD44 divided by MFI of the isotype-matched control) was calculated. In addition, in 4 SM patients with an associated acute myeloid leukemia (SM-AML), expression of CD44 was also examined on $CD45^{dim}/CD34^+/CD38^+$ BM blast cells. Moreover, in 3 SM patients with an associated chronic myelomonocytic leukemia (CMML), we also determined the expression of CD44 on $CD14^+$ BM monocytes. In these experiments, BM cells were stained with peridinin chlorophyll (PerCP)-labeled CD45 mAb (2D1), fluorescein isothiocyanate (FITC)-labeled CD14 mAb (TÜK4), PE-labeled CD44 mAb (515) and an isotype-matched control antibody (Supplemental Table S5). CD44 expression on $CD45^+/CD14^+$ monocytes was examined on a BD FACSCanto II (Becton Dickinson, Franklin Lakes, NJ, USA) and analyzed with FlowJo software Version 8.8.7 (FlowJo LLC, Ashland, OR, USA).

Cell sorting experiments

HMC 1.2 cells and ROSA$^{KIT\ WT}$ cells transduced with lentiviral vectors encoding *K-RAS* or *STAT5* (see below) were sorted for GFP positivity on a BD FACSAria I (Becton Dickinson, Franklin Lakes, NJ, USA) before flow cytometric analysis was performed.

Immunocytochemistry (ICC) and Immunohistochemistry (IHC)

ICC was performed on HMC-1, ROSA, MCPV-1 and K562 cells as well as on primary neoplastic MC obtained from PB and BM samples of two patients with MCL (Supplemental Tables S1 and S4). Cells were spun on cytospin slides, fixed with acetone, and incubated with anti-CD44 mAb (DF1485; Dako, Glostrup, Denmark) dissolved in Tris-buffered saline (TBS) and 10% FCS (work dilution of DF1485, 1:50) at 4°C overnight. Cells were then washed with TBS and incubated with biotinylated goat-anti-mouse IgG (second step antibody) (GM601H; Biocare Medical, Pacheco, CA, USA) for 30 minutes. After washing, cells were incubated with streptavidin-conjugated alkaline phosphatase (AP605H; Biocare Medical, Pacheco, CA, USA) for 30 minutes. Antigen detection was then visualized using New Fuchsin Substrate Kit (Histofine, 415161F, Nichirei Bioscience Inc., Tokyo, Japan) producing a red color staining reaction. Finally, cells were counterstained with Mayer´s Hematoxylin solution (Morphisto, Frankfurt a.M., Germany) and embedded in Aquatex (Merck KGaA, Darmstadt, Germany).

For IHC, BM biopsy material of 12 SM patients (ISM, n=5; SM-AHN, n=2; ASM, n=2; MCL, n=3; Supplemental Tables S1 and S4) and one patient with normal/reactive

BM (Supplemental Figure S4) was examined. BM samples were fixed in neutral-buffered formaldehyde, decalcified in EDTA, and embedded in paraffin. Paraffin-sections were cut (2 µm) and mounted on slides. IHC was performed using the indirect immunoperoxidase staining technique. After deparaffinization (dewaxing), slides were washed and re-hydrated using xylene and graded alcohol series. Then sections underwent antigen-retrieval (antigen unmasking) using heated citrate buffer solution, followed by irreversible blockage of endogenous peroxidase activity. For this purpose, sections were placed in a methanol-based 0.45% hydrogen peroxide solution (Sigma-Aldrich, St. Louis, MO, USA) for 20 minutes. After washing with TBS, sections were incubated with an anti-CD44 mAb (DF1485; Dako, Glostrup, Denmark) dissolved in Renoir Red Diluent (PD904L; Biocare Medical, Pacheco, CA, USA) (work dilution of DF1485, 1:25) or anti-tryptase mAb (G3; Cell Marque, Rocklin, CA, USA) dissolved in TBS/10% FCS (work dilution of G3, 1:50) at 4°C overnight. Thereafter, CD44-stained sections were washed with TBS and then incubated with biotinylated goat-anti-mouse IgG (second step antibody) (GM601H; Biocare Medical, Pacheco, CA, USA) for 30 minutes followed by incubation (for 30 minutes) with streptavidin conjugated to chromogen horseradish peroxidase (HRP) (HP604H; Biocare Medical, Pacheco, CA, USA). Tryptase-stained sections were washed with TBS and then incubated for 30 minutes with second-step biotinylated goat-anti-mouse IgG (BA-9200; Vector Laboratories, Burlingame, CA, USA) dissolved in normal goat serum blocking solution (S-1000; Vector Laboratories, Burlingame, CA, USA) (work dilution of 1:200) followed by incubation (for 30 minutes) with AB-complex solution (consisting of avidin and biotinylated HRP mixed together in order to form large avidin-biotin-enzyme complexes) (Vectastain Elite ABC HRP kit, PK-6100; Vector Laboratories,

Burlingame, CA, USA). For CD44 and tryptase staining, antigen detection was visualized using 3-amino-9-ethylcarbazole (AEC) (A5754; Sigma-Aldrich, St. Louis, MO, USA) dissolved with 30% hydrogen peroxide solution (Sigma-Aldrich, St. Louis, MO, USA) in sodium acetate buffer producing a red color staining reaction. Finally, BM slides were counterstained with Mayer´s Hematoxylin (Morphisto, Frankfurt a.M., Germany) and embedded in Aquatex (Merck KGaA, Darmstadt, Germany).

Isolated cells (ICC) and in situ-stained BM cells (IHC) were analyzed by an Olympus BX50F4 microscope (Olympus Corporation; Shinjuku, Tokyo, Japan) equipped with 60x/0.90 UPlanFL and 100x/1.35 UPlanAPO (Oil Iris) objective lenses. Images of single cells (ICC) or BM sections (IHC) were taken with 1000x or 600x magnifications using an Olympus DP21 camera (Olympus Corporation; Shinjuku, Tokyo, Japan). Images were adjusted by Adobe Photoshop CS5 software Version 12.0.4 (Adobe Systems, San Jose, CA, USA).

Measurement of sCD44 in patients with SM

In the serum of 129 patients with mastocytosis (CM, n=15; ISM, n=74; SSM, n=7; SM-AHN, n=20; ASM, n=9; MCL, n=4; Table 1, Supplemental Tables S1 and S4) and 15 healthy controls (Table 1, Supplemental Table S3), sCD44 levels were examined by a commercial ELISA (Affymetrix eBioscience, Santa Clara, CA, USA). The detection limit for sCD44 in the ELISA was 16 pg/ml.

Quantitative PCR (qPCR)

RNA was isolated from native and lentivirally transduced human MC lines (HMC-1, ROSA, MCPV-1), human CML cell lines (K562, KU812, KCL22), PB MNC isolated

from 6 healthy controls (Supplemental Table S3), BM MNC from 9 patients with normal/reactive BM (Supplemental Table S2), and BM MNC isolated from 12 SM patients (ISM, n=3; SM-AHN, n=4; ASM, n=2; MCL, n=3; Supplemental Tables S1 and S4) using the commercial RNeasy MinEluteCleanupKit (Qiagen, Hilden, Germany). Complementary DNA (cDNA) was synthesized using Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT), Random Primers (3µg/µl), 5x First Strand Buffer, 0.1M DTT, dNTPs (10mM) (all from Invitrogen by Thermo Fisher Scientific, Carlsbad, CA, USA), and RNasin (Promega, Madison, WI, USA) according to the manufacturer`s instructions. PCR was performed using standards (10^3-10^6 cDNA copies) and primers specific for *CD44* and *ABL1* shown in Supplemental Table S8. *ABL1* served as a reference gene. Messenger RNA (mRNA) levels were quantified on the QuantStudio 3 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using iTAq^{TM} Universal SYBR Green Supermix with ROX (Bio-Rad, Hercules, CA, USA). *CD44* mRNA expression levels were normalized to *ABL1* mRNA levels and expressed as percentage of *ABL1* mRNA. Calculations were based on standard curves established for *CD44* and *ABL1* mRNA expression. The same method was applied to confirm the STAT5 knockdown of the lentivirally transduced HMC-1.2 and ROSA$^{KIT\ WT}$ cells using standards (10^3-10^6 cDNA copies) and primers specific for *STAT5* and *ABL1* shown in Supplemental Table S8.

Lentiviral transduction experiments

For *in vitro* studies, the oncogenic RAS mutant *KRAS* G12V and *KRAS* WT as well as the oncogenic STAT5 mutant *STAT5* S710F and *STAT5* WT were expressed by lentiviral-mediated gene delivery in HMC-1.2 and ROSA$^{KIT\ WT}$ cells. For this purpose,

the lentiviral pWPI vector (plasmid) containing the coding sequence of *KRAS* G12V or *KRAS* WT followed by an internal ribosome entry site and the GFP coding sequence was used.[8] Moreover, *STAT5* S710F or *STAT5* WT was cloned into the lentiviral pWPI vector (plasmid) containing the coding sequence *STAT5* S710F or *STAT5* WT followed by an internal ribosome entry site and the GFP coding sequence.[12] In order to knockdown STAT5 in HMC-1.2 and ROSA$^{KIT\ WT}$ cells by lentiviral-mediated gene delivery, short hairpin RNA (shRNA) targeting human/murine STAT5A and STAT5B (Supplemental Table S9) was cloned into a modified pLKO.1 lentivirus vector (plasmid) containing the m-Cherry gene.[12,13] A random sequence shRNA was used as control (Co shRNA).[12,13] For knockdown of CD44 in HMC-1.2 cells, four shRNA targeting human CD44 were cloned into the pRRL based SGEP, a lentiviral vector (plasmid) with an optimized miR-E backbone containing the GFP coding sequence as described.[14] Guide sequences of the four CD44 shRNA are provided in Supplemental Table S9. An shRNA targeting Renilla-luciferase (Ren.713)[14] was used as a control (Co shRNA). Then, recombinant VSV-G pseudotyped lentiviral viruses were produced by transient transfection of HEK 293T-cells as described.[8,12,13] The lentiviral transduction of HMC-1.2 and ROSA$^{KIT\ WT}$ cells was performed by spin infection in the presence of Hexadimethrine bromide (Polybrene) (7µg/ml) for 90 minutes. The knockdown of STAT5 was confirmed by qPCR (Supplemental Figures S2A,B) before analyzing CD44 expression. Moreover, the knockdown of CD44 was confirmed by flow cytometry (Supplemental Figure S3A) and the proliferative potential of these cells was determined by mixing with untransfected native cells (1:1) and analyzing GFP$^+$ cells in culture over 4 weeks. The GFP$^+$ HMC-1.2 and ROSA$^{KIT\ WT}$ cells overexpressing KRAS or STAT5 as well as their empty vector control were finally

purified by cell sorting (see above) and CD44 expression was examined by flow cytometry.

The CD44-knockdown in HMC-1.2 cells for *in vivo* studies (xenograft mouse model) was also achieved by lentiviral-mediated gene delivery through an shRNA-mediated approach. For this purpose, a 65 bp DNA oligomer containing a 19 bp anti-CD44 sequence (Supplemental Table S9, checked for potential off-target effects using PubMed BLAST) or a sequence against Firefly-luciferase as control (Co) shRNA was inserted into the pLVX-Puro vector (with a puromycin-resistance gene coding region) (Clontech, Saint-Germain-en-Laye, France) according to manufacturer's instructions. Lentiviral particles were produced as cell-free supernatants by transient transfection of HEK-293T as described.[15] In brief, lentiviral vectors based on pLVX-Puro (cloned either with CD44 shRNA or Co shRNA) were packaged using the second-generation packaging plasmid psPAX2 (Addgene #12260, Addgene, Teddington, UK) and phCMV-VSV-G[16] expressing the envelope protein of vesicular stomatitis virus (VSV). The supernatant was harvested 24 hours after transfection, 0.45 µm filtered, and stored at -80°C. HMC-1.2 cells were plated at 5×10^4 cells in 0.5 ml medium in each well of a 24 well plate. After addition of viral particle-containing supernatant to HMC-1.2 cells, the medium was replaced the next day, and puromycin (1 µg/ml) was added the second day after transduction. Then, puromycin selection was performed.[17] HMC-1.2 CD44-knockdown cells and control cells were then seeded (3 cells per well) in two 96 well plates each and growing HMC-1.2 sub-clones were screened for CD44 expression by flow cytometry as described.[17] In brief, cells were stained with fluorescein isothiocyanate (FITC)-conjugated anti-human CD44 mAb (B-F24) or the corresponding isotype control (Supplemental Table S5). Stained cells were examined

on CyFlow Cube 8 (Sysmex Partec GmbH, Kobe, Japan). Files were analyzed using FCSExpress 4.0 software (De Novo Software, Glendale, CA, USA). Finally, HMC-1.2 sub-clones expressing Co shRNA with unchanged CD44 surface expression compared to native HMC-1.2 cells and transduced HMC-1.2 CD44 knockdown cells with low CD44 expression (>85 % reduced fluorescence signal compared to the original/native HMC-1.2 cells) were pooled respectively and injected subcutaneously into severe combined immunodeficiency (SCID) mice.

Xenograft mouse model

Pathogen-free Balb/c SCID mice aged 9-14 weeks (weight: 25-30 g/animal) were housed in filter-top cages, providing food and water ad libitum. After subcutaneous injection of HMC-1.2 cells (10^6 per mouse), either transduced with CD44 or Co shRNA (15 SCID mice in each group), animals were inspected daily for tumor development. Moreover, all mice were evaluated by a standardized in-house scoring system based on movement/behavior, weight development, food/water intake and fur condition. Data on survival, ulceration status, and tumor weight were captured. Mice with ulcerated tumors or tumors with an estimated mass exceeding 10% of the animal's body-weight were sacrificed. The mice were killed by cervical dislocation after having been anesthetized by intraperitoneal injection of a weight-adapted dose (10 µl/g bodyweight) of a mixture of 1.2 ml Ketamin (Gräub AG, Bern, Switzerland), 0.8 ml Rompun (Bayer AG, Leverkusen, Germany), and 8 ml saline. DNA was extracted from murine lung tissue, BM and PB as previously described[18,19] and normalized to 30 ng/µl (lung tissue, BM) or 6 ng/µl (PB). 2µl total DNA (i.e. 60ng lung/BM-DNA, 12 ng PB-DNA) was used for each qPCR analysis. Human MC were

quantified by qPCR using established primers specific for human Alu sequences[20] shown in Supplemental Table S8 and numerical data of circulating MC were determined against a standard curve with 10-fold dilution of extracted DNA from $1x10^6$ HMC-1.2 cells to one cell as described.[21] For each sample, analyses were performed in duplicates and as independent experiments twice. Experiments were supervised by the institutional animal welfare officer and approved by the local licensing authority (Behörde für Soziales, Familie, Gesundheit, Verbraucherschutz; Amt für Gesundheit und Verbraucherschutz; Hamburg, Germany) under project number G10/100.

Statistical evaluation of data

To determine the significance of differences in CD44 expression on MC, SC and PC in the patients´ subgroups and controls, the Kruskal-Wallis test followed by Dunn's multiple comparisons test was applied. The same test was applied to determine the significance level in differences of sCD44 levels between various groups of patients and healthy controls. In order to check whether in control BM samples and BM samples obtained from patients with CM, CD44 expression on MC is independent of the MC burden a Spearman's rank correlation analysis was performed. For comparison of matched CD44 expression levels on MC, SC and PC in mastocytosis subgroups and controls, the Friedman test followed by Wilcoxon matched pairs signed rank tests was used. To determine differences in CD44 expression between untreated and drug-treated or lentiviral transduced cells, the unpaired two-sided Student´s t test was applied.

The probability of overall survival (OS), progression-free survival (PFS), and event-free survival (EFS) in patients with mastocytosis was calculated by the product limit method of Kaplan and Meier. Cut-off values were calculated by ROC curve analysis. Statistical significance of differences among patients with different expression levels of surface CD44 and serum levels of sCD44 (high vs. low expression/serum level) concerning OS, PFS and EFS was determined by log-rank test. Moreover, to evaluate the impact of sCD44 serum levels and the WHO classification for mastocytosis on OS and EFS, we used the Cox proportional hazards regression model. For all tests, differences were considered significant when $p<0.05$.

For *in vivo* studies in SCID mice, survival was analyzed according to Kaplan and Meier and statistical significance was determined by log-rank test. For statistical analysis of differences in subcutaneous primary tumor weight between the CD44-knockdown and control group, the Mann-Whitney U test was performed, whereas differences in ulceration rate (percentage of mice affected with ulcerated tumors) were determined by Fisher's exact test. To delineate significant differences in engraftment patterns, the Mann-Whitney U test and a linear prediction model was applied. The Mann-Whitney U test was performed to compare the MC load in various organs (lung, BM, PB) between the CD44-knockdown and control group. Moreover, a mixed-effects model repeated measurement (MMRM) was performed to estimate the effects of the mouse type (CD44-knockdown vs. control group) on the primary tumor weight and the MC load in various organs (lung, BM, PB). The outcome variable ´amount of HMC-1.2 cells´ was logarithmized to fulfill the model assumptions. To allow different effects of these predictors within the resulting subgroups the three-way interaction of these variables and all ancillary terms were included in the model as fixed effects.

Additionally, the potential confounder time until euthanization was included as fixed value. To take the cluster structure of the data into account, we included random intercepts. A backward elimination based on likelihood-ratio tests was performed to select the subset of significant predictors. With the resulting model, adjusted means and corresponding 95%-confidence intervals were calculated and visualized. Nominal p-values were reported without correction for multiplicity. Two-sided p-values <0.05 were considered to indicate statistical significance. All analyses were computed using Stata SE 14.0 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

Supplemental Tables

Supplemental Table S1

Characteristics of patients with mastocytosis

No.	Gender [f/m]	Age* [years]	Diagnosis	*KIT* mutation	Karyotype	Serum Tryptase [ng/ml]	Alkaline Phosphatase [U/L]	% MC in BM smear	% of MC in BM Sections by IHC**	Hemoglobin [g/dL]	Platelet Count [G/L]	White Blood Count [G/L]
1	f	56	CM	neg.	46,XX	5.1	88	<1	<1	14	236	6.1
2	f	73	CM	neg.	n.d.	23.5	40	<1	<1	12.6	233	8.38
3	f	38	CM	neg.	46,XX	10.5	93	<1	<1	13.4	342	7.7
4	f	36	CM	n.d.	46,XX	17.1	45	<1	<1	14.1	254	4.4
5	f	45	CM	neg.	n.d.	6.1	52	<1	<1	13.2	269	5.72
6	m	33	CM	n.d.	n.d.	12.4	88	<1	<1	15.6	150	8
7	m	10	CM	neg.	46,XY	11.8	148	<1	2	14.3	256	7.5
8	f	70	CM	neg.	n.d.	17.0	70	<1	<1	12.9	179	4.57
9	f	35	CM	D816V	n.d.	13.1	75	<1	<1	11.6	233	10.16
10	f	28	CM	n.d.	n.d.	12	75	<1	2	13.8	229	5.7
11	f	76	CM	n.d.	46,XX	8.5	105	<1	<1	12.2	229	3.7
12	m	45	CM	D816V	n.d.	9.2	47	<1	<1	14.7	260	4.72
13	m	49	CM	D816V	46,XY	12	39	<1	<1	15.3	345	4.66
14	f	66	CM	D816V	n.d.	6.5	80	<1	<1	12.9	215	6.86
15	m	42	CM	D816V	45, XY,rob(13;14) (q10;q10)	32.7	132	n.d.	n.d.	16	267	8.7
16	f	49	ISM	neg.	46,XX	22.9	73	1	10	13	251	5.38
17	m	47	ISM	D816V	46,XY	82.5	49	1	5	14.3	354	7.03
18	m	68	ISM	D816V	46,XY	83.3	61	1	10	12.6	363	9.03
19	m	56	ISM	D816V	46,XY	103	60	5	20	15.1	253	7.68
20	f	63	ISM	D816V	46,XX	77.8	91	<1	25	12.7	288	6.46
21	f	68	ISM	neg.	46,XX	47.2	67	<1	10	13.3	236	5.09
22	m	59	ISM	D816V	46,XY	46	75	<1	5	16.9	233	11.95
23	m	52	ISM	D816V	46,XY	234	90	3	25	15.4	256	8.81
24	m	55	ISM	D816V	46,XY	124	66	2	20	13.8	223	6.84
25	f	43	ISM	neg.	46,XX	36.2	61	2	20	12.8	212	6.26
26	f	57	ISM	D816V	46,XX	55.9	87	<1	5	14.3	173	5.54
27	f	46	ISM	D816V	46,XX	11.1	92	<1	5	10.9	362	5.25
28	f	37	ISM	D816V	46,XX	13.2	75	<1	5	12.9	303	10.43
29	f	70	ISM	D816V	46,XX	162	177	6	30	12.8	237	6.8
30	m	39	ISM	neg.	46,XY	35	105	1	10	16	207	4.29
31	f	53	ISM	D816V	46,XX	69.5	48	1	30	13.3	301	5.8
32	m	45	ISM	neg.	46,XY	35.4	59	<1	5	17.6	193	5.81
33	f	47	ISM	D816V	46,XX	34.9	65	<1	10	14.6	286	9.39
34	m	71	ISM	D816V	46,XY	14.5	73	<1	5	13.7	315	7.95
35	f	26	ISM	D816V	n.d.	36.7	48	<1	10	13	381	6.5
36	f	45	ISM	D816V	46,XX	938	155	2	15	14.2	278	9.6
37	f	47	ISM	D816V	46,XX	53.6	85	<1	10	14.2	178	3.78
38	f	34	ISM	D816V	n.d.	44.7	72	1	n.d.	13.2	323	5.7
39	f	52	ISM	D816V	46,XX	23.3	83	2	5	15.4	311	7.26
40	f	37	ISM	D816V	n.d.	69.7	149	1,5	10	12.7	272	7.4

41	m	37	ISM	D816V	46,XY	35.3	93	1	5	14.8	307	7.3
42	f	31	ISM	n.d.	46,XX	17.4	58	<1	5	12.8	200	6.7
43	f	56	ISM	D816V	46,XX	30	109	<1	5	13.7	380	9.66
44	m	64	ISM	D816V	46,XY	164	166	3	15	16.1	206	10.5
45	f	62	ISM	neg.	46,XX	25.6	78	2	10	14.8	257	5.4
46	m	72	ISM	D816V	46,XY	58.3	46	<1	15	14.5	221	5.68
47	m	33	ISM	D816V	n.d.	143	75	n.d.	10	14.3	210	4.41
48	m	36	ISM	n.d.	n.d.	34.4	113	n.d.	10	14.0	319	8.3
49	f	26	ISM	D816V	46,XX	72.3	46	<1	5	13.2	193	3.79
50	f	65	ISM	D816V	46,XX	130	80	<1	20	12.6	254	6.75
51	f	54	ISM	D816V	n.d.	11.9	69	1	5	14.5	173	6.78
52	f	42	ISM	D816V	n.d.	14.8	47	<1	25	13.1	205	4.26
53	f	68	ISM	D816V	46,XX	56.5	60	1	10	11.9	292	14.07
54	m	54	ISM	D816V	46,XY	37.3	50	<1	40	13.4	236	8.27
55	f	33	ISM	D816V	n.d.	15.5	47	n.d.	5	15	251	9.22
56	f	64	ISM	D816V	n.d.	40.1	43	<1	10	11.2	322	4.59
57	f	37	ISM	neg.	n.d.	46.5	158	n.d.	5	13.3	211	4.8
58	f	48	ISM	D816V	46,XY	53.7	130	<1	5	12	212	6.1
59	m	58	ISM	D816V	46,XY	70.7	97	<1	30	13	266	7.1
60	m	39	ISM	D816V	46,XY	70.8	101	<1	10	14.1	218	4.1
61	m	42	ISM	neg.	46,XY	14.5	92	2	5	14.1	279	5.2
62	f	57	ISM	D816V	46,XX	8.8	34	<1	2	14.4	193	6.08
63	m	34	ISM	D816V	46,XY	14	64	1	5	16	214	8.78
64	m	47	ISM	D816V	n.d.	31.4	79	n.d.	n.d.	15	195	6.61
65	m	37	ISM	D816V	46,XY	4.5	51	<1	3	15.2	226	5.95
66	f	40	ISM	D816V	46,XX	17.7	36	<1	5	13.8	185	5.89
67	f	48	ISM	D816V	n.d.	95	94	n.d.	n.d.	12.7	249	4.92
68	f	61	ISM	D816V	n.d.	35.8	75	<1	n.d.	15.5	236	6.05
69	m	45	ISM	D816V	46,XY	33	64	2	4	15.4	225	5.3
70	m	46	ISM	D816V	46,XY	28.8	81	<1	n.d.	16.7	318	7.39
71	f	46	ISM	D816V	46,XX	15.4	50	<1	4	13.8	222	5.87
72	f	59	ISM	D816V	46,XX	19	90	<1	5	15.1	329	10.84
73	f	57	ISM	D816V	46,XX	99.8	54	1	40	13.8	249	6.88
74	m	53	ISM	D816V	46,XY	21	58	4	5	15.4	276	4.22
75	m	30	ISM	D816V	n.d.	68.2	59	2	30	13.5	165	3.52
76	f	24	ISM	D816V	n.d.	4.8	48	<1	3	11.7	231	5.37
77	m	47	ISM	neg.	46, XY	16	65	<1	5	16.2	220	10.69
78	m	29	ISM	n.d.	n.d.	148	97	1	10	15.6	299	12.6
79	m	53	ISM	n.d.	46,XY	50	97	2	15	14.9	238	5.4
80	m	35	ISM	D816V	46,XY	27.1	52	<1	1	15.6	201	5.95
81	m	27	ISM	D816V	46,XY	75.4	79	n.d.	n.d.	16.5	182	3.41
82	m	44	ISM	D816V	46,XY	6.6	64	<1	2	16.1	262	5.46
83	f	22	ISM	D816V	46,XX	9.2	50	<1	3	13.4	228	4.96
84	m	70	ISM	neg.	46,XY	33.6	54	<1	4	13.2	88	2.86
85	f	50	ISM	D816V	46,XX	60.4	73	<1	40	13.5	238	5.35
86	f	29	ISM	D816V	46, XX, inv(2) (p16q11)c	43.4	79	<1	5	13.8	239	7.21
87	m	50	ISM	D816V	46,XY	53.6	76	<1	10	14.7	221	4.24
88	f	36	ISM	D816V	46,XX	29.5	66	<1	30	13.6	255	7.02
89	f	31	ISM	D816V	n.d.	51.2	41	<1	15	13.4	266	7.77
90	m	54	ISM	neg.	n.d.	26.5	80	n.d.	4	15.4	161	6.22
91	f	67	ISM	D816V	46,XX	39	63	1	5	12.6	237	6.54
92	m	28	ISM	D816V	46,XY	68.4	70	<1	10	15.3	217	7.53
93	f	52	ISM	D816V	46,XX	88.1	83	<1	n.d.	13.7	257	6.84
94	m	67	ISM	D816V	46,XY	25.5	32	<1	4	15.9	243	8.23
95	f	53	SSM	D816V	46,XX	352.5	186	2.5	50	12.8	296	4.8
96	f	65	SSM	D816V	46,XX	64.7	55	4	40	13.7	104	4.56
97	m	39	SSM	D816V	46,XY	937	129	2	45	14.2	221	6.2
98	f	35	SSM	n.d.	n.d.	374	81	n.d.	70	13.9	215	8.3
99	f	44	SSM	D816V	46,XX	175	124	1	30	13.2	236	3.4
100	m	74	SSM	D816V	45,X,-Y	252	88	5	30	14.4	196	6.7
101	f	47	SSM	D816V	46,XX	148	161	1	15	13.5	405	30.1
102	m	55	ISM-MGUS	D816V	n.d.	18.8	60	<1	3	16.1	264	9.75

103	m	42	ISM-MGUS	D816V	46,XY	90.6	145	2	25	13.3	204	6.3
104	m	65	ISM-CEL	neg.	46,XY	1.3	73	<1	n.d.	15.3	153	7.01
105	m	60	ISM-CEL	n.d.	n.d.	70.2	86	n.d.	n.d.	12.6	161	14.7
106	m	55	ISM-CLL	D816V	46,XY	534	177	1	15	10.8	162	230.8
107	m	64	ISM-MDS	D816V	46,XY	46	53	1	5	9.5	206	7.5
108	m	66	ISM-MPN	D816V	47,XY,+19	162	126	<1	5	10.8	282	7.5
109	m	64	ISM-MPN	D816V	46,XY	13	59	2	5	14.3	993	4.7
110	m	65	ISM-AML	neg.	46,XY	37.8	68	4	20	10.4	42	1.41
111	m	73	ISM-AML	D816V	45,XY,-7	446	677	<1	5	7.5	24	115
112	m	56	ISM-AML	neg.	46,XY,del(20)(q12;q13.12)	16.8	60	<1	5	6.2	47	1.19
113	f	70	ISM-AML	neg.	46,XX	1.8	70	<1	5	10.3	207	1.73
114	m	57	ISM-AML	n.d.	47,XY,del(20)(q11.2q13),+22	1290	291	3	10	10.6	11	6
115	f	64	ISM-AML	neg.	47,XX,t(9;14)(p?q?;p11-13)x2	8.9	90	n.d.	n.d.	11	48	0.98
116	m	49	SSM-MPN	D816V	46,XY	401	108	n.d.	60	15.8	160	12.34
117	m	55	ASM-CEL	D816V	46,XY	193	155	<1	10	12.1	247	8.9
118	m	76	ASM-CMML	D816V	46,XY	220	212	5	65	11.4	273	9.6
119	m	64	ASM-CMML	D816V	46,XY	220	308	5	50	10.8	42	2.9
120	m	72	ASM-CMML	D816V	46,XY,9ph+	38	695	1	30	10.3	55	17
121	m	78	ASM-CMML	D816V	46,XY	110	80	5	10	11.3	48	6.2
122	m	71	ASM-CMML	D816V	46,XY	73.7	215	2	10	11.1	64	4.06
123	m	67	ASM-CMML	D816V	n.d.	164	226	<1	10	9.9	90	15.11
124	m	53	ASM-CMML	D816V	46,XY	615	944	5	70	8.8	53	28
125	f	70	ASM-CMML	D816V	46,XX	30	122	5	10	11.5	184	17.65
126	m	78	ASM-MDS/MPN	D816V	45,XY,-7	45.9	155	1	10	8.2	76	27.5
127	m	63	ASM-MDS/MPN	D816V	46,XY	80	743	<1	25	10.9	63	33.5
128	f	81	ASM-MDS/MPN	D816V	46,XX	284	90	<1	50	9.7	103	6.7
129	f	71	ASM-MPN	D816V	46,XX	113	165	3	20	10.9	179	4.9
130	m	75	ASM-MPN	D816V	46,XY	172	60	5	5	12.1	188	13.4
131	m	62	ASM-MPN	D816V	46,XY	427	244	10	35	8	374	8.7
132	m	74	ASM-MPN	D816V	46,XY	71	170	5	5	13.4	168	4.2
133	f	58	ASM-AML	D816V	46,XX	173	61	<1	20	7.8	6	3.8
134	m	77	ASM-AML	D816V	46,XY	101	66	5	50	7.9	52	35
135	f	81	ASM-AML	D816V	47,XX,t(5;10)(q34;p15),+8,der(19)t(3;19)(q11;q13)	38	628	<1	20	7.5	11	12.3
136	m	63	ASM-AML	D816V	46,XY	33.9	133	<1	15	8.8	17	18.2
137	f	54	ASM-AML	D816V	46,XX	1510	88	2	50	10.4	51	23.4
138	m	71	ASM-AML	D816V	46,XY	31.8	86	2	10	11.7	73	48.1
139	m	81	MCL-MM	neg.	n.d.	180	59	50	50	10.5	24	2.46
140	m	73	MCL-MDS/MPN	D816V	46,XY	280	259	30	20	8.9	32	49.2
141	f	62	MCL-AML	D816V	46,XX,del(5q13q31)	547	469	50	70	9.4	64	30
142	f	57	ASM	D816V	46,XX	87.9	218	2	50	9	98	3.1
143	m	74	ASM	D816V	46,XY	152	129	<1	10	9.3	140	6.83
144	f	56	ASM	D816V	46,XX	150	166	5	30	12.4	75	4.2
145	f	54	ASM	D816V	45,X	31.9	496	<1	35	11.4	113	3.68
146	m	89	ASM	D816V	46,XY	125	121	2	20	10.9	196	17.13
147	m	28	ASM	D816V	46,XY	14	169	5	10	12.4	99	3.9
148	m	59	ASM	D816V	46,XY	285	494	14	70	9.6	115	6.8
149	m	21	ASM	neg.	46,XY	72	270	37	80	12.9	269	5.0

150	m	72	ASM	D816V	46,XY	199	97	<1	60	9.6	95	4.86
151	f	34	ASM	neg.	46,XX	256	158	21	10	11.3	486	7.21
152	m	71	ASM	D816V	46,XY	88.6	620	n.d.	15	8.3	47	16.16
153	m	61	ASM	D816V	46,XY	102	288	<1	15	9	41	7.23
154	f	54	MCL	D816H	46,XX,del(7)(q22?),der(10)t(7;10)(q22?;q26?)	904	188	60	80	8.4	63	54.64
155	m	58	MCL	D816H	46,XY	151	96	20	25	10.8	87	3.6
156	m	90	MCL	D816V	46,XY	330	223	25	35	9.5	32	8.23
157	f	49	MCL	S476I	46,XX	533	55	75	70	10.6	190	4.43
158	m	64	MCL	neg.	46,XY	767	104	50	80	8.7	57	2.7
159	f	80	MCL	D816V	46,XX	151	98	20	15	10.1	78	2.4
160	m	62	MCL	D816V	46,XY	309	895	28	80	9.9	55	12.5
161	m	25	MCL	neg.	46,XY	1910	86	58	100	13.3	58	6.1

*Age at the time of bone marrow (BM) or serum sampling; in most cases, BM was obtained at diagnosis. **The percentage of mast cells (MC) of all nucleated cells in the BM was determined by immunohistochemistry using an antibody against tryptase. Abbreviations: No., number; m, male; f, female; MC, mast cells; BM, bone marrow; IHC, immunohistochemistry; CM, cutaneous mastocytosis; ISM, indolent systemic mastocytosis; SSM, smoldering systemic mastocytosis; ASM, aggressive systemic mastocytosis; MCL, mast cell leukemia; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; CEL, chronic eosinophilic leukemia; CLL, chronic lymphocytic leukemia; CMML, chronic myelomonocytic leukemia; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; AML, acute myeloid leukemia; n.d., not determined.

Supplemental Table S2

Characteristics and CD44 expression of control patients with mainly lymphoproliferative disorders

No.	Gender [f/m]	Age* [years]	Diagnosis	Hemoglobin [g/dL]	Platelet Count [G/L]	White Blood Count [G/L]	% MC in BM**	CD44 SI** of BM-MC ($CD45^{+}/CD117^{++}/CD34^{-}$)	CD44 SI** of BM-SC ($CD45^{+}/CD34^{+}/CD38^{-}$)	CD44 SI** of BM-PC ($CD45^{+}/CD34^{+}/CD38^{+}$)	CD44 mRNA Analysis [qPCR]
1	m	33	RBM	11.2	189	4.84	n.d.	n.d.	4.94	6.15	qPCR
2	m	64	RBM	12.4	337	9.30	0.001	6.46	13.80	9.86	n.d.
3	m	68	RBM	9.4	250	11.56	0.001	3.64	8.83	9.38	qPCR
4	m	74	RBM	8.8	199	3.59	0.004	6.82	9.06	11.96	n.d.
5	m	80	RBM	12.6	190	6.07	0.002	4.24	10.25	15.57	n.d.
6	m	21	ICUS	16.6	83	3.73	0.007	2.15	9.72	12.45	n.d.
7	m	61	ICUS	14.3	86	3.31	0.02	6.22	10.42	17.37	qPCR
8	f	64	ICUS	10.5	374	14.05	0.001	6.85	8.50	7.94	n.d.
9	f	38	HD	9.6	614	10.98	n.d.	n.d.	11.43	14.08	qPCR
10	f	41	HD	10.8	379	13.3	0.002	9.22	12.82	15.84	qPCR
11	f	68	MGUS, IgA-kappa	9.2	172	5.57	0.002	5.06	8.14	15.46	qPCR
12	m	75	MGUS, IgA-lambda	15.7	259	6.53	0.01	4.94	9.29	10.89	qPCR
13	m	58	MGUS, IgG-kappa	16.2	222	9.46	0.002	2.28	7.39	8.30	qPCR
14	m	55	MGUS, IgG-lambda	13.9	191	11.21	0.002	5.61	8.69	13.29	n.d.
15	m	56	AL-amyloidosis-kappa	12.7	241	8.71	n.d.	n.d.	4.91	3.99	n.d.
16	f	71	AL-amyloidosis-lambda	11.8	316	6.73	0.005	3.48	11.15	14.25	n.d.
17	m	18	B-NHL (DLBCL)	12.2	362	9.51	n.d.	n.d.	4.29	4.90	n.d.
18	m	34	B-NHL (DLBCL)	12.6	206	7.80	n.d.	n.d.	13.05	15.91	n.d.
19	f	42	B-NHL (DLBCL)	9.8	182	9.84	0.004	7.03	14.33	16.44	n.d.
20	m	45	B-NHL (DLBCL)	14.0	375	8.68	n.d.	n.d.	6.26	10.52	n.d.
21	m	49	B-NHL (DLBCL)	8.0	22	1.48	0.013	2.73	2.93	1.15	n.d.
22	m	50	B-NHL (DLBCL)	13.1	275	10.27	0.005	4.82	5.78	8.00	n.d.
23	f	56	B-NHL (DLBCL)	10.1	155	35.92	0.002	4.93	1.52	9.62	n.d.
24	f	59	B-NHL (DLBCL)	8.6	17	8.30	0.01	6.09	n.d.	11.04	n.d.
25	f	59	B-NHL (DLBCL)	13.3	450	8.22	0.005	6.76	9.15	11.55	n.d.
26	m	70	B-NHL (DLBCL)	13.6	228	8.45	n.d.	n.d.	6.78	9.64	n.d.
27	m	70	B-NHL (DLBCL)	11.3	422	6.89	0.002	4.71	4.53	6.30	n.d.
28	m	76	B-NHL (DLBCL)	12.0	266	15.06	0.001	5.98	11.79	14.95	n.d.
29	m	79	B-NHL (DLBCL)	10.2	197	7.81	0.008	3.31	3.66	6.20	n.d.
30	m	85	B-NHL (DLBCL)	13.6	284	6.29	0.002	3.36	6.68	5.58	n.d.
31	f	87	B-NHL (DLBCL)	12.9	297	9.83	n.d.	n.d.	4.71	11.71	n.d.
32	m	36	B-NHL (FL)	15.1	245	7.02	n.d.	n.d.	12.50	11.70	n.d.
33	m	50	B-NHL (FL)	14	84	6.73	n.d.	n.d.	7.25	10.34	n.d.
34	m	66	B-NHL (IC, IgM-kappa)	10.0	178	5.54	0.03	5.21	n.d.	n.d.	n.d.
35	f	73	B-NHL (IC, IgM-kappa)	9.4	245	30.34	0.003	3.46	7.82	10.54	n.d.
36	m	70	B-NHL (IC, IgM-kappa/lambda)	9.7	108	2.04	0.01	5.40	13.23	8.81	n.d.
37	m	63	B-NHL (IC, IgM-lambda)	13.5	149	10.77	0.004	5.14	11.89	14.26	n.d.
38	m	73	B-NHL (IC, IgM-lambda)	9.7	345	8.12	n.d.	n.d.	10.55	15.37	n.d.
39	m	87	B-NHL (IC, IgM-lambda)	13.3	206	5.48	0.006	5.89	9.65	16.59	n.d.
40	m	75	B-NHL (MM, IgA-kappa)	11.4	203	6.60	0.002	6.11	10.20	13.57	n.d.
41	f	41	B-NHL (MM, IgG-kappa)	7.9	152	6.84	0.003	5.29	n.d.	n.d.	n.d.
42	m	69	B-NHL (MM, IgG-kappa)	11.0	70	2.87	0.009	3.32	8.26	12.06	n.d.
43	m	69	B-NHL (MM, IgG-kappa)	7.7	294	13.18	0.001	5.30	9.64	8.16	n.d.
44	m	50	B-NHL (MM, IgG-lambda)	13.4	246	5.00	0.001	5.24	11.08	14.67	n.d.
45	m	56	B-NHL (MM, IgG-lambda)	15.8	277	9.92	0.002	3.60	8.40	9.19	n.d.
46	f	67	B-NHL (MM, IgG-lambda)	9.9	275	6.24	0.002	4.46	6.34	11.13	n.d.

47	f	71	B-NHL (MM, IgG-lambda)	10.7	337	7.27	0.002	4.54	6.15	8.78	n.d.
48	m	75	B-NHL (MM, IgG-lambda)	9.7	414	9.67	0.001	6.01	11.51	15.68	n.d.
49	f	57	B-NHL (MM, LC-kappa)	11.9	111	4.35	0.005	5.56	4.07	7.74	n.d.
50	m	74	B-NHL (MM, LC-lambda)	15.2	157	6.53	0.003	5.28	10.04	15.18	n.d.
51	m	65	B-NHL (SLL)	13.8	202	7.48	0.007	7.15	15.56	19.26	n.d.
52	f	58	B-NHL (NOS)	9.5	368	7.97	0.008	9.35	10.49	16.49	n.d.
53	f	22	T-NHL (ALCL)	13.5	312	9.99	0.002	5.44	11.68	15.00	n.d.
54	f	37	T-NHL (GD-TCL)	11.9	171	2.45	n.d.	n.d.	12.07	10.90	n.d.
55	f	58	T-NHL (SPTCL)	12.3	238	4.85	n.d.	n.d.	12.17	14.40	n.d.
56	m	51	T-NHL (T-LBL)	15.1	257	46.8	0.01	6.06	8.61	7.29	qPCR
57	m	42	T-NHL (T-LGL)	14.9	291	12.48	0.001	8.20	8.66	11.28	n.d.

*Age at the time of bone marrow (BM) sampling; in most cases, BM was obtained at diagnosis. **The percentage of mast cells (MC) of all nucleated cells in the BM as well as the Staining Index (SI) of the 3 cell populations analyzed was determined using flow cytometry. Abbreviations: No, number; m, male; f, female; MC, mast cells; BM, bone marrow; SI, Staining Index; SC, stem cells; PC, progenitor cells; qPCR, quantitative polymerase chain reaction; RBM, normal reactive bone marrow (suspected lymphoproliferative disorder); ICUS, idiopathic cytopenia of undetermined significance; HD, Hodgkin's disease (without BM involvement); MGUS, monoclonal gammopathy of undetermined significance; AL-amyloidosis, amyloid light-chain amyloidosis; B-NHL, B-Non-Hodgkin lymphoma; DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; IC, immunocytoma (Waldenström's macroglobulinemia, lymphoplasmacytic lymphoma); MM, multiple myeloma; LC, light chain; SLL, small lymphocytic lymphoma; NOS, not otherwise specified; T-NHL, T-Non-Hodgkin lymphoma; ALCL, anaplastic large cell lymphoma; GD-TCL, primary cutaneous gamma/delta T-cell lymphoma; SPTCL, subcutaneous panniculitis-like T-cell lymphoma; T-LBL, T-cell lymphoblastic lymphoma; T-LGL, T-cell large granular lymphocyte leukemia; n.d., not determined.

Supplemental Table S3

Characteristics and soluble CD44 (sCD44) serum levels of healthy controls

No.	Gender [f/m]	Age* [years]	Diagnosis	Serum sCD44** [ng/ml]	CD44 mRNA Analysis [qPCR]
1	m	32	Healthy Control	181.865	qPCR
2	f	35	Healthy Control	187.100	n.d.
3	f	31	Healthy Control	188.630	n.d.
4	f	35	Healthy Control	160.416	qPCR
5	f	29	Healthy Control	123.593	n.d.
6	m	36	Healthy Control	102.334	n.d.
7	f	32	Healthy Control	194.937	qPCR
8	f	32	Healthy Control	180.415	n.d.
9	f	34	Healthy Control	116.147	n.d.
10	f	32	Healthy Control	152.986	n.d.
11	m	27	Healthy Control	150.678	qPCR
12	m	27	Healthy Control	141.115	qPCR
13	f	54	Healthy Control	99.126	n.d.
14	f	26	Healthy Control	177.821	qPCR
15	m	50	Healthy Control	160.014	n.d.

*Age at the time of serum sampling. **soluble CD44 (sCD44) was analyzed by ELISA. Abbreviations: No, number; m, male; f, female; sCD44, soluble CD44; qPCR, quantitative polymerase chain reaction; n.d., not determined.

Supplemental Table S4

CD44 expression and soluble CD44 serum levels of mastocytosis patients´ cohort

No.	Gender [f/m]	Age* [years]	Diagnosis	CD44 SI** of BM-MC ($CD45^{+}$/ $CD117^{++}$/ $CD34^{-}$)	CD44 SI** of BM-SC ($CD45^{+}$/$CD34^{+}$/$CD38^{-}$)	CD44 SI** of BM-PC ($CD45^{+}$/$CD34^{+}$/$CD38^{+}$)	CD44 Immunostaining [IHC/ICC]	CD44 mRNA Analysis [qPCR]	Serum sCD44*** [ng/ml]
1	f	56	CM	4.77	9.85	11.67	n.d.	n.d.	143.177
2	f	73	CM	4.79	10.77	13.48	n.d.	n.d.	125.359
3	f	38	CM	n.d.	n.d.	n.d.	n.d.	n.d.	182.856
4	f	36	CM	n.d.	n.d.	n.d.	n.d.	n.d.	141.868
5	f	45	CM	n.d.	n.d.	n.d.	n.d.	n.d.	156.614
6	m	33	CM	n.d.	n.d.	n.d.	n.d.	n.d.	130.138
7	m	10	CM	n.d.	n.d.	n.d.	n.d.	n.d.	165.477
8	f	70	CM	n.d.	n.d.	n.d.	n.d.	n.d.	188.484
9	f	35	CM	n.d.	n.d.	n.d.	n.d.	n.d.	155.418
10	f	28	CM	n.d.	n.d.	n.d.	n.d.	n.d.	112.833
11	f	76	CM	n.d.	n.d.	n.d.	n.d.	n.d.	103.337
12	m	45	CM	n.d.	n.d.	n.d.	n.d.	n.d.	128.760
13	m	49	CM	n.d.	n.d.	n.d.	n.d.	n.d.	185.360
14	f	66	CM	n.d.	n.d.	n.d.	n.d.	n.d.	109.233
15	m	42	CM	n.d.	n.d.	n.d.	n.d.	n.d.	204.136
16	f	49	ISM	25.02	20.27	34.11	n.d.	n.d.	236.129
17	m	47	ISM	55.40	84.57	81.17	n.d.	qPCR	154.982
18	m	68	ISM	11.85	10.21	13.82	n.d.	n.d.	145.782
19	m	56	ISM	6.57	8.91	7.15	n.d.	n.d.	n.d.
20	f	63	ISM	20.09	15.97	20.86	n.d.	n.d.	246.587
21	f	68	ISM	22.28	25.37	44.40	n.d.	n.d.	215.733
22	m	59	ISM	18.70	3.32	3.89	n.d.	n.d.	244.319
23	m	52	ISM	10.28	9.24	11.65	IHC	n.d.	212.269
24	m	55	ISM	29.18	32.95	38.96	IHC	n.d.	n.d.
25	f	43	ISM	23.60	38.97	36.07	IHC	n.d.	n.d.
26	f	57	ISM	11.67	18.29	23.08	IHC	n.d.	248.135
27	f	46	ISM	16.73	12.83	12.18	n.d.	n.d.	172.300
28	f	37	ISM	18.58	19.27	18.00	n.d.	n.d.	212.197
29	f	70	ISM	10.79	11.80	24.82	n.d.	n.d.	215.649
30	m	39	ISM	3.24	2.56	3.74	IHC	n.d.	n.d.
31	f	53	ISM	3.11	2.92	6.51	n.d.	n.d.	n.d.
32	m	45	ISM	6.87	5.34	5.59	n.d.	qPCR	223.326
33	f	47	ISM	17.57	17.76	20.50	n.d.	qPCR	218.481
34	m	71	ISM	8.00	8.04	8.59	n.d.	n.d.	260.916
35	f	26	ISM	6.66	9.57	12.43	n.d.	n.d.	169.361
36	f	45	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	150.947
37	f	47	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	160.035
38	f	34	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	148.919
39	f	52	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	282.285
40	f	37	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	185.484
41	m	37	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	140.883
42	f	31	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	118.324
43	f	56	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	237.664
44	m	64	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	200.770
45	f	62	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	185.381
46	m	72	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	178.822
47	m	33	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	150.145
48	m	36	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	164.243

49	f	26	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	133.257
50	f	65	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	237.488
51	f	54	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	240.225
52	f	42	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	150.190
53	f	68	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	184.470
54	m	54	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	181.775
55	f	33	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	180.345
56	f	64	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	239.692
57	f	37	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	133.981
58	f	48	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	150.582
59	m	58	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	117.768
60	m	39	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	139.457
61	m	42	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	176.614
62	f	57	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	179.577
63	m	34	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	143.435
64	m	47	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	137.637
65	m	37	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	161.469
66	f	40	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	183.153
67	f	48	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	157.690
68	f	61	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	169.217
69	m	45	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	158.315
70	m	46	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	134.981
71	f	46	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	153.971
72	f	59	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	178.649
73	f	57	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	261.572
74	m	53	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	147.041
75	m	30	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	119.958
76	f	24	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	136.555
77	m	47	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	189.639
78	m	29	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	236.719
79	m	53	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	159.331
80	m	35	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	149.673
81	m	27	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	230.292
82	m	44	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	165.278
83	f	22	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	169.564
84	m	70	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	155.883
85	f	50	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	128.167
86	f	29	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	95.255
87	m	50	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	136.636
88	f	36	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	184.013
89	f	31	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	181.526
90	m	54	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	231.762
91	f	67	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	188.703
92	m	28	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	150.308
93	f	52	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	174.769
94	m	67	ISM	n.d.	n.d.	n.d.	n.d.	n.d.	168.204
95	f	53	SSM	n.d.	n.d.	n.d.	n.d.	n.d.	202.000
96	f	65	SSM	n.d.	n.d.	n.d.	n.d.	n.d.	270.031
97	m	39	SSM	n.d.	n.d.	n.d.	n.d.	n.d.	321.132
98	f	35	SSM	n.d.	n.d.	n.d.	n.d.	n.d.	297.803
99	f	44	SSM	n.d.	n.d.	n.d.	n.d.	n.d.	225.520
100	m	74	SSM	n.d.	n.d.	n.d.	n.d.	n.d.	274.603
101	f	47	SSM	n.d.	n.d.	n.d.	n.d.	n.d.	283.205
102	m	55	ISM-MGUS	n.d.	n.d.	n.d.	n.d.	n.d.	294.427
103	m	42	ISM-MGUS	n.d.	n.d.	n.d.	n.d.	n.d.	221.894
104	m	65	ISM-CEL	n.d.	n.d.	n.d.	n.d.	n.d.	190.183
105	m	60	ISM-CEL	n.d.	n.d.	n.d.	n.d.	n.d.	303.327
106	m	55	ISM-CLL	n.d.	n.d.	n.d.	n.d.	n.d.	841.067
107	m	64	ISM-MDS	12.92	16.31	16.08	n.d.	n.d.	n.d.
108	m	66	ISM-MPN	8.85	7.07	11.09	n.d.	n.d.	n.d.
109	m	64	ISM-MPN	39.28	n.d.	n.d.	n.d.	n.d.	n.d.
110	m	65	ISM-AML	8.05	4.00	4.07	IHC	n.d.	455.355
111	m	73	ISM-AML	n.d.	n.d.	n.d.	n.d.	n.d.	1002.171
112	m	56	ISM-AML	n.d.	n.d.	n.d.	n.d.	n.d.	163.945

113	f	70	ISM-AML	n.d.	n.d.	n.d.	n.d.	n.d.	189.949
114	m	57	ISM-AML	n.d.	n.d.	n.d.	n.d.	n.d.	294.514
115	f	64	ISM-AML	n.d.	n.d.	n.d.	n.d.	n.d.	208.677
116	m	49	SSM-MPN	n.d.	n.d.	n.d.	n.d.	n.d.	173.163
117	m	55	ASM-CEL	37.51	75.56	56.25	n.d.	n.d.	n.d.
118	m	76	ASM-CMML	11.35	8.57	7.59	n.d.	n.d.	n.d.
119	m	64	ASM-CMML	11.40	7.68	8.46	n.d.	n.d.	n.d.
120	m	72	ASM-CMML	41.55	49.67	39.82	n.d.	qPCR	n.d.
121	m	78	ASM-CMML	27.30	11.75	18.30	n.d.	n.d.	n.d.
122	m	71	ASM-CMML	n.d.	n.d.	n.d.	n.d.	n.d.	241.461
123	m	67	ASM-CMML	n.d.	n.d.	n.d.	n.d.	n.d.	411.614
124	m	53	ASM-CMML	n.d.	n.d.	n.d.	n.d.	n.d.	401.084
125	f	70	ASM-CMML	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
126	m	78	ASM-MDS/MPN	41.60	73.20	79.02	IHC	n.d.	589.430
127	m	63	ASM-MDS/MPN	49.11	55.86	45.19	n.d.	qPCR	n.d.
128	f	81	ASM-MDS/MPN	32.60	18.41	23.26	n.d.	n.d.	n.d.
129	f	71	ASM-MPN	35.43	16.61	17.12	n.d.	n.d.	n.d.
130	m	75	ASM-MPN	11.06	26.46	16.71	n.d.	n.d.	n.d.
131	m	62	ASM-MPN	35.38	17.50	17.36	n.d.	n.d.	n.d.
132	m	74	ASM-MPN	6.46	8.80	9.50	n.d.	n.d.	n.d.
133	f	58	ASM-AML	n.d.	n.d.	n.d.	n.d.	n.d.	303.429
134	m	77	ASM-AML	15.84	52.25	52.65	n.d.	n.d.	n.d.
135	f	81	ASM-AML	10.01	19.98	12.71	n.d.	n.d.	n.d.
136	m	63	ASM-AML	35.83	198.75	254.19	n.d.	qPCR	518.027
137	f	54	ASM-AML	n.d.	n.d.	n.d.	n.d.	n.d.	817.714
138	m	71	ASM-AML	n.d.	n.d.	n.d.	n.d.	n.d.	627.441
139	m	81	MCL-MM	14.72	4.31	10.23	n.d.	n.d.	n.d.
140	m	73	MCL-MDS/MPN	22.28	22.17	17.92	n.d.	n.d.	n.d.
141	f	62	MCL-AML	27.13	152.39	65.94	n.d.	qPCR	n.d.
142	f	57	ASM	16.46	17.46	37.72	n.d.	n.d.	211.267
143	m	74	ASM	22.54	35.58	73.38	IHC	n.d.	569.490
144	f	56	ASM	9.78	12.62	15.06	n.d.	n.d.	n.d.
145	f	54	ASM	37.65	49.36	43.04	IHC	qPCR	n.d.
146	m	89	ASM	44.31	107.27	130.29	n.d.	qPCR	466.999
147	m	28	ASM	45.32	22.16	21.09	n.d.	n.d.	n.d.
148	m	59	ASM	n.d.	n.d.	n.d.	n.d.	n.d.	166.815
149	m	21	ASM	n.d.	n.d.	n.d.	n.d.	n.d.	278.414
150	m	72	ASM	n.d.	n.d.	n.d.	n.d.	n.d.	347.747
151	f	34	ASM	n.d.	n.d.	n.d.	n.d.	n.d.	500.395
152	m	71	ASM	n.d.	n.d.	n.d.	n.d.	n.d.	486.401
153	m	61	ASM	n.d.	n.d.	n.d.	n.d.	n.d.	373.794
154	f	54	MCL	72.96	95.37	46.35	IHC/ICC	qPCR	n.d.
155	m	58	MCL	19.76	20.52	30.55	n.d.	n.d.	n.d.
156	m	90	MCL	10.43	51.40	132.06	IHC	n.d.	672.157
157	f	49	MCL	125.73	43.29	47.95	IHC/ICC	qPCR	353.987
158	m	64	MCL	59.02	24.67	21.57	n.d.	qPCR	n.d.
159	f	80	MCL	45.86	19.02	23.22	n.d.	n.d.	n.d.
160	m	62	MCL	n.d.	n.d.	n.d.	n.d.	n.d.	318.428
161	m	25	MCL	n.d.	n.d.	n.d.	n.d.	n.d.	336.024

*Age at the time of bone marrow (BM) or serum sampling; in most cases, BM was obtained at diagnosis. **The Staining Index (SI) of the 3 cell populations analyzed was determined using flow cytometry. ***soluble CD44 (sCD44) was analyzed by ELISA. No., number; m, male; f, female; SI, Staining Index; MC, mast cells; BM, bone marrow; SC, stem cells; PC, progenitor cells; IHC, immunohistochemistry; ICC, immunocytochemistry; qPCR, quantitative polymerase chain reaction; sCD44, soluble CD44; CM, cutaneous mastocytosis; ISM, indolent systemic mastocytosis; SSM, smoldering SM; ASM, aggressive SM; MCL, mast cell leukemia; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; CEL, chronic eosinophilic leukemia; CLL, chronic lymphocytic leukemia; CMML, chronic myelomonocytic leukemia; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; AML, acute myeloid leukemia; n.d., not determined.

Supplemental Table S5

Specification of antibodies used in flow cytometric experiments

CD	Antigen	Clone	Fluorochrome Conjugate	Species, Isotyp	Manufacturer*
CD14	LPSRr	TÜK4	FITC	Mouse, IgG2a	Dako
CD34	HPCA-1	581	Pacific Blue	Mouse, IgG1	BioLegend
CD38	ADP-ribosyl cyclase 1	HIT-2	APC	Mouse, IgG1	BD Biosciences
CD44	Pgp-1/ HCAM	515	PE	Mouse, IgG1	BD Biosciences
CD44	Pgp-1/ HCAM	B-F24	FITC	Mouse, IgG1	Diaclone
CD44	Pgp-1/ HCAM	BJ18	APC	Mouse, IgG1	BioLegend
CD45	PTPRC	HI30	APC-Cyanine7	Mouse, IgG1	BioLegend
CD45	PTPRC	2D1	PerCP	Mouse, IgG1	BD Biosciences
CD117	KIT	104D2	PE-Cyanine7	Mouse, IgG1	eBioscience

*Manufacturers´ locations: Dako: Glostrup, Denmark; BioLegend: San Diego, CA, USA; Becton Dickinson (BD): Franklin Lakes, NJ, USA; Diaclone SAS, Besancon Cedex, France; Affymertix eBiosciences, Santa Clara, CA, USA. Abbreviations: CD, cluster of differentiation; LPSRr, lipopolysaccharide receptor-related antigen; HPCA-1: hematopoietic progenitor cell antigen-1; Pgp-1, phagocytic glycoprotein-1; HCAM, homing cell adhesion molecule. PTPRC, protein tyrosine phosphatase receptor type C; FITC, fluorescein isothiocyanate; APC, allophycocyanin; PE, phycoerythrin; PerCP, peridinin chlorophyll; IgG, immunoglobulin G; BD, Becton Dickinson.

Supplemental Table S6

In control cases, CD44 expression on BM MC is independent of the MC load in the BM samples

Spearman's rank correlation in control cases and patients with CM	n	Correlation coefficient	95% CI	p-Value
Percentage of MC in the BM vs. CD44 expression on MC [SI]	46	-0.06620	-0.3576 to 0.2369	0.6620

In 44 control cases without mastocytosis (Supplemental Table S2) and 2 patients with CM (Supplemental Tables S1 and S4) BM MC were stained with an antibody against CD44. A Spearman's rank correlation analysis was performed to determine the correlation between the numbers of MC in the samples and expression levels of CD44 on MC. As visible, no correlation was found between the percentage of BM MC and CD44 expression on MC (shown as SI) ($p>0.05$). Abbreviations: BM, bone marrow; MC, mast cells; CM, cutaneous mastocytosis; SI, Staining Index; n, total number; CI, confidence interval.

Supplemental Table S7

In control cases, expression levels of CD44 on BM MC are independent of the underlying diagnosis

Diagnosis	n	Median SI (CD44 on MC)	95% CI of the Median
RBM	4	5.350	3.639 – 6.823
ICUS	3	6.223	2.146 – 6.854
MGUS/AL-amyloidosis	5	4.944	2.276 – 5.610
NHL	32	5.297	4.821 – 6.014
CM	2	4.782	4.771 – 4.793
Kruskal-Wallis test in control cases and patients with CM		**Number of values**	**p-Value**
RBM, ICUS, MGUS/AL-amyloidosis, NHL, and CM vs. CD44 expression on MC [SI]		46	0.5891

In 44 control cases without mastocytosis (Supplemental Table S2) and 2 patients with CM (Supplemental Tables S1 and S4) expression levels of CD44 on BM MC (shown as SI) were determined by multicolor flow cytometry. To define the level of significance in differences of expression of CD44 on BM MC in various subgroups (RBM, ICUS, MGUS/AL-amyloidosis, NHL, CM), a Kruskal-Wallis test was performed. As visible, no significant differences in CD44 expression on BM MC (median SI) between these 5 groups were found. Abbreviations: BM, bone marrow; MC, mast cells; n, number of cases; SI, Staining Index; CI, confidence interval; RBM, normal/reactive bone marrow; ICUS, idiopathic cytopenia of undetermined significance; MGUS, monoclonal gammopathy of undetermined significance; AL-amyloidosis, amyloid light-chain amyloidosis; NHL, Non-Hodgkin lymphoma; CM, cutaneous mastocytosis.

Supplemental Table S8

Sequences of human oligonucleotide primers used for qPCR

Gene	Sequence
CD44-fwd	5’-CGGACACCATGGACAAGTTT-3’
CD44-rev	5’-GCAAAGCGGCAGGTTATATT-3’
STAT5-fwd	5’-CCAGGAGAGCCTGAGGATCCAA-3’
STAT5-rev	5’-TCTGCTGGAGGGCCGTCTCC-3’
ABL1-fwd	5’-TGTATGATTTTGTGGCCAGTGGAG-3’
ABL1-rev	5’-GCCTAAGACCCGGAGCTTTTCA-3’
Alu-fwd	5’-TGGCTCACGCCTGTAATCCCA-3’
Alu-rev	5’-GCCACTACGCCCGGCTAATTT-3’

Abbreviations: qPCR, quantitative polymerase chain reaction; fwd, forward; rev, reverse; ABL1, Abelson murine leukemia virus oncogene homolog 1; Alu, Arthrobacter luteus.

Supplemental Table S9

CD44 shRNA and STAT5 shRNA sequences used for knockdown in human mast cell lines for *in vitro* and *in vivo* studies

shRNA	Sequence*
(1) CD44.492	5'-TATATTCAAATCGATCTGCGCC-3'
(2) CD44.488	5'-TTCAAATCGATCTGCGCCAGGC-3'
(3) CD44.584	5'-TTGAAAGCCTTGCAGAGGTCAG-3'
(4) CD44.586	5'-TATTGAAAGCCTTGCAGAGGTC-3'
STAT5A and STAT5B	5'-GCAGCAGACCATCATCCTG-3'
CD44 (for xenograft mouse model)	5'-GGCGCAGATCGATTTGAAT-3'

*Sequences shown are the 19-22 bp long guide sequences. Abbreviations: shRNA, short hairpin ribonucleic acid.

Supplemental Table S10

Interventional therapy in patients with mastocytosis

No.*	Gender [f/m]	Age [years] at diagnosis	Diagnosis	Initial interventional treatment	Age [years] at progression	Progression	Interventional treatment after progression
18	m	64	ISM	prednisolone (25 mg/day)	69	ASM	midostaurin (100 mg/day), prednisolone (12.5 mg/day)
45	f	62	ISM	imatinib (200 mg/day)	none	none	none
111	m	72	ISM-CMML	busulfan (8 mg/day), prednisolone (50 mg/day), 6 cycles of etoposide (200-500 mg/cycle)	73	ISM-AML	hydroxyurea (1000 mg/day), busulfan (6-8 mg/day)
124	m	53	ASM-CMML	IFN-α (3 x 10^6 IU, 3 times a week), prednisolone (50 mg/day)	55	ASM-AML	hydroxyurea (1000 mg/day), 1 induction poly-CT (DAV 3+5+7 protocol)
129	f	71	ASM-MPN	ruxolitinib (10 mg/day), 4 cycles of 2CdA (10mg, days 1-3), midostaurin (200 mg/day)	none	none	none
142	f	57	ASM	5 cycles of 2CdA (0.14 mg/kg, days 1-5)	58	ASM-AML	2 induction poly-CT (3+7 protocol and MIDAC) + midostaurin (100 mg/day), 1 cycle of azacytidine (75 mg/m^2, days 1-7) + thalidomide (100 mg/day)
146	m	81	ISM	prednisolone (5 mg/day)	89	ASM	prednisolone (50 mg/day); 2 cycles of brentuximab vedotin (135 mg, day 1).
149	m	21	ASM	prednisolone (50 mg/day), IFN-α (3 x 10^6 IU, every second day), 3 cycles of 2CdA (0.14 mg/kg, days 1-5) + prednisolone (12.5 mg/day)	25	MCL	imatinib (400 mg/day) + prednisolone (25 mg/day); 1 induction poly-CT (DAV 3+5+7 protocol) + prednisolone (12.5 mg/day)

153	m	61	ASM	6 cycles of 2CdA (0.14 mg/kg, days 1-5)	63	ASM-AML	2 induction poly CT (FLAG)
154	f	54	MCL	1 induction poly-CT (FLAG), followed by 3 cycles of consolidation with FLAG; one day clofarabine + cyclophosphamide	none	none	none
156	m	90	MCL	prednisolone (25 mg/day), 7 cycles of 2CdA (0.14 mg/kg, days 1-5), midostaurin (50-100 mg/day)	none	none	none

*For each patient listed in this table a time dependent graph including serum tryptase levels, CD44 expression, and all interventional therapies administered is shown in Supplemental Figures S8, S9, and S10. Abbreviations: No., number; m, male; f, female; ISM, indolent systemic mastocytosis; ASM, aggressive systemic mastocytosis; MCL, mast cell leukemia; CMML, chronic myelomonocytic leukemia; MPN, myeloproliferative neoplasm; AML, acute myeloid leukemia; IFN-α, interferon-alpha; IU, international units; 2CdA, cladribine; CT, chemotherapy; DAV 3+5+7 protocol, daunorubicin (45 mg/m^2, days 1-3), etoposide (100 mg/m^2, days 1-5) and cytarabine (2 x 100 mg/m^2, days 1-7); 3+7 protocol, daunorubicin (60 mg/m^2, days 1- 3) and cytarabine (100 mg/m^2, days 1-7); MIDAC, mitoxantrone (12 mg/m², days 3-5) and cytarabine (2 x 1000 mg/m², days 1-4); FLAG, fludarabine (30 mg/m^2, days 1-5), cytarabine (2000 mg/m^2, days 1-5), and granulocyte colony-stimulating factor, G-CSF (30 x 10^6 international units, from day 6 until neutrophil recovery).

Supplemental Table S11

Cox proportional hazards regression model for overall and event-free survival

	Univariate Analysis			**Multivariate Analysis**		
OS	**Hazard Ratio**	**95% CI**	**p-Value**	**Hazard Ratio**	**95% CI**	**p-Value**
sCD44 per additional ng/ml	1.006	1.004 – 1.007	<0.0001	1.003	1.001 – 1.005	0.007
WHO classification			<0.0001			<0.0001
EFS	**Hazard Ratio**	**95% CI**	**p-Value**	**Hazard Ratio**	**95% CI**	**p-Value**
sCD44 per additional ng/ml	1.006	1.004 – 1.007	<0.0001	1.002	1.000 – 1.005	0.022
WHO classification			<0.0001			<0.0001

Abbreviations: OS, overall survival; CI, confidence interval; sCD44, soluble CD44; WHO, World Health Organization; EFS, event-free survival.

Supplemental Figures

Supplemental Figure S1

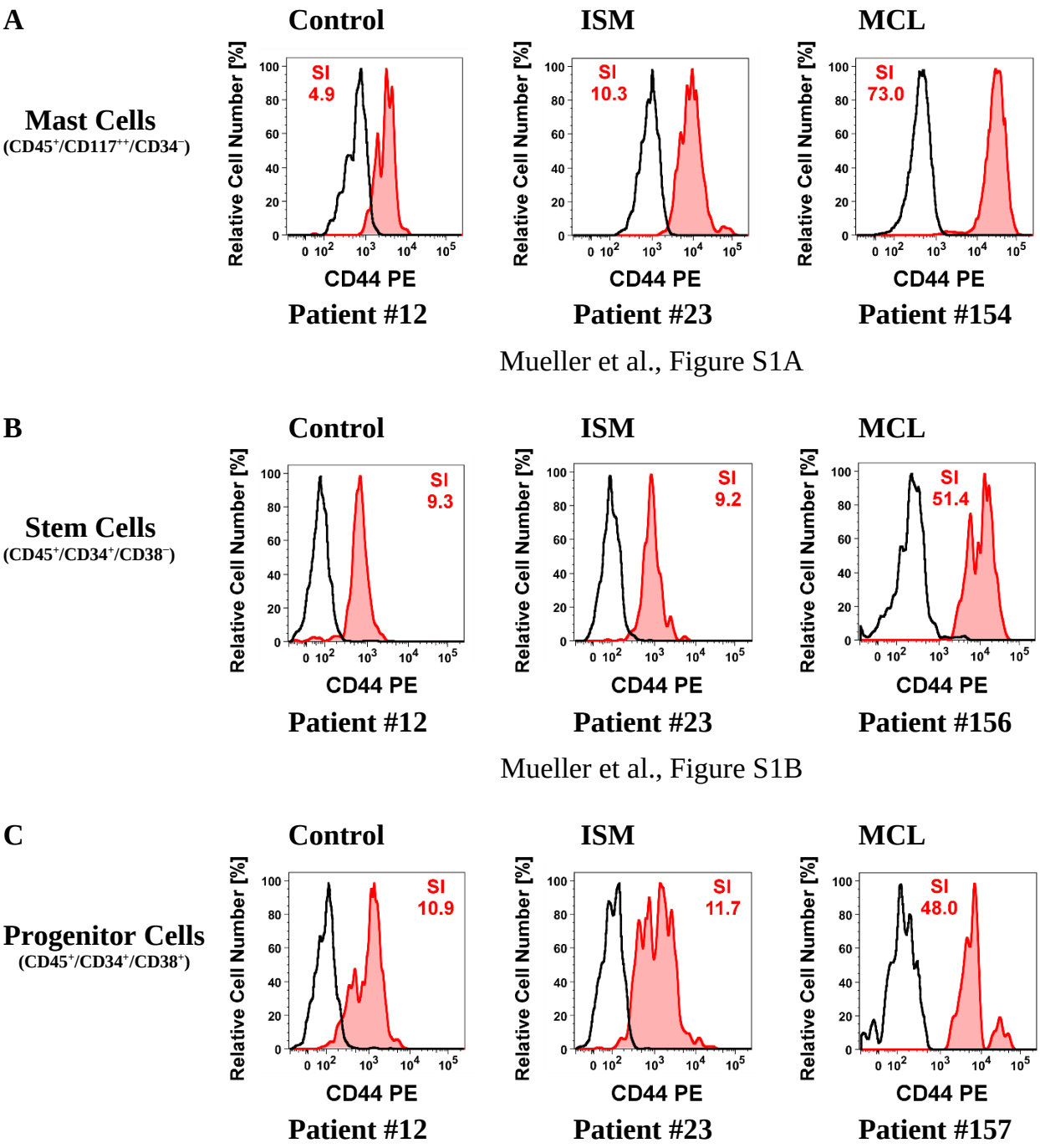
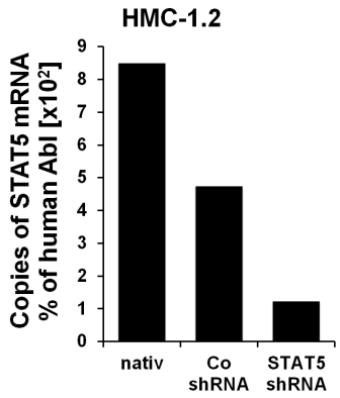


Figure S1: Evaluation of CD44 expression on neoplastic cells by flow cytometry
Bone marrow (BM) samples obtained from patients with monoclonal gammopathy of undetermined significance (MGUS) (Control, left histograms), indolent systemic mastocytosis (ISM, central histograms) and mast cell leukemia (MCL, right histograms) were examined by multicolor flow cytometry. $CD45^{+}/CD117^{++}/CD34^{-}$ mast cells (A), $CD45^{+}/CD34^{+}/CD38^{-}$ stem cells (B) and $CD45^{+}/CD34^{+}/CD38^{+}$

progenitor cells (C) were examined for expression of CD44 (red histograms). The isotype-matched control antibody is also shown (open histograms). The Staining Index (SI), indicated in each histogram (red color), was calculated as median fluorescence intensity (MFI) produced by CD44 antibody divided by the MFI of the isotype-control antibody.

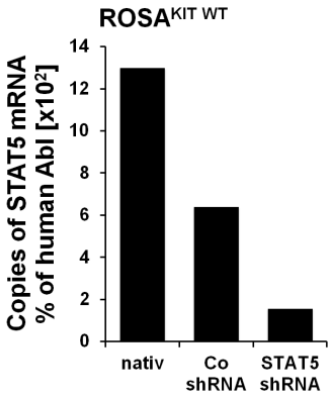
Supplemental Figure S2

A



Mueller et al., Figure S2A

B



Mueller et al., Figure S2B

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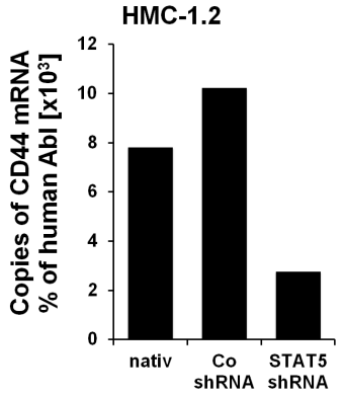
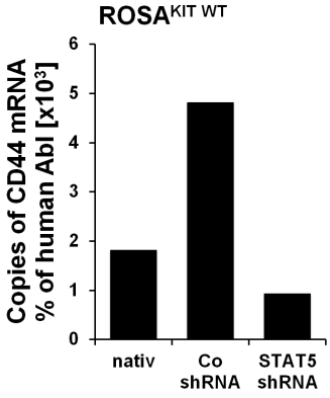


Mueller et al., Figure S2C

D

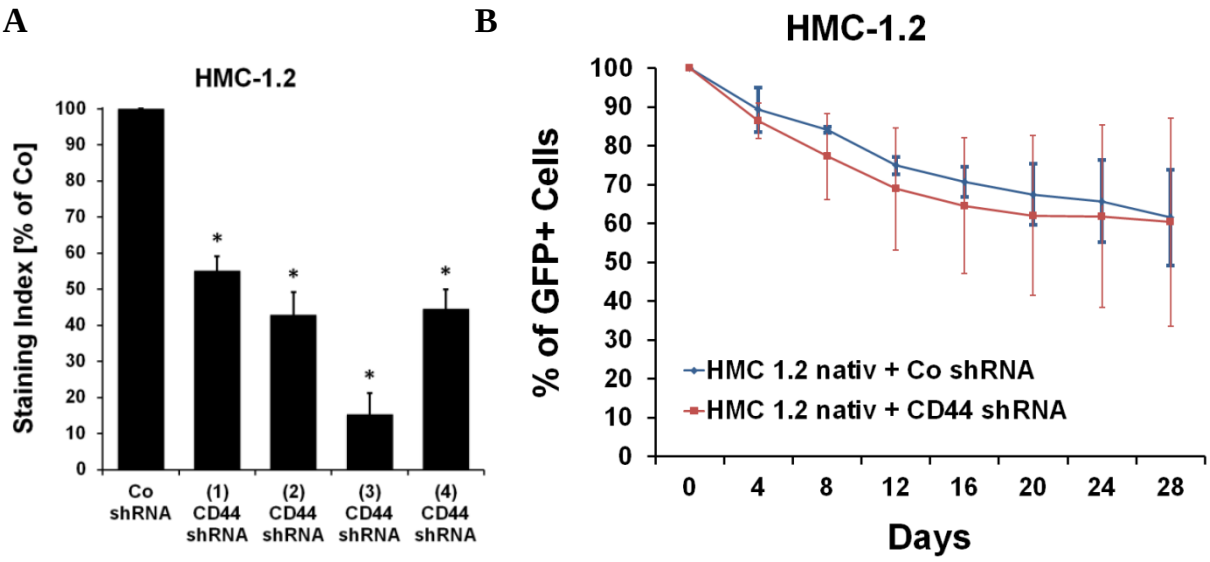


Mueller et al., Figure S2D

Figure S2: STAT5-knockdown downregulates expression of CD44 in neoplastic mast cells

HMC-1.2 (A, C) and ROSA$^{KIT\ WT}$ (B, D) cells were lentivirally transduced with an shRNA against STAT5 or control (Co) shRNA as described in the text. Then, cells were subjected to RNA isolation at day 4 after transduction and *STAT5* (A, B) as well as *CD44* (C, D) mRNA levels were measured by qPCR. Bars represent percentage of *STAT5* and *CD44* mRNA copies relative to *ABL1* mRNA levels.

Supplemental Figure S3



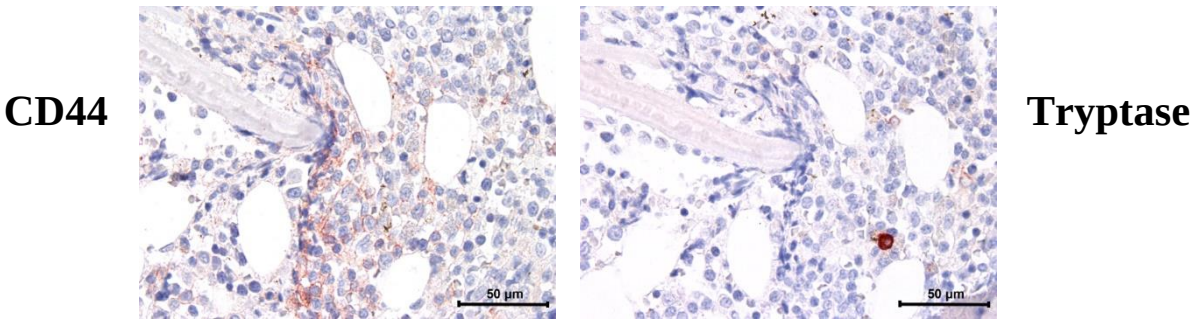
Mueller et al., Figure S3A

Mueller et al., Figure S3B

Figure S3: The effect of shRNA-mediated CD44-knockdown on the proliferation in HMC-1.2 neoplastic mast cells *in vitro*

A: HMC-1.2 cells were lentivirally transduced with 4 different green fluorescent protein (GFP)-labeled shRNA against CD44 (indicated with numbers 1-4) and Renilla-luciferase as control (Co) as described in the text. CD44 expression of these 4 cell clones and their related Co was analyzed by flow cytometry. Cells were gated for GFP positivity. Bars represent the expression of CD44 (Staining Index) as percentage of Co and are expressed as mean±S.D. of 3 independent experiments. Asterisk (*): $p<0.05$ compared to Co (Student´s t test). B: The proliferative potential of the CD44-knockdown clones and their related Co was determined by mixing each clone and the Co separately with untransfected native cells (1:1). Afterwards, the percentage of GFP^+ cells was analyzed in each cell culture over 28 days. The graph shows the percentage of GFP^+ cells in the CD44-knockdown cell cultures (averaged value of 4 clones, red curve) in direct comparison to the Co cell culture (blue curve) as mean±S.D. of 3 independent experiments.

Supplemental Figure S4

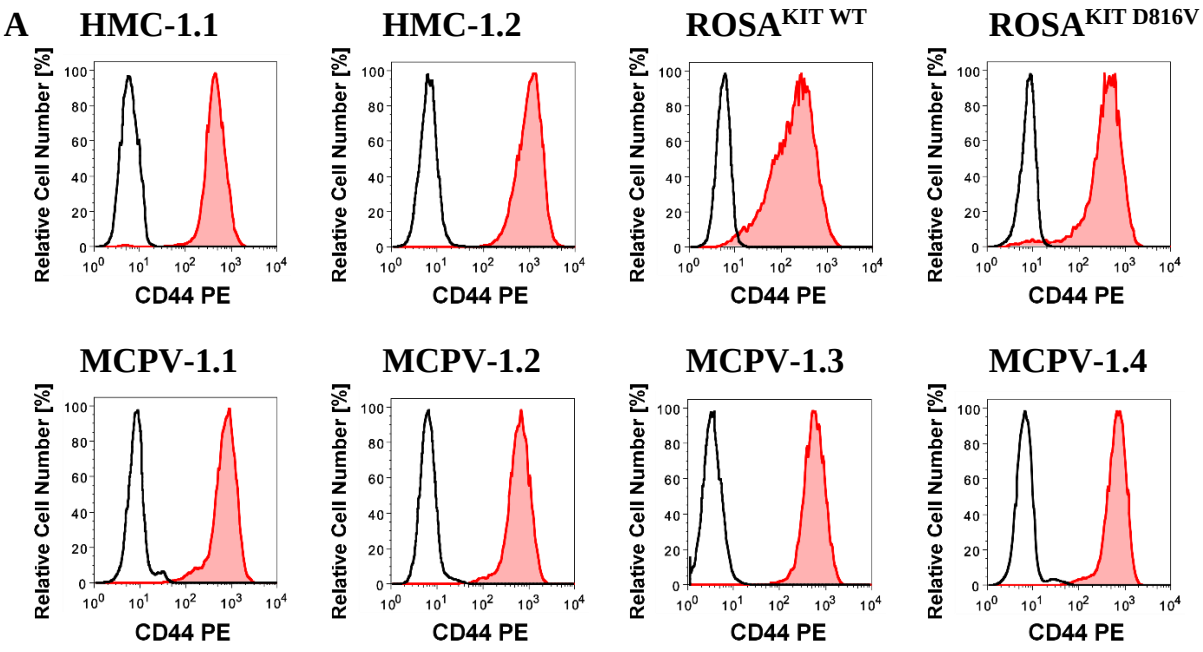


Mueller et al., Figure S4

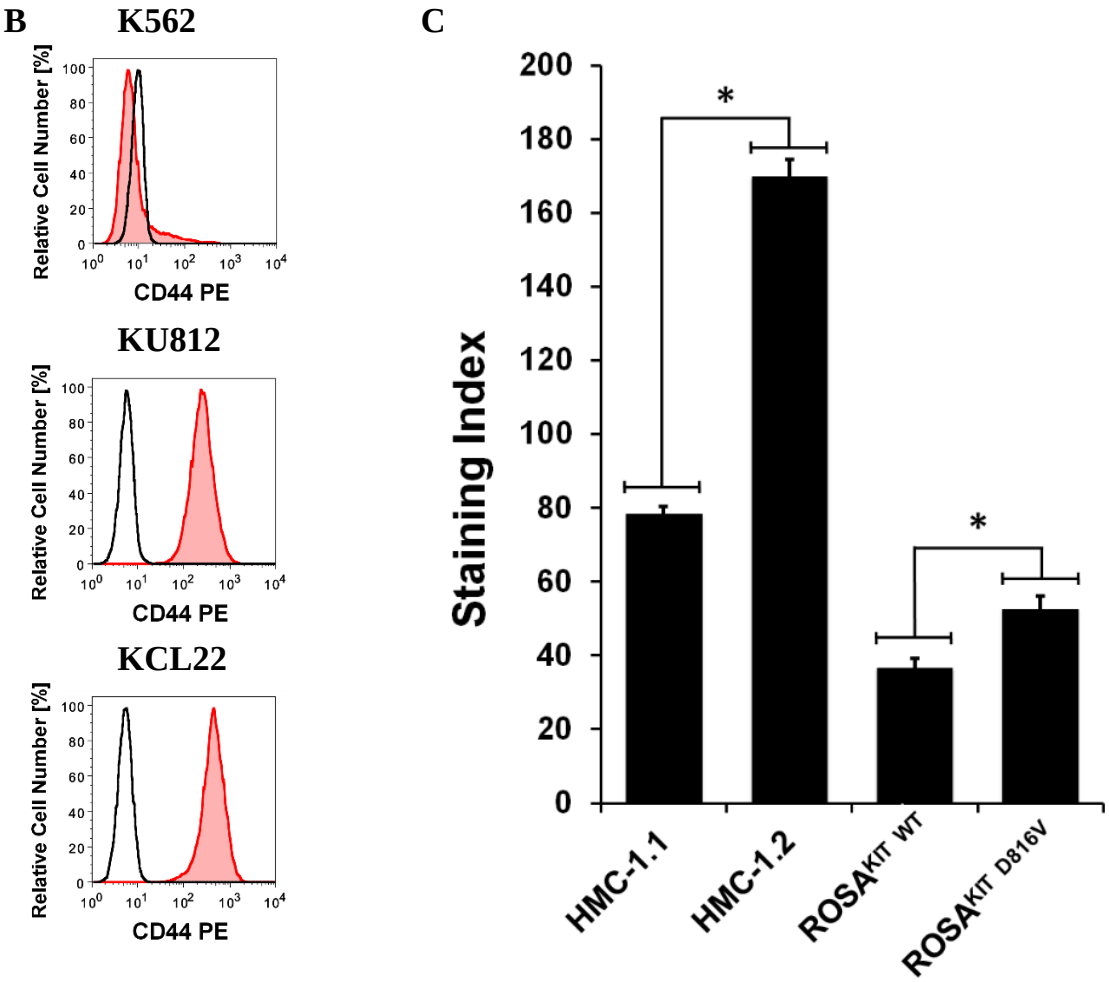
Figure S4: Detection of CD44 in normal/reactive bone marrow (BM) by immunostaining

BM sections of a female patient with normal/reactive BM at the age of 39 were immunohistochemically stained with an antibody against CD44 (left panel) and tryptase (right panel). As expected, the number of tryptase^{+} non-neoplastic mast cells co-expressing CD44 is very low. Slides were examined using an Olympus DP21 camera connected to an Olympus BX50F4 microscope (Olympus Corporation; Shinjuku, Tokyo, Japan) equipped with 60x/0.90 UPlanFL objective lens. Images of BM sections were taken with 600x magnification and adjusted by Adobe Photoshop CS5 software Version 12.0.4 (Adobe Systems, San Jose, CA, USA).

Supplemental Figure S5



Mueller et al., Figure S5A



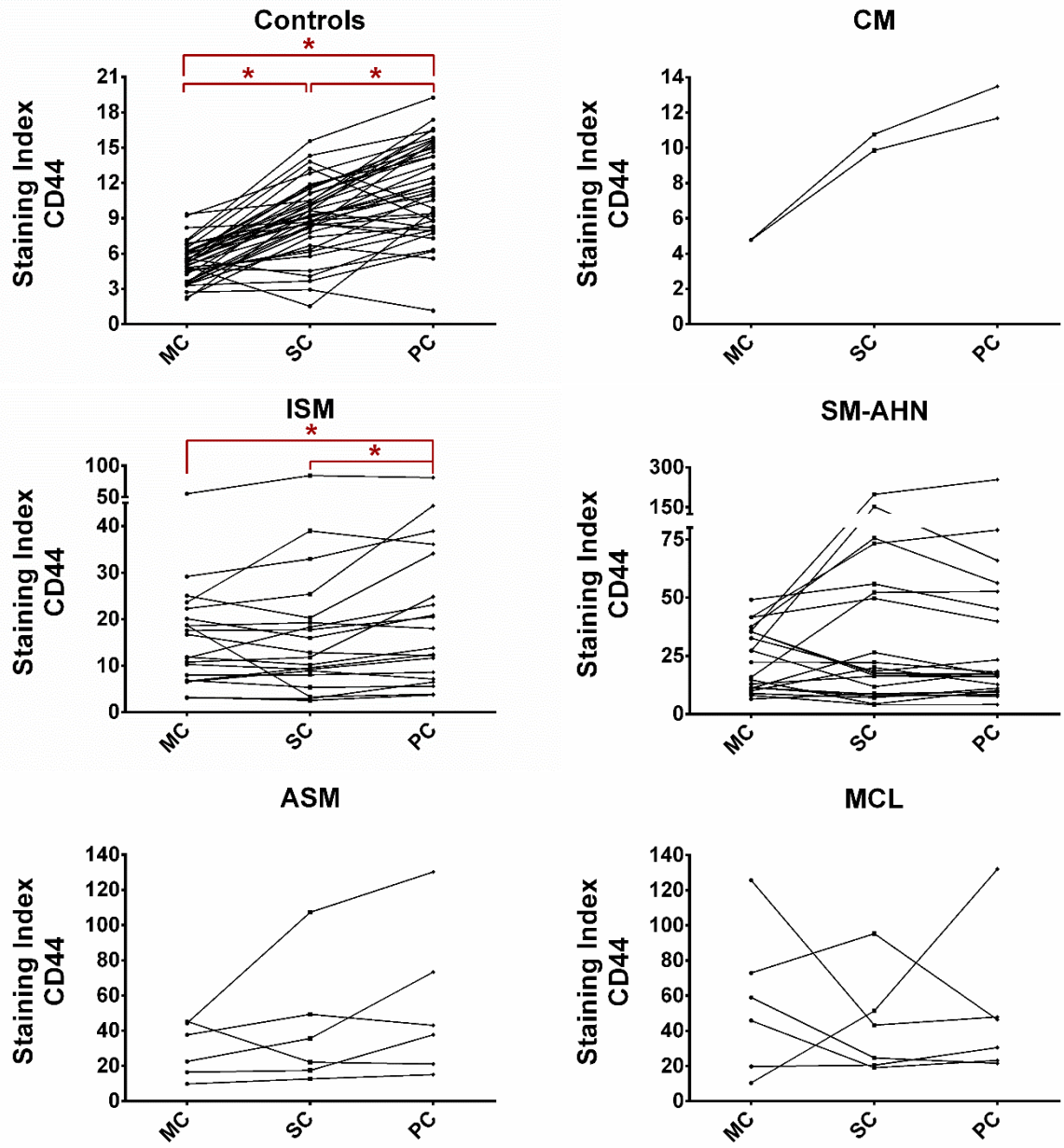
Mueller et al., Figure S5B

Mueller et al., Figure S5C

Figure S5: Detection of CD44 on human mast cell (MC) lines and chronic myeloid leukemia (CML) cell lines by flow cytometry

A,B: CD44 surface expression (red histograms) on human MC lines (HMC-1, ROSA and MCPV-1) (A) and on human non-MC lines (CML cell lines K562, KU812 and KCL22) (B) was analyzed by flow cytometry using a phycoerythrin (PE)-labeled antibody against CD44. The isotype-matched control antibody is also shown (open histograms). C: Bars represent the expression level of CD44 on KIT *D816V*-mutated human MC lines (HMC-1.2 and ROSA$^{\text{KIT D816V}}$) and on their related non-KIT D816V-mutated controls (HMC-1.1 and ROSA$^{\text{KIT WT}}$) as Staining Index. Results are shown as mean±S.D. of 3 independent experiments. Asterisk (*): $p<0.05$ compared to non-KIT D816V-mutated control (Student´s t test).

Supplemental Figure S6



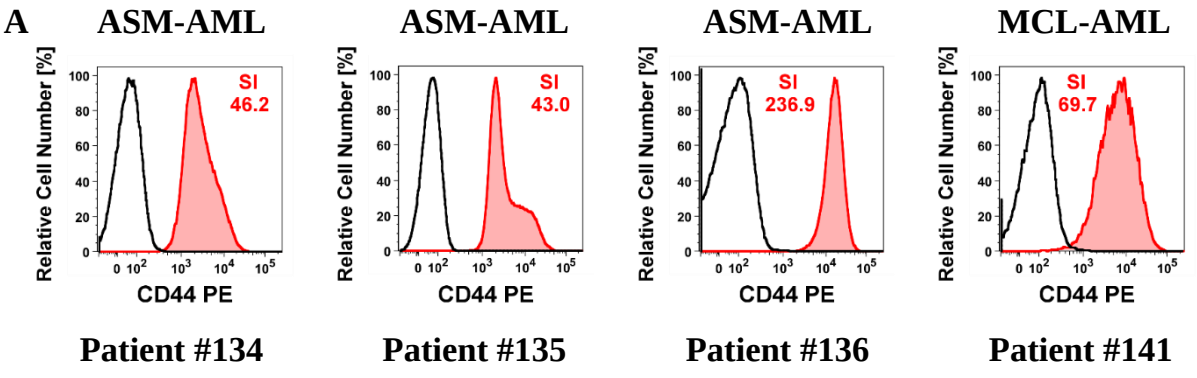
Mueller et al., Figure S6

Figure S6: Detection of CD44 on the surface of mast cells (MC), stem cells (SC) and progenitor cells (PC)

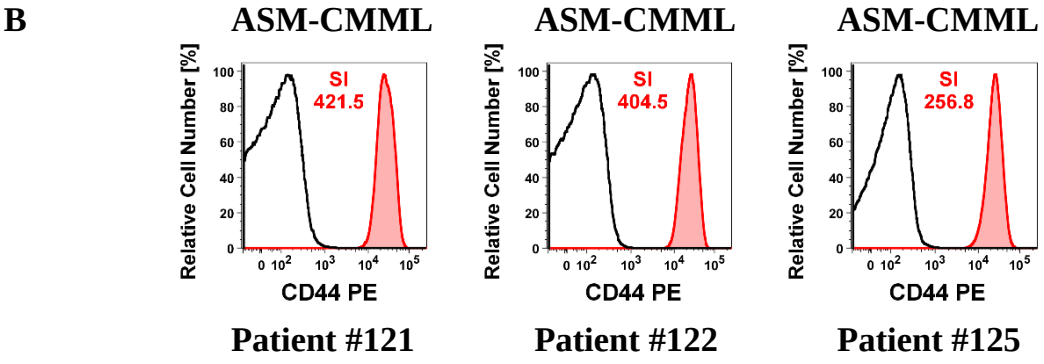
Bone marrow (BM) cells obtained from controls (Co: patients with lympho-proliferative neoplasms or normal/reactive BM, n=41) and patients with cutaneous mastocytosis (CM, n=2), indolent systemic mastocytosis (ISM, n=20), systemic mastocytosis with an associated hematologic neoplasm (SM-AHN, n=21), aggressive systemic mastocytosis (ASM, n=6), or MC leukemia (MCL, n=6) were analyzed by multicolor flow cytometry. Expression of CD44 on $CD45^{+}/CD117^{++}/CD34^{-}$ MC, $CD34^{+}/CD38^{-}$ SC and $CD34^{+}/CD38^{+}$ PC is expressed as Staining Index (median fluorescence intensity (MFI) produced by CD44 antibody divided by MFI of the

isotype-control antibody). Results are shown as graphs showing CD44 surface expression on MC, SC and PC for each individual patient separately. Asterisk (*): $p<0.05$ (Wilcoxon matched pairs signed rank test). As visible, no significant differences in CD44 expression were seen when comparing MC, SC and PC in patients with advanced SM (SM-AHN, ASM, MCL), whereas in ISM and control patients, significant differences in CD44 expression were found when comparing MC, SC and PC. The highest levels of surface CD44 were measured on PC.

Supplemental Figure S7



Mueller et al., Figure S7A

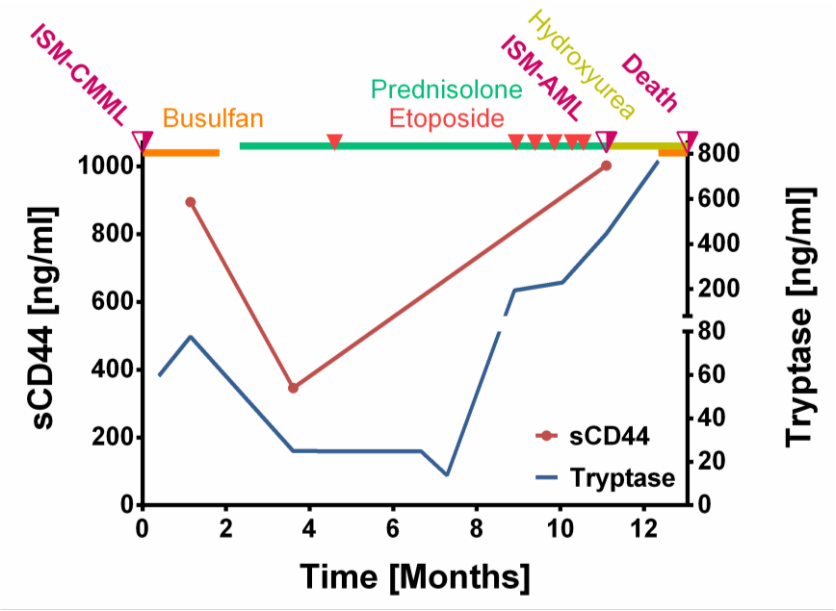


Mueller et al., Figure S7B

Figure S7: Evaluation of CD44 expression on neoplastic cells in patients with systemic mastocytosis with an associated hematologic neoplasm (SM-AHN)
Bone marrow (BM) cells obtained from patients with SM-AHN were examined by multicolor flow cytometry. CD45dim/CD34$^+$/CD38$^+$ blast cells (A) and CD45$^+$/CD14$^+$ monocytes (B) were examined for CD44 expression (red histograms) in 4 SM patients with an associated acute myeloid leukemia (AML – blast cells) and 3 SM patients with associated chronic myelomonocytic leukemia (CMML – monocytes). The Staining Index (SI) is indicated in each histogram (red color). The isotype-matched control antibody is also shown (black open histograms). As visible, AML blasts as well as the CD14$^+$ monocytes stained clearly positive for CD44 in all patients tested.

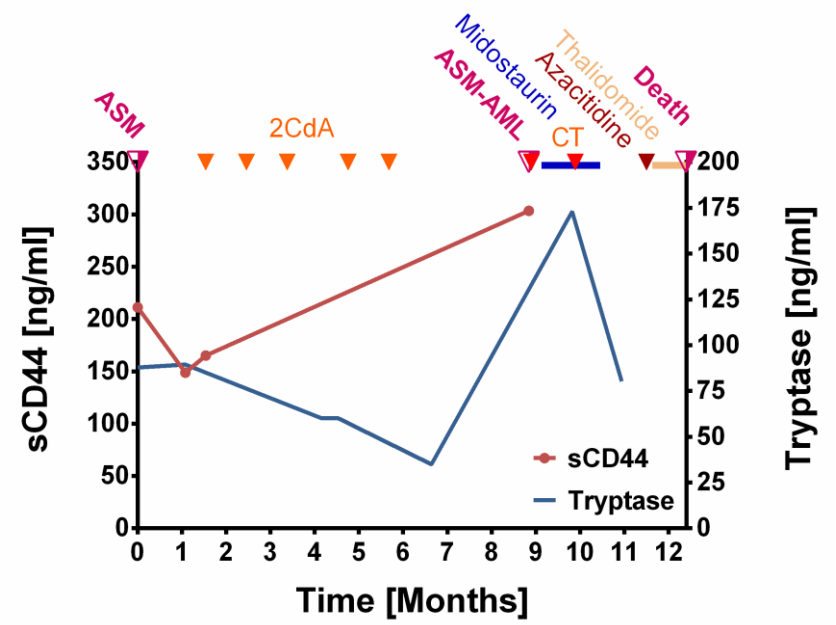
Supplemental Figure S8

A



sCD44 (ng/ml) and serum tryptase (ng/ml) were measured over 13 months in the sera of a 72-year-old male patient with indolent systemic mastocytosis (ISM) and associated chronic myelomonocytic leukemia (ISM-CMML; #111 in Supplemental Table S1), who progressed to acute myeloid leukemia (ISM-AML). Initially, the patient received busulfan (8 mg/day) which resulted in a transient decrease in tryptase and sCD44. Later (because of resistance) the patient received prednisolone (50 mg/day) and 6 cycles of etoposide (200-500 mg/cycle). However, despite therapy, tryptase and sCD44 increased and the patient progressed to ISM-AML. At that time treatment was switched to hydroxyurea (1000 mg/day). Because of comorbidities and clinical deterioration, no intensive therapy could be administered and the patient received palliative busulfan (6-8 mg/day). AML further progressed and the patient died 1.9 months after the diagnosis AML had been established.

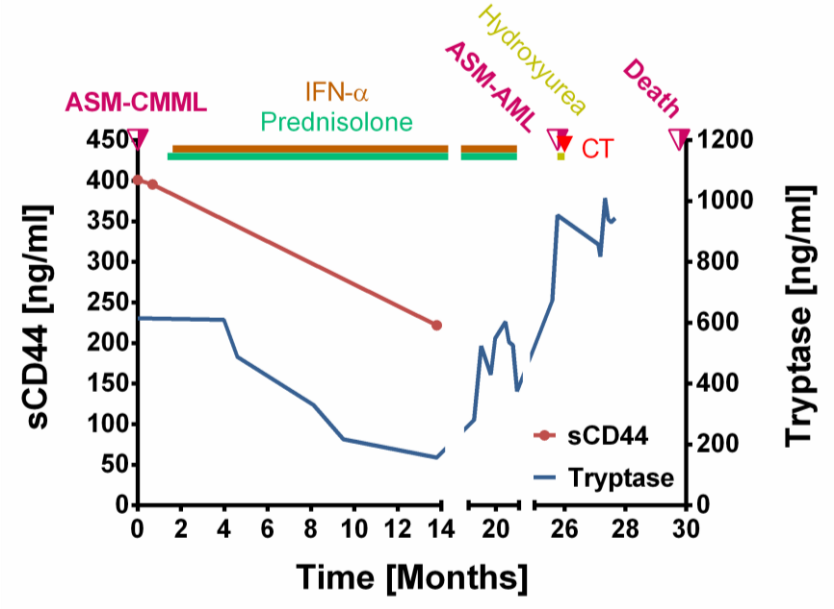
B



sCD44 (ng/ml) and tryptase (ng/ml) were measured over 12.4 months in the sera of a 57-year-old female patient with KIT D816V^{+} aggressive systemic mastocytosis (ASM; #142 in Supplemental Table S1) in whom treatment with 5 cycles of cladribine (2CdA; 0.14 mg/kg, days 1-5) was administered. However, despite initial disease-stabilization (best response: stable disease) the patient later progressed to acute myeloid leukemia (ASM-AML). During treatment with 2CdA, tryptase levels decreased transiently. sCD44 levels were measured before and after therapy with 2CdA. At the time of progression to AML, sCD44 levels were higher compared to pre-therapy levels. After progression to AML, induction poly-chemotherapy (CT) with daunorubicin (60 mg/m^2, days 1-3)

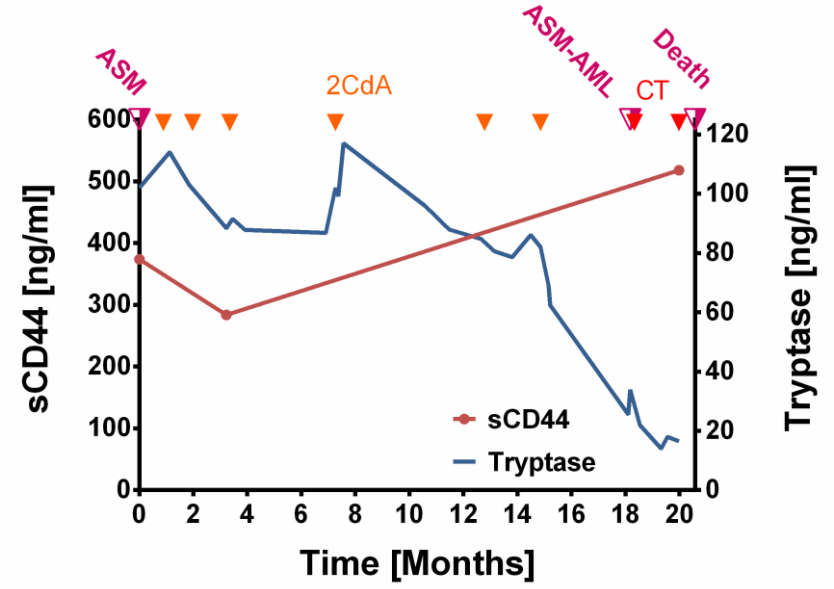
and cytarabine (100 mg/m^2, days 1-7) (3+7 protocol) was administered, but no remission was obtained. Therefore, the patient received a second induction poly-CT cycle, consisting of mitoxantrone (12 mg/m^2, days 3-5) and cytarabine (2 x 1000 mg/m^2, days 1-4) (MIDAC). In addition, the patient received midostaurin (PKC412) (100 mg/day). However, no response was seen. Thereafter, the patient received one cycle of azacytidine (75 mg/m^2, days 1-7) combined with thalidomide (100 mg/day). However, AML further progressed and the patient died 3.6 months after the diagnosis AML had been established.

C



sCD44 (ng/ml) and tryptase (ng/ml) were measured over 29.8 months in the sera of a 53-year-old male patient with aggressive systemic mastocytosis with associated chronic myelomonocytic leukemia (ASM-CMML; #124 in Supplemental Table S1) in whom treatment with interferon-alpha (IFN-α; 3 x 10^6 international units, 3 times a week) and prednisolone (50 mg/day) was administered. During therapy, both tryptase and sCD44 levels decreased and the patient´s best response was classified as major clinical response (seen after 3 month). However, after 14 months, tryptase levels increased and the patient progressed to acute myeloid leukemia (ASM-AML). After progression to AML, the patient received hydroxyurea for cytoreduction (1000 mg/day) and was then treated with induction poly-chemotherapy (CT) consisting of daunorubicin (45 mg/m^2, days 1-3), etoposide (100 mg/m^2, days 1-5) and cytarabine (2 x 100 mg/m^2, days 1-7), (DAV 3+5+7 protocol). However, no remission was obtained and the patient died 4 months after the diagnosis AML had been established.

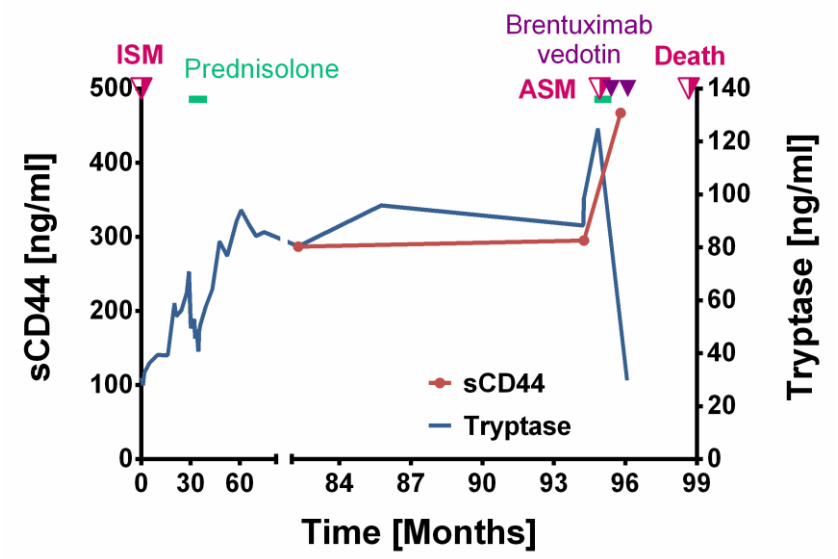
D



sCD44 (ng/ml) and tryptase (ng/ml) were measured over 20.6 months in the sera of a 61-year-old male patient with aggressive systemic mastocytosis (ASM; #153 in Supplemental Table S1) in whom treatment with 6 cycles

of cladribine (2CdA; 0.14 mg/kg, days 1-5) were administered. During therapy with 2CdA, serum tryptase levels and the sCD44 level initially decreased. However, no sustained response was achieved (best response: stable disease). Later, the patient progressed to acute myeloid leukemia (ASM-AML). The patient then received 2 induction poly-chemotherapy (CT) cycles with fludarabine (30 mg/m^2, days 1-5), cytarabine (2000 mg/m^2, days 1-5), and granulocyte colony-stimulating factor, G-CSF (30 x 10^6 international units, from day 6 until neutrophil recovery) (FLAG). However, no remission was obtained and sCD44 levels substantially increased under progression. Finally, the patient died 2.4 months after the diagnosis AML had been established.

E



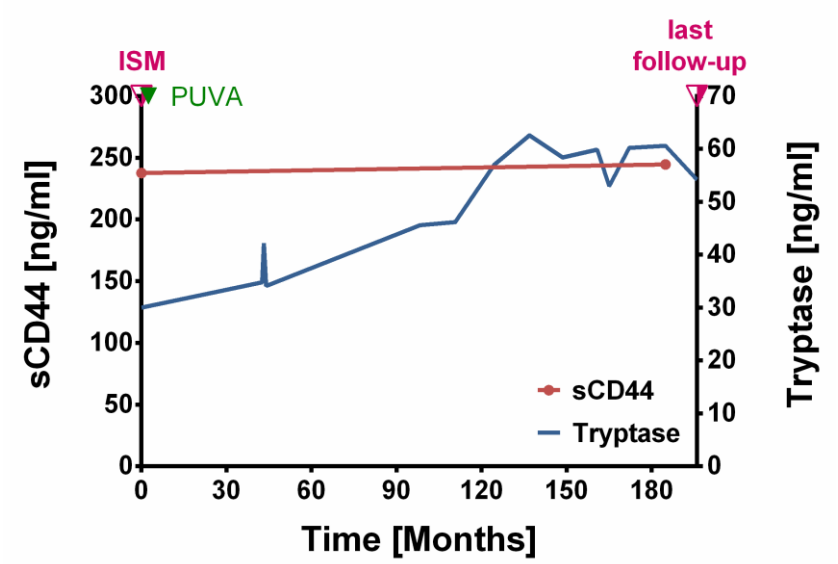
sCD44 (ng/ml) and tryptase (ng/ml) were measured over 98.7 months in the sera of a 81-year-old male patient with indolent systemic mastocytosis (ISM; #146 in Supplemental Table S1). Because of mediator-related symptoms and an increase in serum tryptase, the patient initially received short-term prednisolone (5 mg/day). Despite the initial (massive) increase in serum tryptase and discontinuation of prednisolone, the patient had then a stable course for over 7.9 years. However, at the age of 89, the patient suddenly developed an aggressive SM (ASM). Because of age, comorbidities and his reduced performance status, no intensive therapy could be administered. After confirming that the neoplastic mast cells express CD30, the patient received prednisolone (50 mg/day) and 2 cycles of brentuximab vedotin (135 mg, day 1). However, no remission was obtained and the patient died 3.7 months after the diagnosis of ASM had been established.

Figure S8: Detection of soluble CD44 (sCD44) in the sera of patients with advanced SM before and during interventional therapy

sCD44 (ng/ml) levels and serum tryptase levels (ng/ml) were measured in the sera of patients who had or developed advanced SM, including SM with an associated hematologic neoplasm (ISM-AHN, patient #111; ASM-AHN, patient #124) (A,C), ASM progressing to ASM-AML (patients #142, #153) (B,D), and ISM progressing to ASM (patient #146) (E). sCD44 levels were quantified by ELISA and serum tryptase levels by a fluoroenzyme immunoassay (ImmunoCAP, ThermoFisher, Uppsala, Sweden). Abbreviations: SM, systemic mastocytosis; ISM, indolent SM; AHN, associated hematologic neoplasm; ASM, aggressive SM; AML, acute myeloid leukemia.

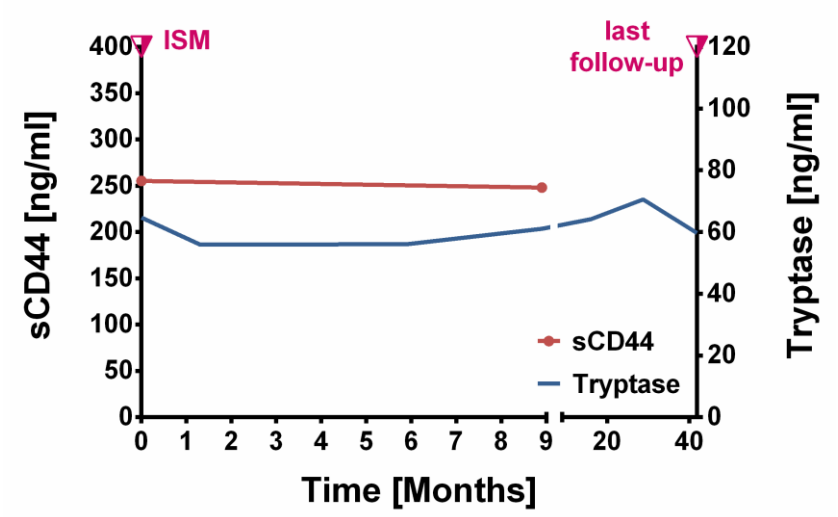
Supplemental Figure S9

A



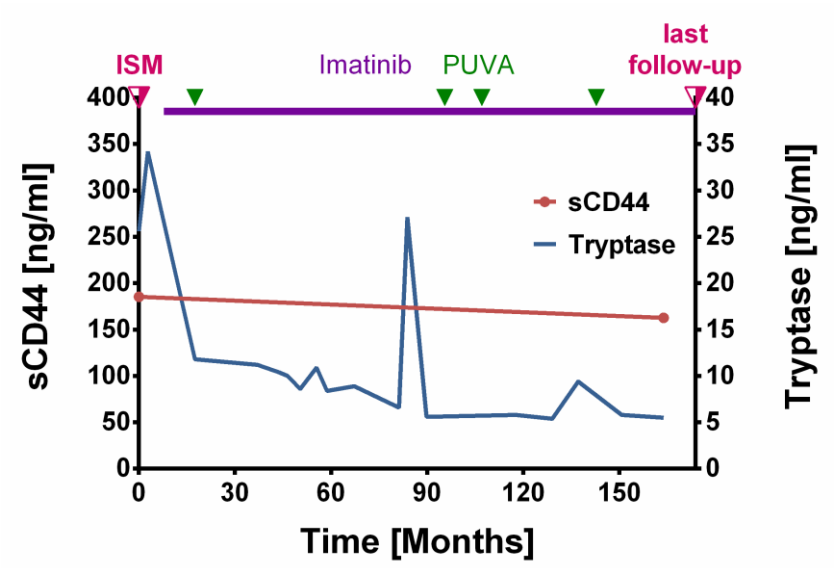
sCD44 (ng/ml) and tryptase (ng/ml) were measured over 195.9 months in the sera of a 56-year-old female patient with indolent systemic mastocytosis (ISM; #43 in Supplemental Table S1), who had a stable clinical course over 16.3 years. Initially, the patient was treated with ultraviolet light and psoralen (PUVA). During the observation period, serum tryptase levels slightly increased. sCD44 levels measured at diagnosis and after 15.4 years were almost identical. The patient is still alive.

B



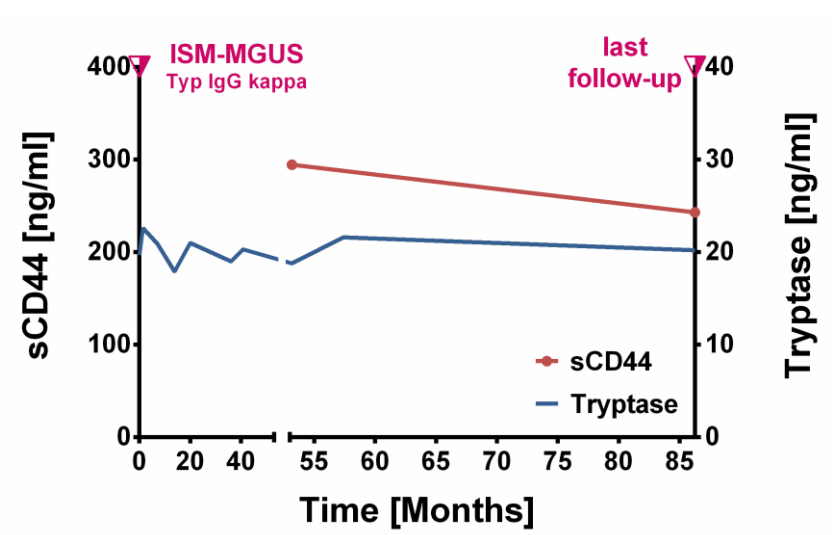
sCD44 (ng/ml) and tryptase (ng/ml) were measured over 41.8 months in the sera of a 57-year-old female patient with indolent systemic mastocytosis (ISM; #26 in Supplemental Table S1), who had a stable clinical course over 3.5 years. During the observation period, serum tryptase levels did not increase substantially. sCD44 levels were measured at diagnosis and after 8.9 months and were found to be almost identical. The patient is still alive.

C



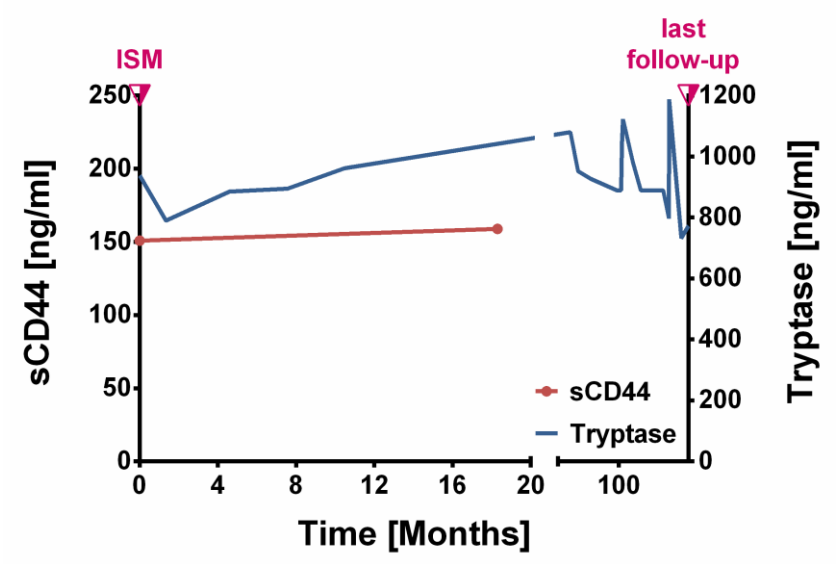
sCD44 (ng/ml) and tryptase (ng/ml) were measured over 173.7 months in the sera of a 62-year-old female patient with KIT D816V-negative indolent systemic mastocytosis (ISM; #45 in Supplemental Table S1) who had a stable clinical course (concerning hematologic parameters) over 14.5 years. The patient suffered from severe skin lesions that improved substantially during long-term treatment with imatinib (200 mg/day) and intermittent ultraviolet light and psoralen (PUVA). During therapy, serum tryptase levels decreased substantially and the skin lesions disappeared. sCD44 levels were measured at diagnosis and after 13.6 years and was slightly lower after therapy compared to the pre-imatinib time point. The patient is still alive.

D



sCD44 (ng/ml) and tryptase (ng/ml) were measured over 86.2 months in the sera of a 50-year-old male patient (patient #102, Supplemental Table S1) with indolent systemic mastocytosis (ISM) with concomitant monoclonal gammopathy of undetermined significance (MGUS) who had a stable clinical course over 7.2 years. The patient received only supportive therapy but no interventional therapy. During the observation period, serum tryptase levels remained stable. sCD44 levels were measured after 4.4 and 7.2 years. The sCD44 level determined during the last follow-up was slightly lower compared to the sCD44 level measured after 4.4 years. The patient is still alive.

E



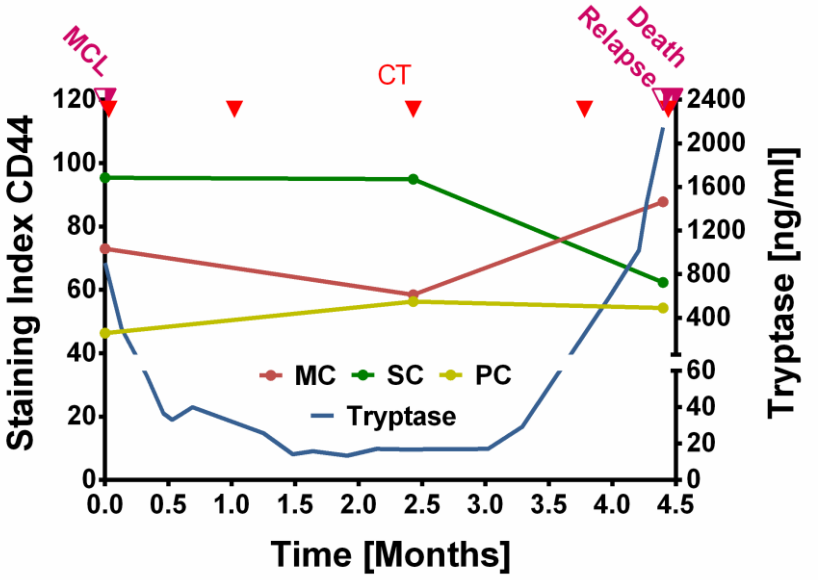
sCD44 (ng/ml) and tryptase (ng/ml) were measured over 191.4 months in the sera of a 45-year-old female patient with indolent systemic mastocytosis (ISM; #36 in Supplemental Table S1) and massively elevated tryptase. Despite the massively elevated tryptase, the patient showed a stable clinical course over 16.0 years. During the observation period serum tryptase levels slightly increased, but no progression occurred. sCD44 levels were measured at diagnosis and after 1.5 years and were found to be almost identical. The patient is still alive.

Figure S9: Detection of soluble CD44 (sCD44) in the sera of patients with indolent systemic mastocytosis (ISM) during follow-up

sCD44 (ng/ml) and tryptase (ng/ml) were measured in the sera of patients with ISM (patients #43, #26, #45, #36 and #102) (A-E). Patient #102 had an ISM with concomitant monoclonal gammopathy of undetermined significance (ISM-MGUS) (D). As visible, serum tryptase levels did not substantially change or slightly increased during follow-up, except in one patient with KIT D816V-negative ISM who received imatinib therapy (patient #45) (C). sCD44 levels were quantified by ELISA and serum tryptase levels by a fluoroenzyme immunoassay (ImmunoCAP, ThermoFisher, Uppsala, Sweden).

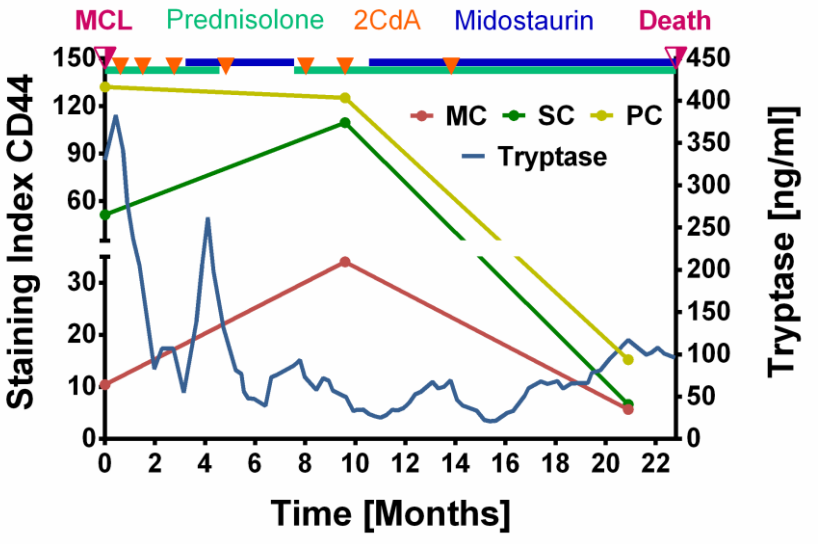
Supplemental Figure S10

A



Surface expression of CD44 on bone marrow (BM) mast cells (MC), stem cells (SC) and progenitor cells (PC) (shown as Staining Index) and serum tryptase (ng/ml) were measured over 4.5 months in a 54-year-old female patient with acute mast cell leukemia (MCL; #154 in Supplemental Table S1). Because of a hyper-acute clinical course, the patient received induction poly-chemotherapy (CT) consisting of fludarabine (30 mg/m^2, days 1-5), cytarabine (2000 mg/m^2, days 1-5) and granulocyte colony-stimulating factor, G-CSF (30 x 10^6 international units, from day 6 until neutrophil recovery) (FLAG), followed by 3 cycles of consolidation with FLAG. Initially, the patient responded well and entered complete remission (CR). However, after several months, the patient relapsed and despite started treatment with clofarabine and cyclophosphamide the patient died. During initial therapy (FLAG), serum tryptase levels decreased substantially. However, the levels of CD44 on MC, SC and PC remained essentially unchanged during therapy and at the time of relapse when serum tryptase levels again increased.

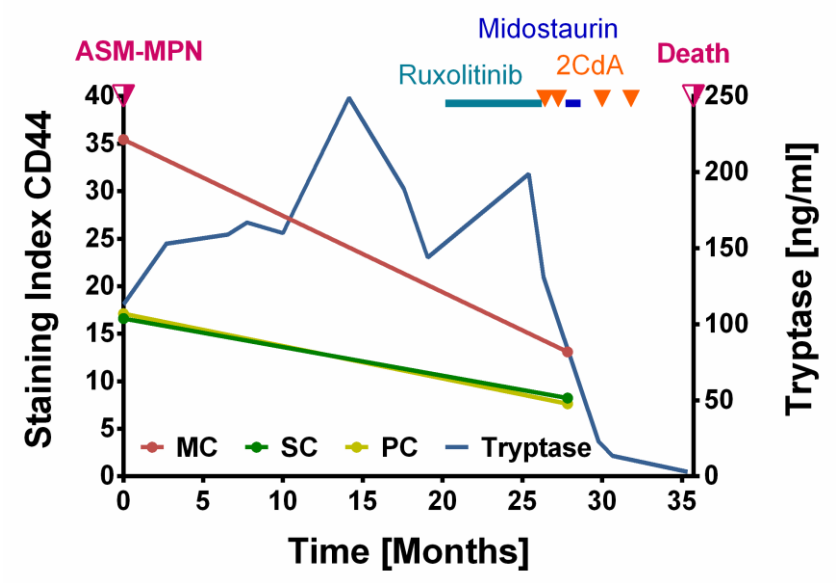
B



Surface expression of CD44 on bone marrow (BM) mast cells (MC), stem cells (SC) and progenitor cells (PC) (shown as Staining Index) and serum tryptase (ng/ml) were measured over 22.8 months in a 90-year-old male patient with mast cell leukemia (MCL; #156 in Supplemental Table S1) in whom treatment with prednisolone (25 mg/day), 7 cycles of cladribine (2CdA; 0.14 mg/kg, days 1-5), and midostaurin (PKC412) (100 mg/day) were administered. During therapy with 2CdA and midostaurin (drug was temporarily stopped due to side effects) serum tryptase levels substantially decreased. However, no clinical response was achieved and the levels of CD44 on MC, SC and PC after 9.6 months remained essentially unchanged (PC) or even increased (MC and SC) during therapy. Interestingly, after 20.9 months, CD44 expression on MC, SC and PC dramatically

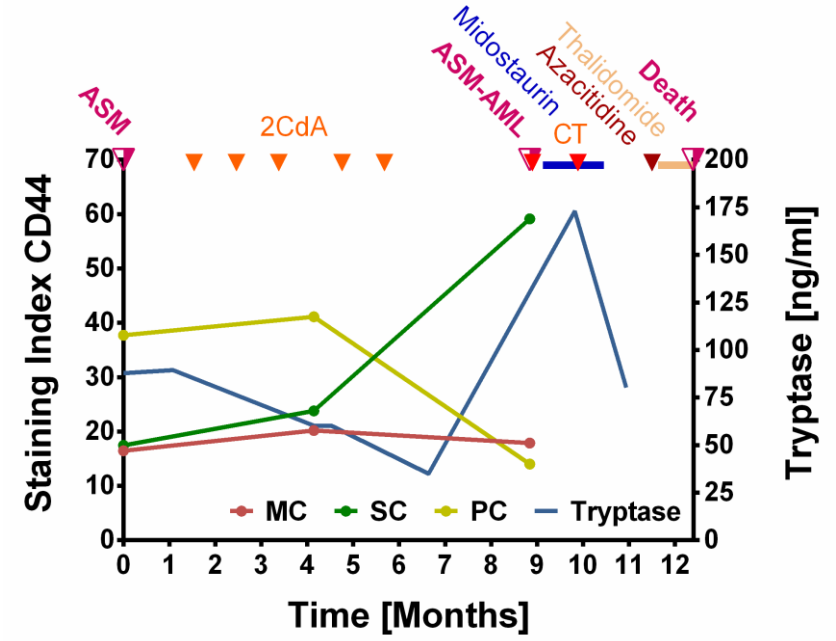
decreased in the follow-up bone marrow biopsy, although serum tryptase levels increased again under treatment with prednisolone (25 mg/day) and midostaurin (PKC412) (50 mg/day). After 1.9 months the patient died at the age of 92 years.

C



Surface expression of CD44 on bone marrow (BM) mast cells (MC), stem cells (SC) and progenitor cells (PC) (shown as Staining Index) and serum tryptase (ng/ml) were measured over 35.7 months in a 71-year-old female patient with an aggressive systemic mastocytosis with an associated myeloproliferative neoplasm (ASM-MPN; #129 in Supplemental Table S1). An initial cytoreductive treatment was refused by the patient. Due to an initially stable clinical course and the patient's preference to watch and wait, a first treatment with the janus kinase inhibitor ruxolitinib (10 mg/day) started 20.2 months after initial diagnosis. However, no sustained response was achieved and serum tryptase levels even increased. The patient then received 4 cycles of cladribine (2CdA; 10mg, days 1-3). Midostaurin (PKC412) (200 mg/day) was shortly applied but the drug was stopped due to side effects. Treatment was accompanied by a substantial decrease in serum tryptase. Interestingly, after 2 cycles of 2CdA, CD44 expression on the surface of MC, SC and PC decreased substantially in a follow-up bone marrow biopsy 2.3 years after initial diagnosis. Despite normal serum tryptase levels the patient died 7.9 months later.

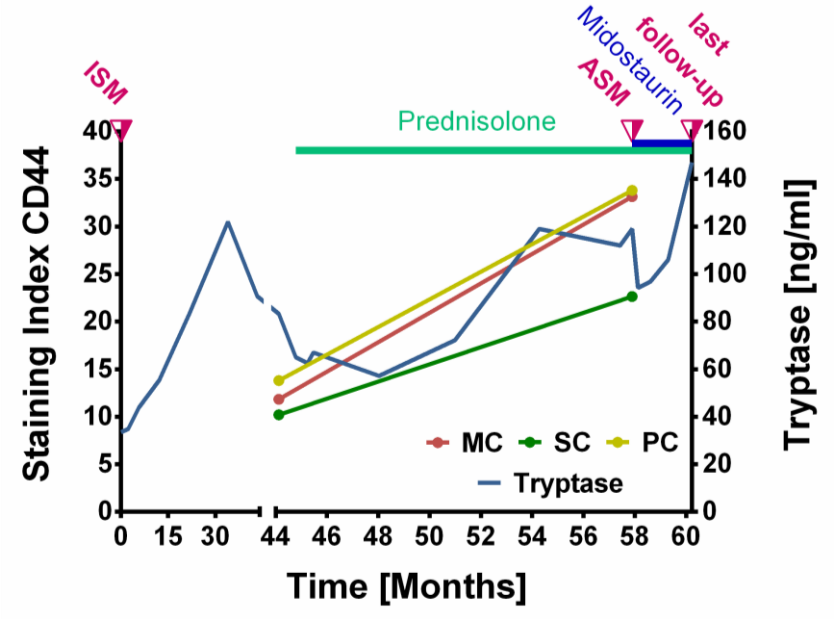
D



Surface expression of CD44 on bone marrow (BM) mast cells (MC), stem cells (SC) and progenitor cells (PC) (shown as Staining Index) and serum tryptase (ng/ml) were measured over 12.4 months in a 57-year-old female patient with KIT D816V^{+} aggressive systemic mastocytosis (ASM; #142 in Supplemental Table S1) in whom treatment with 5 cycles of cladribine (2CdA; 0.14 mg/kg, days 1-5) was administered. However, despite initial

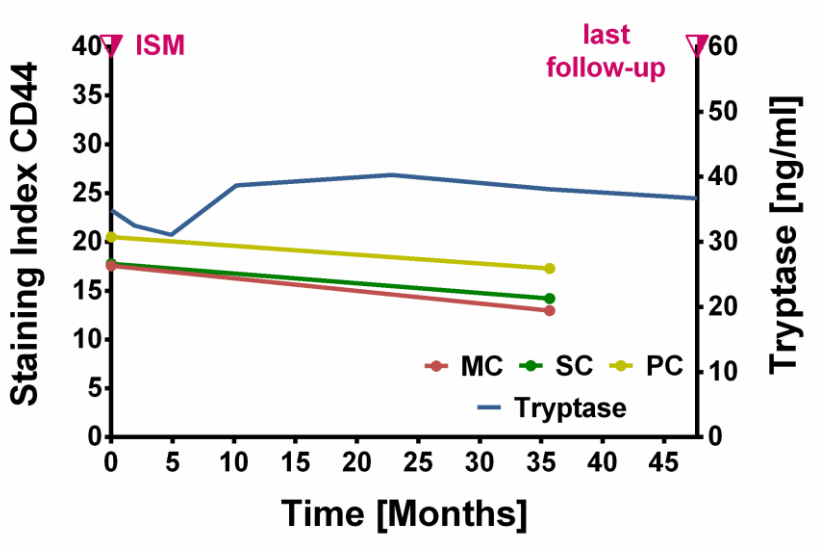
disease-stabilization (best response: stable disease) the patient later progressed to acute myeloid leukemia (ASM-AML). During treatment with 2CdA, tryptase levels decreased transiently. CD44 surface expression levels were measured before, during and after therapy with 2CdA. At the time of progression to AML, CD44 surface expression on SC substantially increased, while the expression levels on MC remained stable. After progression to AML, induction poly-chemotherapy (CT) with daunorubicin (60 mg/m^2, days 1-3) and cytarabine (100 mg/m^2, days 1-7) (3+7 protocol) was administered, but no remission was obtained. Therefore, the patient received a second induction poly-CT cycle, consisting of mitoxantrone (12 mg/m^2, days 3-5) and cytarabine (2 x 1000 mg/m^2, days 1-4) (MIDAC). In addition, the patient received midostaurin (PKC412) (100 mg/day). However, no response was seen. Thereafter, the patient received one cycle of azacytidine (75 mg/m^2, days 1-7) combined with thalidomide (100 mg/day). However, AML further progressed and the patient died 3.6 months after the diagnosis AML had been established.

E



Surface expression of CD44 on bone marrow (BM) mast cells (MC), stem cells (SC) and progenitor cells (PC) (shown as Staining Index) and serum tryptase (ng/ml) were measured over 60.2 months in a 64-year-old male patient with indolent systemic mastocytosis (ISM; #18 in Supplemental Table S1). Because of mediator-related symptoms and an increase in serum tryptase, the patient initially received prednisolone (25 mg/day). Despite the (massive) increase in tryptase, the patient had a stable course for over 4.8 years. However, at the age of 69, the patient developed an aggressive SM (ASM). After progression to ASM, the patient received treatment with midostaurin (PKC412) (100 mg/day) and prednisolone (12.5 mg/day). CD44 surface expression levels on MC, SC, and PC were measured after 3.7 and 4.8 years and were found to be substantially increased with disease progression. The patient is still alive.

F



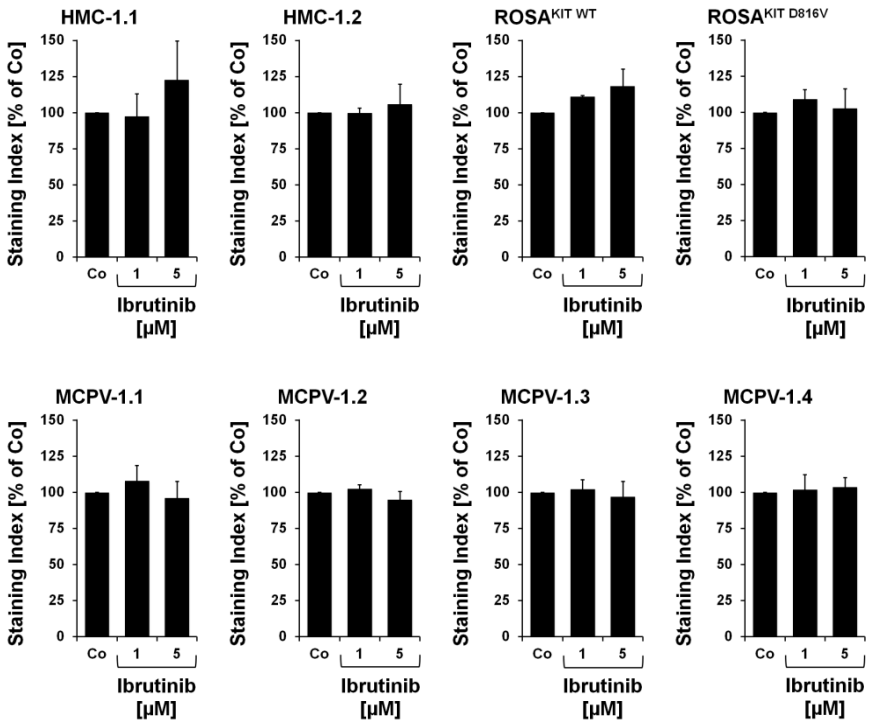
Surface expression of CD44 on bone marrow (BM) mast cells (MC), stem cells (SC) and progenitor cells (PC) (shown as Staining Index) and serum tryptase (ng/ml) were measured over 47.7 months in a 47-year-old female patient with indolent systemic mastocytosis (ISM; #33 in Supplemental Table S1), who had a stable clinical course over 4.0 years. The patient received only supportive therapy but no interventional therapy. During the observation period, serum tryptase levels remained stable. CD44 surface expression levels on all 3 cell types were measured at diagnosis and after 3.0 years and were found to be slightly lower after 3.0 years compared to initial time point at diagnosis. The patient is still alive.

Figure S10: Detection of CD44 expression on BM MC, SC and PC in SM patients during follow-up

Surface expression of CD44 on BM MC, SC and PC (shown as Staining Index) and serum tryptase (ng/ml) were measured in patients who had or developed advanced SM, including MCL (patients #154, #156) (A,B), SM with an associated hematologic neoplasm (ASM-AHN, patient #129) (C), ASM progressing to ASM-AML (patient #142) (D), and ISM progressing to ASM (patient #18) (E). In addition, CD44 levels on MC, SC and PC were determined in one ISM patient with stable clinical course (patient #33) (F). CD44 expression levels on MC, SC and PC were determined by multicolor flow cytometry and serum tryptase levels by a fluoroenzyme immunoassay (ImmunoCAP, ThermoFisher, Uppsala, Sweden). Abbreviations: BM, bone marrow; MC, mast cells; SC, stem cells; PC, progenitor cells; SM, systemic mastocytosis; MCL, MC leukemia; ASM, aggressive SM; AHN, associated hematologic neoplasm; AML, acute myeloid leukemia; ISM, indolent SM.

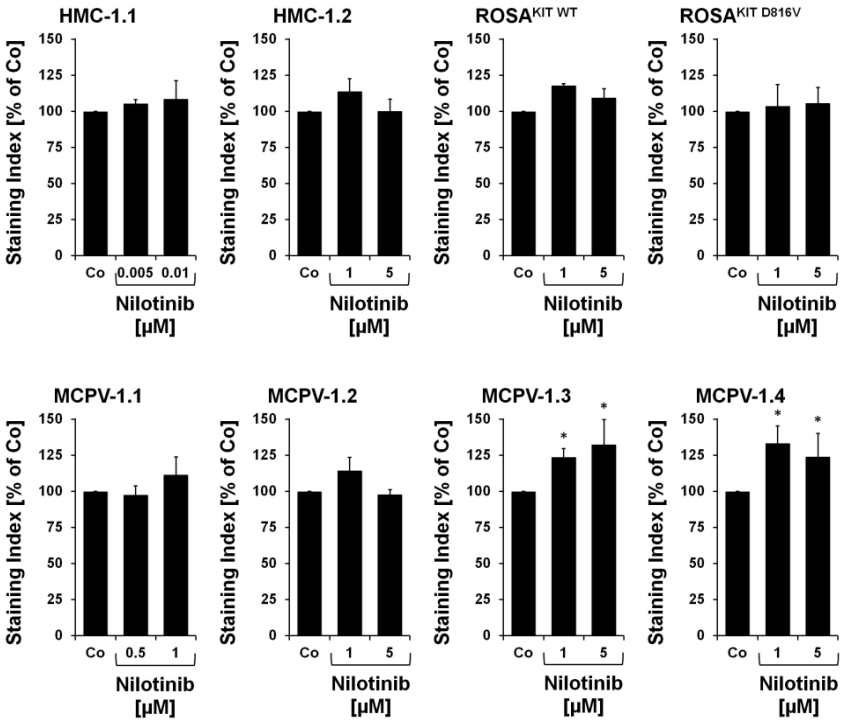
Supplemental Figure S11

A



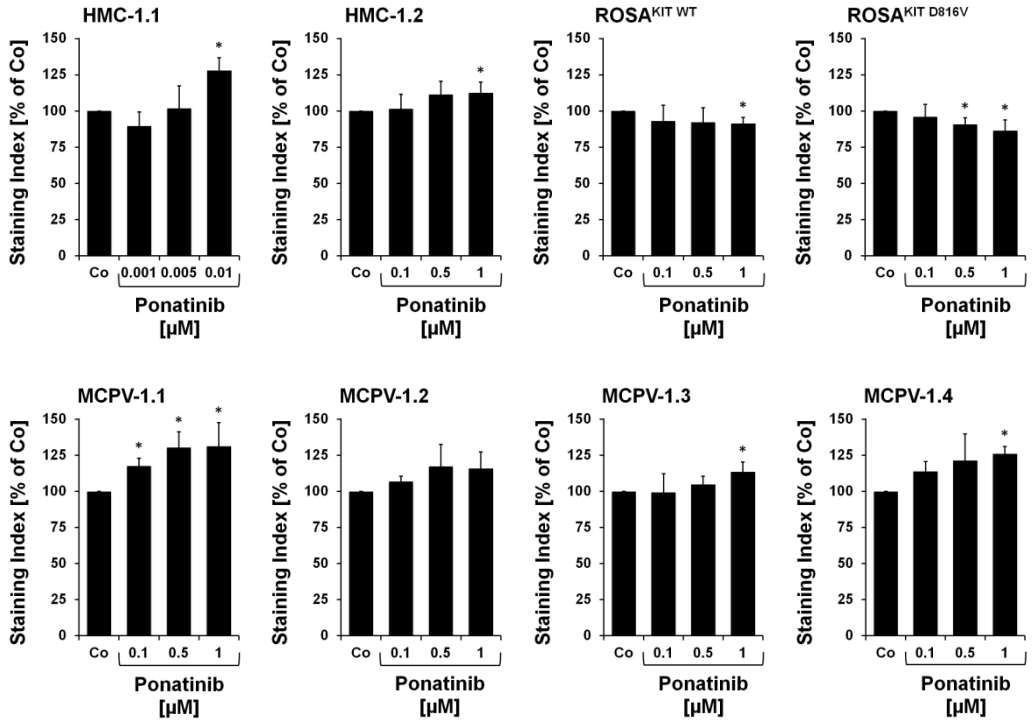
Mueller et al., Figure S11A

B



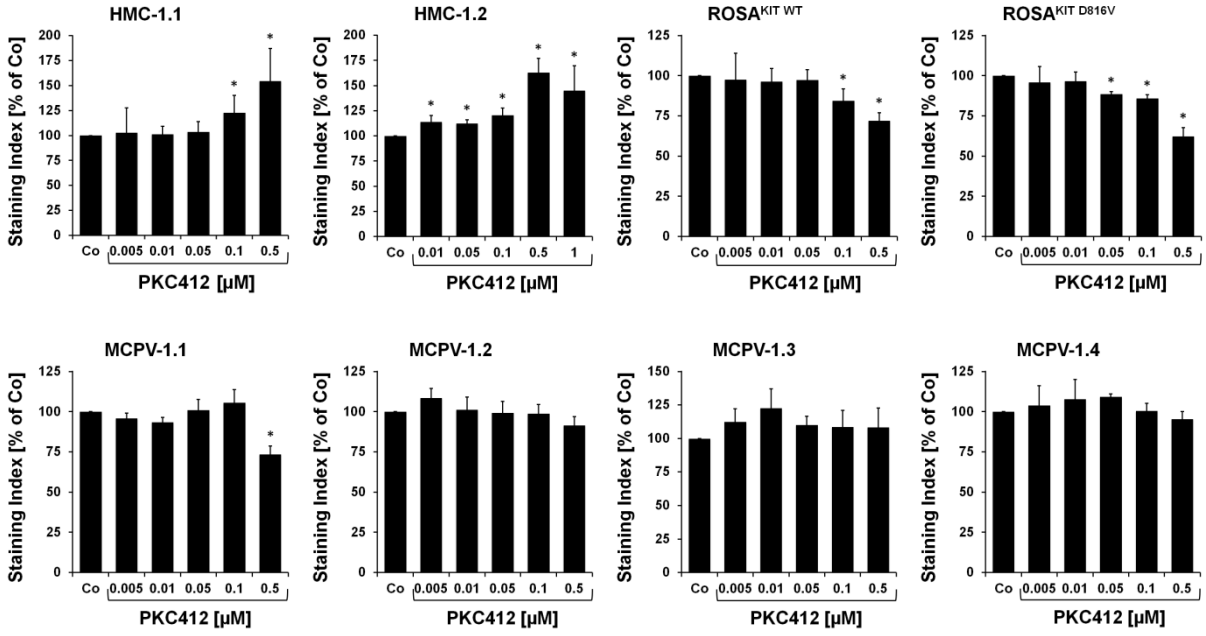
Mueller et al., Figure S11B

C



Mueller et al., Figure S11C

D



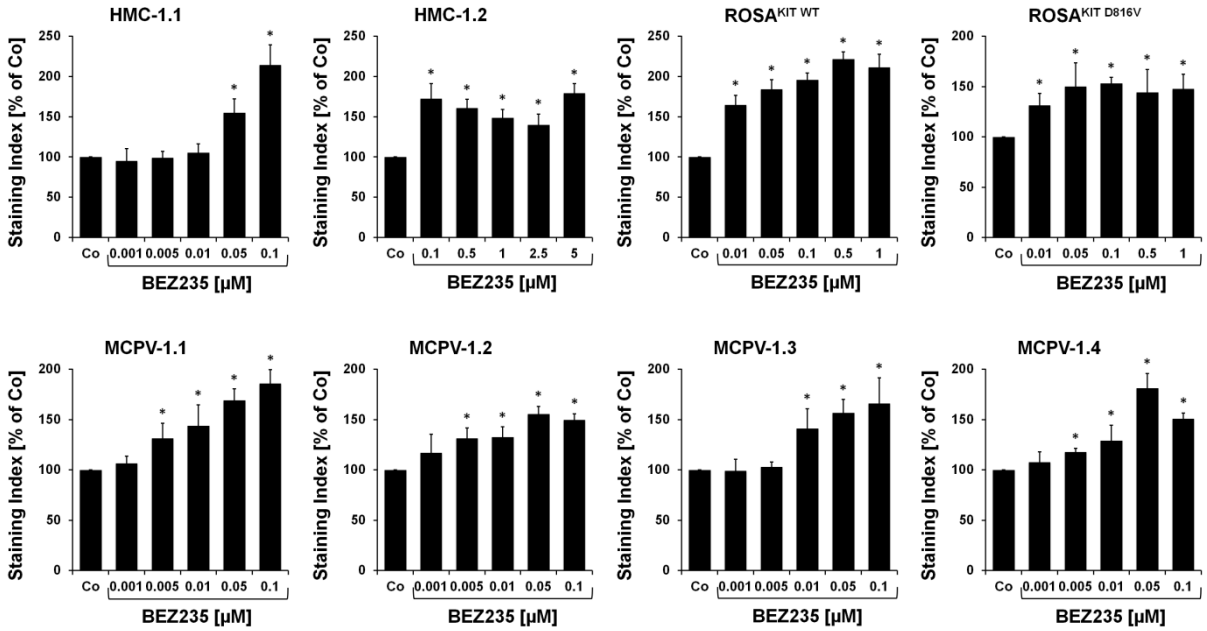
Mueller et al., Figure S11D

Figure S11: The effects of various tyrosine kinase-inhibitors on the expression of CD44 in neoplastic mast cells

HMC-1, ROSA and MCPV-1 cells were incubated with the BTK (Bruton's tyrosine kinase)-inhibitor ibrutinib (1 and 5µM) (A), the TKI (tyrosine kinase inhibitor) nilotinib (0.005-5µM) (B), the multi-targeted TKI ponatinib (0.001-1µM) (C), or

multi-targeted KIT inhibitor PKC412 (midostaurin) (0.005-1µM) (D) at 37°C for 48 hours. Then, expression of CD44 was analyzed by flow cytometry. Bars represent the expression levels of CD44 (Staining Index) as percentage of DMSO control (Co) and are expressed as mean±S.D. of at least 3 independent experiments. Asterisk (*): $p<0.05$ compared to Co (Student´s t test).

Supplemental Figure S12



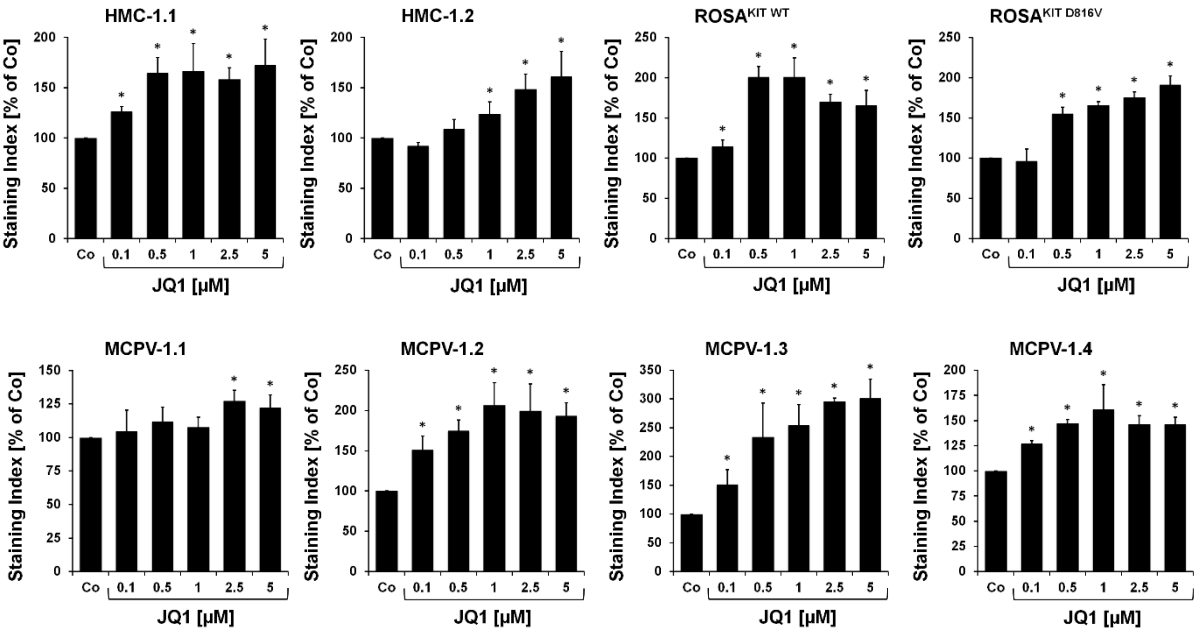
Mueller et al., Figure S12

Figure S12: The effect of the dual PI3-Kinase/mTOR inhibitor BEZ235 on the expression of CD44 in neoplastic mast cells

HMC-1, ROSA and MCPV-1 cells were incubated with the PI3-Kinase/mTOR inhibitor BEZ235 (0.001-5µM) at 37°C for 48 hours. Then, expression of CD44 was analyzed by flow cytometry. Bars represent the expression of CD44 (Staining Index) as percentage of DMSO control (Co) and are expressed as mean±S.D. of at least 3 independent experiments. Asterisk (*): $p<0.05$ compared to Co (Student´s t test).

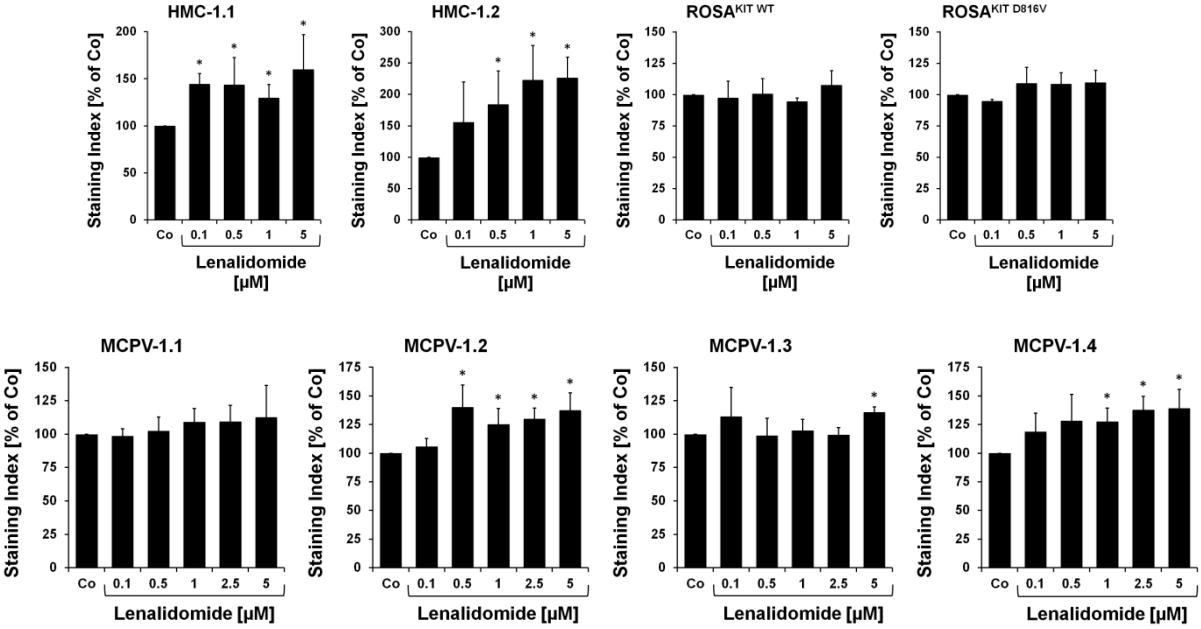
Supplemental Figure S13

A



Mueller et al., Figure S13A

B



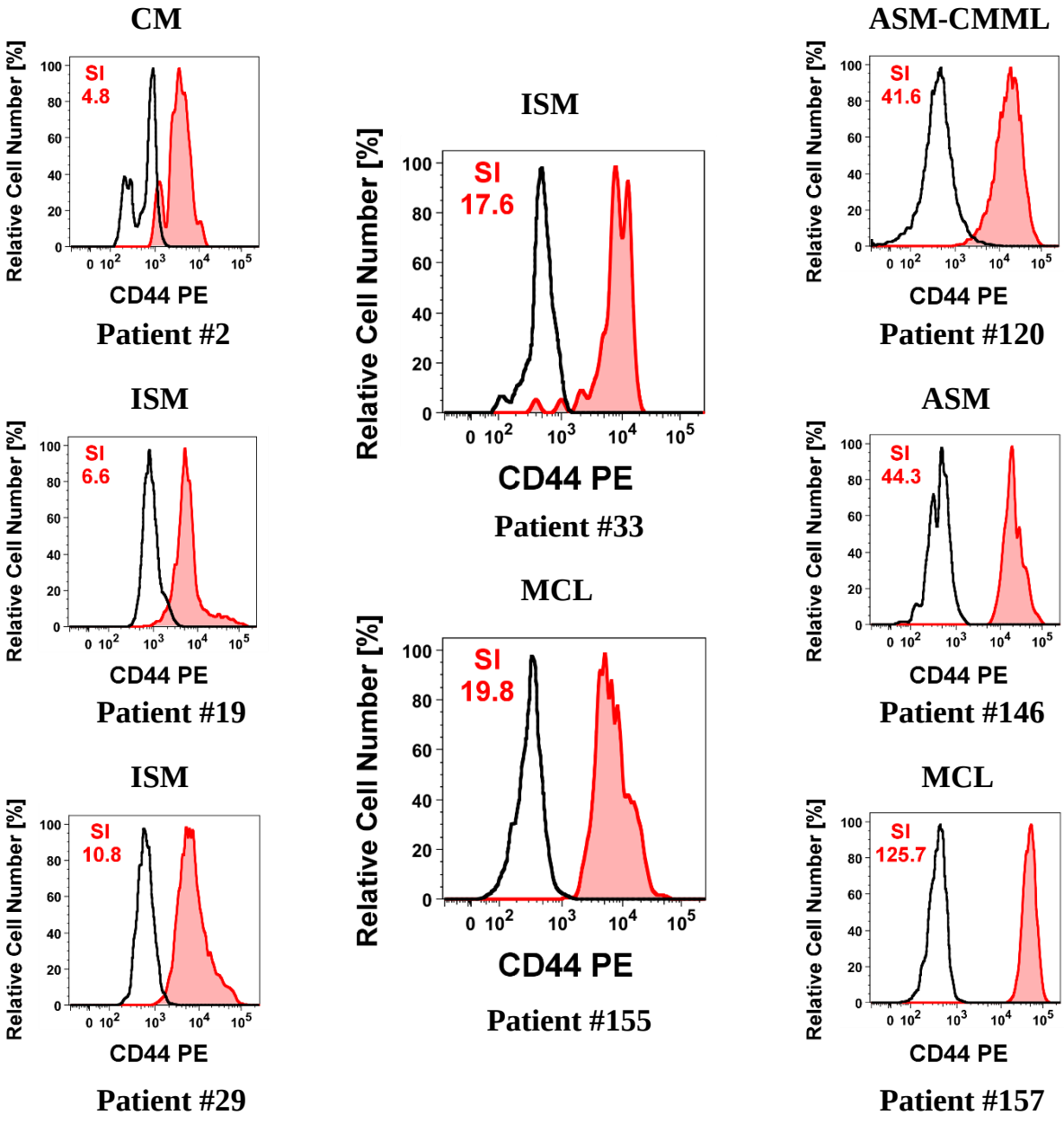
Mueller et al., Figure S13B

Figure S13: The effects of JQ1 and lenalidomide on the expression of CD44 in neoplastic mast cells

HMC-1, ROSA and MCPV-1 cells were incubated with the BET inhibitor JQ1 (0.1-5µM) (A) or lenalidomide (0.1-5µM) (B) at 37°C for 96 hours. Then, expression of CD44 was analyzed by flow cytometry. Bars represent the expression of CD44

(Staining Index) as percentage of DMSO control (Co) and are expressed as mean±S.D. of at least 3 independent experiments. Asterisk (*): $p<0.05$ compared to Co (Student´s t test).

Supplemental Figure S14



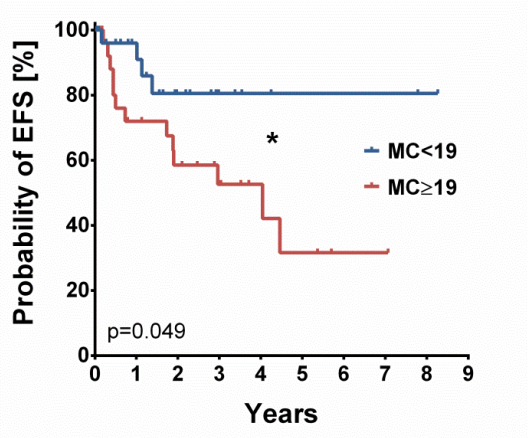
Mueller et al., Figure S14

Figure S14: Evaluation of CD44 expression on mast cells (MC) by flow cytometry
$CD45^{+}/CD117^{++}/CD34^{-}$ bone marrow (BM) MC obtained from patients with cutaneous mastocytosis (CM) and different variants of systemic mastocytosis (SM), including indolent SM (ISM), aggressive SM (ASM), SM with an associated hematologic neoplasm (SM-AHN) such as chronic myelomonocytic leukemia

(CMML), and MC leukemia (MCL) were examined by multicolor flow cytometry for expression of CD44 (red histograms). In addition, the isotype-matched control antibody (open histograms) and the Staining Index (SI) are shown. Representative histograms with an SI around 19, illustrating the calculated cut-off value for CD44 surface expression on MC used in our Kaplan Meier survival analysis (Figure 7, Supplemental Figures S15 and S16), are shown in the central column. On the left- and right-hand side, 3 patients each with clearly lower SI (left column) or higher SI (right column) are displayed.

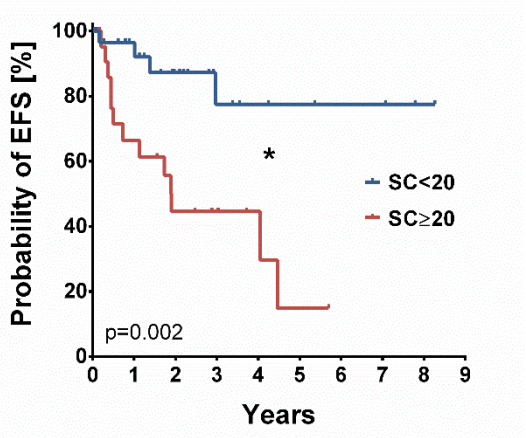
Supplemental Figure S15

A



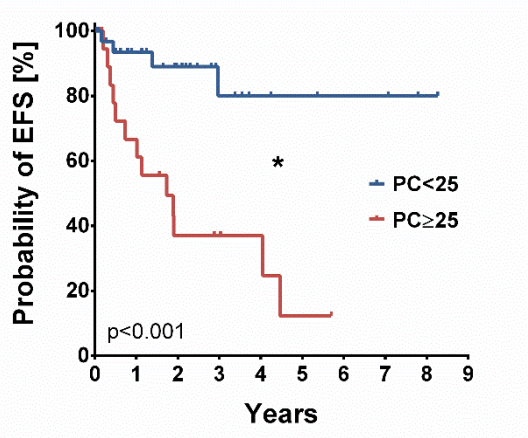
Mueller et al., Figure S15A

B



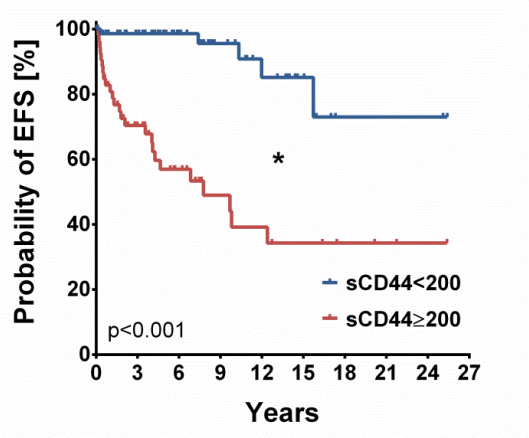
Mueller et al., Figure S15B

C



Mueller et al., Figure S15C

D



Mueller et al., Figure S15D

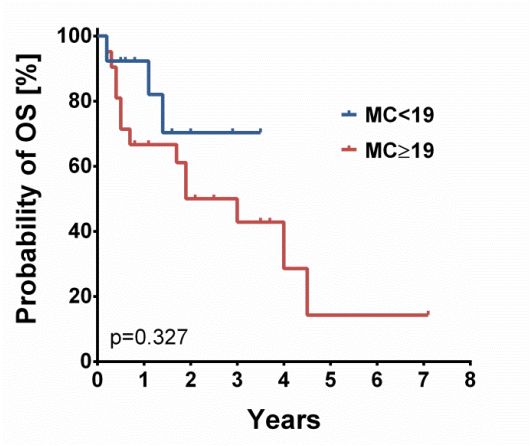
Figure S15: Prognostic impact of CD44 in patients with mastocytosis

A-C: The probability of event-free survival (EFS) was determined for subgroups of patients with mastocytosis. Patients were split into subgroups based on higher (red

curves) and lower expression (blue curves) levels of CD44 on $CD117^{++}/CD34^{-}$ mast cells, MC (A), $CD34^{+}/CD38^{-}$ stem cells, SC (B), and $CD34^{+}/CD38^{+}$ progenitor cells, PC (C). Cut-off values of Staining Indices to define higher and lower CD44 surface expression levels based on ROC curve analyses were: 19 for MC, 20 for SC, and 25 for PC. The total patient sample (n=56) consisted of 2 with cutaneous mastocytosis (CM), 20 with indolent systemic mastocytosis (ISM), 22 with systemic mastocytosis with an associated hematologic neoplasm (SM-AHN), 6 with aggressive systemic mastocytosis (ASM), and 6 with mast cell leukemia (MCL). The median follow-up of our patients was 2.8 years. The probability of EFS was calculated by the product limit method of Kaplan and Meier. The differences in EFS in the subgroups defined by higher or lower surface expression of CD44 were significant by log-rank test. Asterisk (*): $p<0.05$. D: The probability of EFS was determined in 129 patients with mastocytosis, consisting of 15 with CM, 74 with ISM, 7 with smoldering systemic mastocytosis, 20 with SM-AHN, 9 with ASM, and 4 with MCL. Based on ROC curve analysis, patients were split into those with higher levels (≥200 ng/ml) of soluble CD44 (sCD44) (red curves) or lower levels of sCD44 (<200 ng/ml) (blue curves). The median follow-up of our patients was 6.6 years. The probability of EFS was calculated by the product limit method of Kaplan and Meier. The difference in EFS in the subgroups defined by higher or lower levels of sCD44 was significant by log-rank test. Asterisk (*): $p<0.05$.

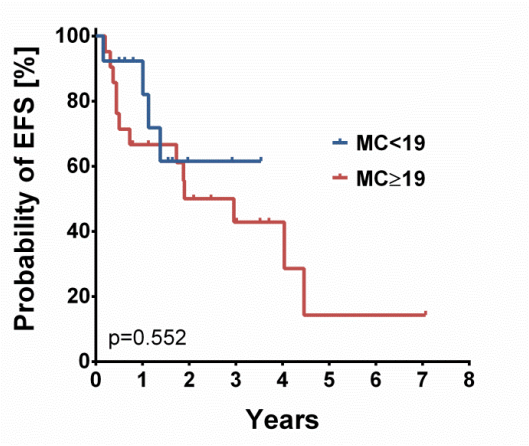
Supplemental Figure S16

A



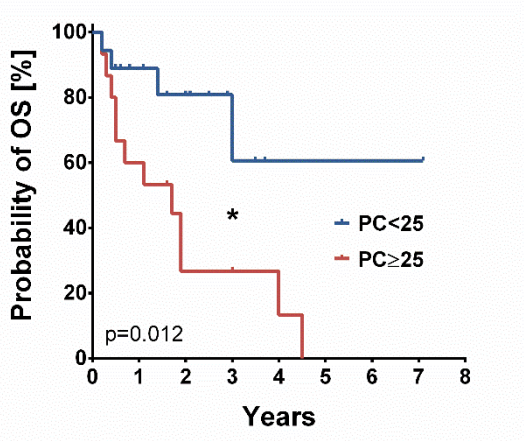
Mueller et al., Figure S16A

B



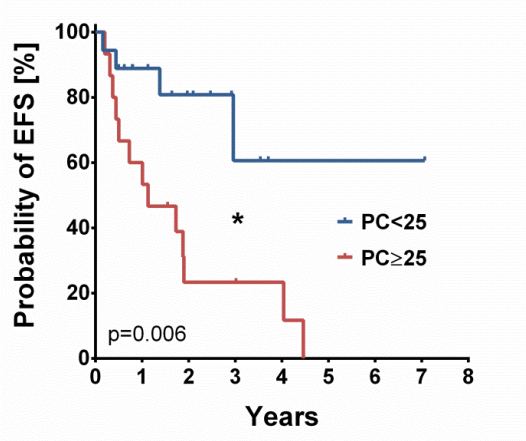
Mueller et al., Figure S16B

C



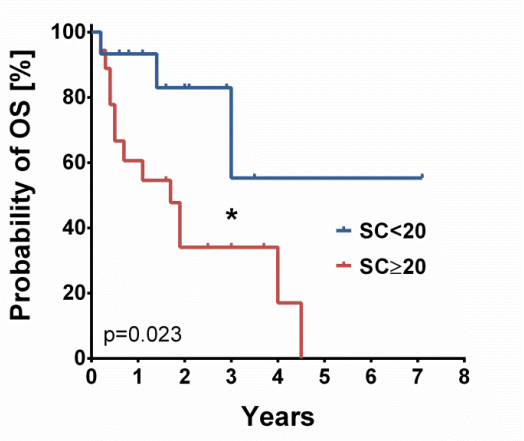
Mueller et al., Figure S16C

D



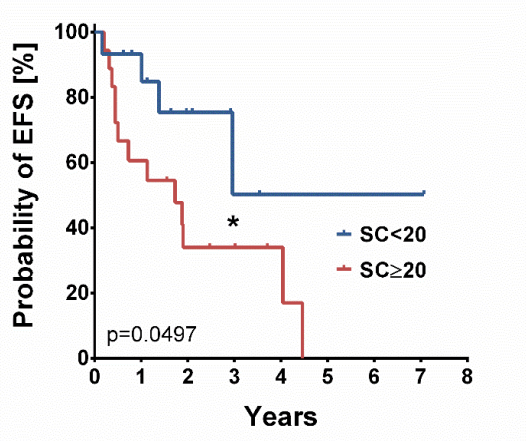
Mueller et al., Figure S16D

E



Mueller et al., Figure S16E

F

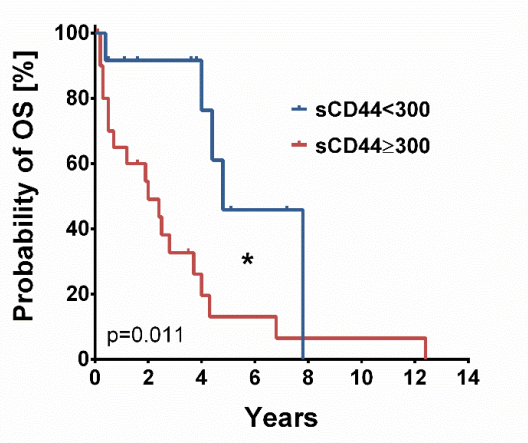


Mueller et al., Figure S16F

Figure S16: Prognostic impact of CD44 in patients with advanced mastocytosis
The probability of overall survival (OS) and event-free survival (EFS) was determined for subgroups of patients with advanced mastocytosis. Patients were split into subgroups based on higher (red curves) and lower expression (blue curves) levels of CD44 on $CD117^{++}/CD34^{-}$ mast cells, MC (A,B), $CD34^{+}/CD38^{-}$ stem cells, SC (C,D), and $CD34^{+}/CD38^{+}$ progenitor cells, PC (E,F). Cut-off values of Staining Indices to define higher and lower CD44 surface expression levels based on ROC curve analyses were: 19 for MC, 20 for SC, and 25 for PC. The total patient sample (n=34) consisted of 22 with systemic mastocytosis with an associated hematologic neoplasm (SM-AHN), 6 with aggressive systemic mastocytosis (ASM), and 6 with mast cell leukemia (MCL). The median survival of our patients was 3.0 years. The probability of OS (A,C,E) and EFS (B,D,F) was calculated by the product limit method of Kaplan and Meier. For SC and PC, the differences in OS and EFS in the subgroups defined by higher or lower expression of CD44 were significant by log-rank test. Asterisk (*): $p<0.05$.

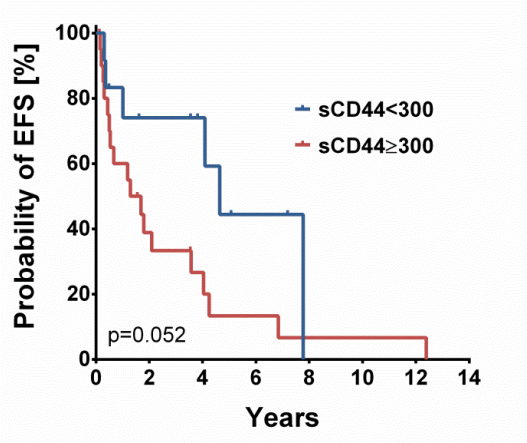
Supplemental Figure S17

A



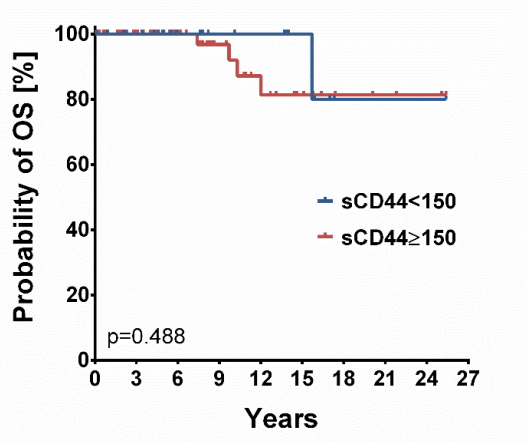
Mueller et al., Figure S17A

B



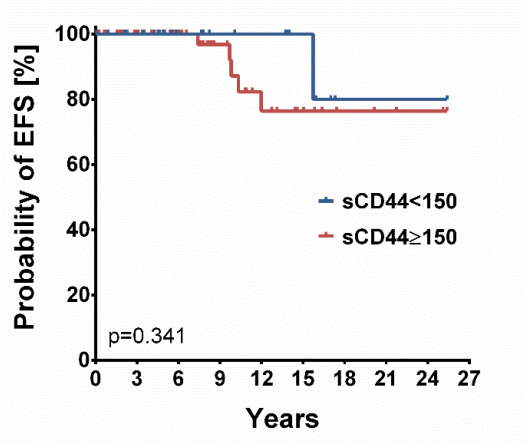
Mueller et al., Figure S17B

C



Mueller et al., Figure S17C

D



Mueller et al., Figure S17D

Figure S17: Prognostic impact of soluble CD44 (sCD44) in patients with advanced and indolent forms of mastocytosis

A,B: Probability of overall survival (OS) and event-free survival (EFS) was determined in 33 patients with advanced forms of mastocytosis, consisting of 20 with systemic mastocytosis with an associated hematologic neoplasm (SM-AHN), 9 with aggressive systemic mastocytosis (ASM), and 4 with mast cell leukemia (MCL). Based on ROC curve analysis, patients were split into those with higher levels (≥300 ng/ml) of soluble CD44 (sCD44) (red curves) or lower levels of sCD44 (<300 ng/ml) (blue curves). The median survival of our patients was 3.7 years. C,D: The probability of OS and EFS was determined in 96 patients with indolent forms of mastocytosis, consisting of 15 with cutaneous mastocytosis (CM), 74 with indolent systemic mastocytosis (ISM), and 7 with smoldering systemic mastocytosis. Based on ROC curve analysis, patients were split into those with higher levels (≥150 ng/ml) of soluble CD44 (sCD44) (red curves) or lower levels of sCD44 (<150 ng/ml) (blue curves). The

median follow-up of our patients was 6.6 years. The probability of OS (A,C) and EFS (B,D) was calculated by the product limit method of Kaplan and Meier. In patients with advanced SM, the difference in OS in the subgroups defined by higher or lower levels of sCD44 was significant by log-rank test. Asterisk (*): $p<0.05$.

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