Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Selectin-Dependent Organ Invasion and Prognostic Impact of Loss of CD2 in Systemic Mastocytosis

Peter Valent

peter.valent@meduniwien.ac.at

Medical University of Vienna https://orcid.org/0000-0003-0456-5095

Emir Hadzijusufovic

Medical University of Vienna, Ludwig Boltzmann Cluster Oncology https://orcid.org/0000-0001-7409-4204

Nina Nehmann

University Cancer Center Hamburg, University Medical Center Hamburg Eppendorf, Hamburg, Germany

Hanna Maar

University of Lübeck and University Medical Center Schleswig-Holstein, Campus Lübeck, Lübeck, Germany

Gerit Schernthaner

Medical University of Vienna

Daniela Berger

Medical University of Vienna

Irena Angelova-Fischer

Kepler University Hospital, Johannes Kepler University

Alex Stefan

Klinik für Innere Medizin 3 - Schwerpunkt Hämatologie und Onkologie, Kepler Universitätsklinikum

Isma Ishaq-Parveen

Medical University of Vienna, Vienna, Austria

Johannes Woltsche

Univ.Klinik für Dermatologie, Medical University of Graz

Karoline Gleixner

Medical University of Vienna

Martin Bilban

Medical University of Vienna

Wolfgang Sperr

Medical University of Vienna

Hans-Peter Horny

Ludwig-Maximilians-Universität Muenchen

Udo Schumacher

Institute for Anatomy and Experimental Morphology, University Cancer Center Hamburg, University Medical Center Hamburg Eppendorf, Hamburg, Germany

Daniel Wicklein

Medical School Hamburg, University of Applied Sciences and Medical University, Institute for Molecular Medicine, Hamburg, Germany

---

Article

Keywords: mastocytosis, mast cell leukemia, adhesion receptors, selectins, LFA-2, CD44

Posted Date: November 1st, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11354632/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: There is **NO** conflict of interest to disclose.

Abstract

In non-advanced systemic mastocytosis (SM), mast cells (MC) express CD2 and CD58 and form focal tissue-aggregates, whereas in advanced SM, MC often lack CD2 and produce diffuse infiltration. To explore the role of CD2 in SM progression, CD2 + and CD2 − subclones of HMC-1.2 cells were generated and injected intraperitoneally into pfp/rag2 mice and scid mice. CD2 + HMC-1.2 cells formed focal MC lesions in the peritoneum and lungs of pfp/rag2 mice, while CD2 − HMC-1.2 cells produced more diffuse infiltration, resulting in reduced survival. To explore the mechanisms of organ invasion, E- and P-selectin-deficient scid mice (scid-select) were employed. While infiltrates of CD2 + and CD2 − HMC-1.2 cells were detected in scid mice, organ-infiltration was markedly reduced or absent in scid-select mice. Moreover, scid-select mice injected with CD2 + or CD2 − HMC-1.2 cells showed prolonged survival compared to control mice ($p < 0.01$). Correspondingly, CD2 was expressed more frequently on MC in patients with non-advanced SM compared to advanced SM, and patients with CD2 + SM were found to have prolonged survival compared to patients with CD2 − SM ($p < 0.05$). Together, E- and P-selectins mediate organ-invasion of neoplastic MC, whereas loss of CD2 contributes to a diffuse leukemic spread of MC and thus SM progression with reduced survival.

Background

Mastocytosis is a term collectively used for a group of myeloid neoplasms characterized by abnormal proliferation and accumulation of mast cells (MC) in one or multiple organs, encompassing the bone marrow (BM), spleen, gastrointestinal (GI) tract, and skin.[1–8] Cutaneous mastocytosis is usually detected in childhood and shows a benign course.[1,4,5,8] Systemic mastocytosis (SM) may develop at any age throughout lifetime and is characterized by persistent disease and involvement of one or more internal organs with or without cutaneous involvement.[1–4,6–8] In a vast majority of these patients, the driver mutation *KIT* D816V is detected in neoplastic cells.[1,4,6–15]

The course, prognosis, and survival of patients with SM vary, depending on the disease variant, genetic landscape, presence of organomegaly, and phenotype of MC.[1,4,8,11–13,16–21] Based on the World Health Organization (WHO), SM is divided into BM mastocytosis (BMM), indolent SM (ISM), smoldering SM (SSM), SM with an associated hematologic neoplasm (SM-AHN), aggressive SM (ASM), and MC leukemia (MCL).[22–25] Whereas BMM, ISM and SSM are collectively called non-advanced SM, patients with SM-AHN, ASM and MCL are often referred to as advanced SM.[4,7,8,24,25]

The formation of multifocal dense aggregates of MC in hematopoietic tissues is the pathologic hallmark of SM.[2,4,26–28] Whereas in non-advanced SM, most MC are packed within coherent focal cell aggregates in the BM and other organs, MC in advanced SM are often “mobilized” and may be detected in the peripheral blood (PB).[2,4,6,26–30] In MCL, the leukemic spread of MC is massive and is a diagnostic criterion.[6,22–26,31] In these patients, a devastating diffuse infiltration of multiple organs by atypical, mostly immature, MC is often seen.[6,26–31] However, little is known about mechanisms and molecules

contributing to organ invasion of MC in advanced SM and the leukemic spread of MC that obviously contributes to disease progression.

A number of previous and more recent data suggest that various adhesion receptors contribute to homing, distribution, and aggregation of normal and neoplastic MC.[4,8,32–37] Indeed, neoplastic MC in SM express multiple adhesion receptors, including leukocyte function antigen-2 (LFA-2 = CD2), Hermes invasion receptor CD44, ICAM-1 (CD54), and LFA-3 (CD58), a recognition molecule that binds LFA-2.[21,32–37]

The observation that MC in patients with SM express CD2 in an aberrant manner is remarkable.[35,36] CD2 is expressed physiologically on thymocytes, circulating (activated) T cells, and a subset of NK cells, whereas normal MC and other myeloid cells are usually CD2-negative.[33–36] In patients with MCL, the levels of CD2 on MC are often lower or undetectable compared to indolent SM.[21,38–40] From these observations, the hypothesis was raised that CD2-CD58-binding contributes to the formation of MC aggregates in indolent SM, and that a loss of CD2 during progression to MCL is associated with decreased cluster-formation and thus a leukemic spread of MC.[4,21,36]

However, so far, this hypothesis has not been tested formally. Another open question is how neoplastic MC and their progenitor cells invade in various tissues and organs during disease progression. In this study, we examined the mechanisms of invasion and cluster formation of neoplastic MC in a xenotransplantation model using the human MCL-like cell line HMC-1.2 and severe immunodeficient (scid) mice.

Materials and Methods

Monoclonal antibodies (mAb) and other reagents

A number of mAb were used to characterize CD2 + and CD2 − HMC-1.2 subclones by flow cytometry. A specification of these mAb is provided in Supplemental Table S1. Table S2 features the antibodies used to stain cells by immunocytochemistry (ICC). Fetal calf serum (FCS), Iscove´s Modified Dulbecco´s medium (IMDM), penicillin, and streptomycin were purchased from PAA Laboratories (Pasching, Austria). Additional reagents used in this study are described in the supplement.

Culture of HMC-1.2 cells and generation of CD2 + and CD2 − subclones

The MCL-like cell line HMC-1.2[41] was cultured in IMDM with L-glutamine, antibiotics, and 10% FCS at 37°C. HMC-1.2 cells display *KIT* D816V and consist of a CD2 + and a CD2 − fraction.[36] To generate subclones expressing or lacking CD2, HMC-1.2 cells were sorted using the PE-conjugated mAb RPA-2.10 (CD2) and a FACS Star Plus (Becton Dickinson) as described.[36] Then, limiting dilution was performed. The two resulting CD2 − subclones were designated HMC-1.2 − 2fC7 and HMC-1.2 − 3rD2, and the three CD2 + subclones were termed HMC-1.2 + 3rE8, HMC-1.2 + 1rH3, and HMC-1.2 + 2fE8. All subclones were

found to express tryptase and KIT (Supplemental Figure S1) and retained both *KIT* mutations, D816V and V560G. Stable expression of CD2 in these clones was tested at certain time intervals by flow cytometry to confirm phenotypic stability. Subclones were stored in liquid nitrogen until used.

Flow cytometry and immunocytochemistry (ICC)

HMC-1.2 subclones and parental cells were stained with fluorochrome-labeled mAb shown in Supplemental Table S1 and analyzed by flow cytometry as reported.[36,37] ICC was performed on cytospin preparations of parental HMC-1.2 cells and CD2 + and CD2 − HMC-1.2 subclones using antibodies listed in Table S2 following standard methods. Details about flow cytometry and ICC are described in the supplement.

Whole transcriptome array profiling studies

Total RNA was extracted from sorted CD2 + and CD2 − HMC-1.2 cells using an RNeasy kit (Qiagen). Then, total RNA (5 µg each) was used for GeneChip analysis. Preparation of terminal-labeled cRNA, hybridization to genome-wide human U133 2.0 plus GeneChips (Affymetrix, Santa Clara, CA, USA) and scanning of the arrays were carried out according to the manufacturer's protocols (https://www.affymetrix.com). Robust microarray analysis (RMA), signal extraction, and mRNA level normalization were performed as described previously (http://www.bioconductor.org/).[42] The filtering criteria required a minimal signal of 50 in at least one sample tested. A complete list of genes examined by RMA (mRNA species) is available at Gene Expression Omnibus under: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE33504. Differences in detected mRNA levels were expressed as ratio of CD2 + versus CD2 − HMC-1.2 cells. Expression of top regulated genes of interest (encoding surface antigens) at the mRNA level was confirmed by qPCR using primers depicted in Table S3A. In addition, we compared gene array studies with flow cytometry staining results using antibodies against various adhesion receptors. Details are described in the supplement.

Laminar flow assay

To study adhesion of CD2 + and CD2 − HMC-1.2 cells to endothelial cells under flow conditions, a laminar flow adhesion assay was employed using human umbilical vein endothelial cells (HUVEC). Technical details are described in the supplement.

Mouse experiments

To define the role of adhesion molecules in HMC-1.2 cell invasion and expansion, 3 mouse xenotransplantation models were employed. Pore forming gene/recombinase activating gene (pfp/rag2) mice and severe combined immunodeficiency (scid) mice were purchased from Taconic (Ry, Denmark). E- and P-selectin-deficient scid mice (scid select) were generated as described previously.[43] All mice were housed in ventilated cages and supplied with sterile food and water ad libitum. Experiments were supervised by the institutional animal welfare officer and approved by the local licensing authority

(Behörde für Soziales, Gesundheit, Familie, Verbraucherschutz; Amt für Gesundheit und Verbraucherschutz, Hamburg, Germany, project number #G36/06). CD2 + and CD2 − HMC-1.2 cells were suspended in 1 mL medium without FCS. A total volume of 200 µl medium containing 1 x 10^6 HMC-1.2 cells were injected intraperitoneally (i.p.) into scid mice, scid-select mice, or pfp/rag2 mice (10 mice per group) following published protocols.[44–47] Animals were sacrificed when their overall condition became critical according to an in-house scoring system. Survival was defined as time between injection and sacrifice. Lungs and intraperitoneal mastocytoma (including invaded organs and surrounding tissue) were prepared, fixed in 4% buffered formaldehyde and processed to paraffin histology. BM was not investigated because this study focused on extramedullary organ invasion by MC after i.p. injection.

Histopathological examination of mouse tissues and immunohistochemistry (IHC)

Organ specimens were cut and embedded in 4% agar (Agar Noble, Difco Laboratories, Detroit, MI, USA). Agar blocks were routinely processed for wax histology and cut into serial sections. The number and size of lung metastases were quantified in Hematoxylin & Eosin-stained sections according to a standard protocol.[45,46] Staining for human CD44 expression by engrafted HMC-1.2 cells in paraffin-embedded tissue was performed as described.[47] Briefly, after 85°C water bath pretreatment with citrate buffer at pH 6.0, an anti-human CD44 mAb (BD Biosciences, Heidelberg, Germany, 550392) and an isotype-matched control IgG2b mAb (Dako, Copenhagen, Denmark; X0944) were applied followed by application of goat anti-mouse-biotin diluted in TBS (LS-Bio, Lynnwood, WA, USA; LS-C149505), ABC-AP and Permanent Red (Biozol, Eching, Germany). Sections were counterstained with hematoxylin. Negative control lung sections were treated without specific antibodies. This omission control and the isotype antibody control showed no specific staining reactions. Stained sections were examined and photographed under a Zeiss Axioplan photomicroscope (Zeiss Jena, Germany).

Evaluation of patients with SM according to expression or lack of CD2 in mast cells

A total of 113 patients with SM (females: n = 51; males: n = 62; f/m ratio, 1:1.2) seen and followed at the Medical University of Vienna, Medical University of Graz, or Kepler University Linz between 2015 and 2024 were examined. The patients´ median age was 52 years (range: 20–90 years; IQR: 40–61 years). Patients were diagnosed according to diagnostic WHO criteria.[22–25] BM studies included histology, immunohistochemistry, flow cytometry, and PCR testing for *KIT* D816V. All studies were performed according to generally accepted guidelines and methodologies.[12,26–28,39,48,49] Staging investigations included ultrasound and/or computed tomography examinations of the liver, spleen, and lymph nodes, blood counts, and serum chemistry, including basal serum tryptase levels. Expression of CD2, CD25, and/or CD30 in BM MC was examined by multi-color flow cytometry and/or immunohistochemistry.[27,39,49] Patients were only enrolled when MC had been tested for CD2 expression. The median observation time was 5.58 years. Progression-free survival (PFS) and overall survival (OS) were analyzed in all cases. All patients provided written informed consent to participate. The study was performed in accordance with the declaration of Helsinki and approved by the local ethics committee (EC) of the Medical University of Vienna (EC number 1549/2012).

Statistical analysis

To determine the levels of significance in our *in vitro* and *in vivo* studies, appropriate statistical tests were applied. To study the differences in growth rates and expression levels of individual surface proteins in CD2 + and CD2 − HMC-1 cells, the paired student´s t-test was employed. The significance level of differences in the percentage of patients with CD2 + MC compared to CD2 − MC in patients with non-advanced SM versus advanced SM was calculated by chi square test. Results were considered significant when the p value was < 0.05. To estimate the probability of OS and PFS in our SM patients, the method of Kaplan & Meier was applied. The significance levels in differences of PFS and OS in the two patient cohorts was determined by the Gehan-Breslow-Wilcoxon test. We also established Kaplan & Meier estimates to determine differences in the survival of mice. The resulting significance levels were calculated using GraphPad Prism software (Version 5.03, La Jolla, CA USA).

Results

Phenotypic characterization of CD2 + and CD2 − HMC-1.2 subclones

Sorted CD2 + and CD2 − HMC-1.2 cells were found to exhibit an identical morphology on Wright-Giemsa-stained slides. In 3 independent experiments, sorted CD2 + and CD2 − HMC-1.2 cells were cultured separately for more than 10 weeks. In all 3 experiments, both cell populations showed comparable growth characteristics and phenotypic stability. In particular, the sorted CD2 + cells remained CD2-positive and the sorted CD2 − cells all remained CD2-negative. The same clonal stability was found in the two CD2 + and the three CD2 − HMC-1 sub-clones (Figure S2). Apart from CD2, the CD2 + and the CD2 − HMC-1.2 sub-clones displayed an almost identical phenotype in our flow cytometry studies (Table 1). In addition, CD2 + and CD2 − HMC-1.2 sub-clones exhibited an identical pattern of cytoplasmic antigens as determined by ICC (Figure S1, Table 2). In particular, all sub-clones expressed tryptase, histidine decarboxylase, vascular endothelial growth factor (VEGF) and heme oxygenase-1 without visible differences in expression levels (Figure S1, Table 2).

CD2 + and CD2 − HMC-1.2 subclones express an almost identical profile of classical cell surface adhesion receptors

As determined by flow cytometry, the CD2 + and CD2 − HMC-1.2 sub-clones were found to display an almost identical profile of cell surface adhesion receptors, except for CD2-expression and lower expression of intercellular adhesion molecules-1 (ICAM-1) (CD54) in the CD2 + sub-clones (Table 1). All HMC-1.2 sub-clones were found to express substantial amounts of the homing (invasion) receptor Hermes (CD44) and the CD2-recognition receptor LFA-3 (CD58) without substantial differences in surface expression levels (Table 1). Corresponding results were obtained in our gene array studies (Table S4). In addition, all HMC-1.2 sub-clones expressed P-selectin ligand PSGL-1 (CD162) on their surface (Table 1). Expression of adhesion receptors was also confirmed at the mRNA level by qPCR

(Table S3B). By contrast, none of the HMC-1.2 sub-clones tested expressed substantial amounts of surface E-Selectin (CD62E) or P-Selectin (CD62P) (Table 1). We also examined dynamic adhesion of CD2 + and CD2 − HMC-1.2 cells on endothelial cells (HUVEC) under physiological flow conditions. In these experiments we found that CD2 − HMC-1.2 cells produce significantly more firm adhesion signals on HUVEC than the CD2 + HMC-1 cells (Figure S3).

mRNA profiling reveals overexpression of additional adhesion and tissue matrix-relevant receptors in CD2 + HMC-1.2 cells compared to CD2 − HMC-1.2 cells

To compare more adhesion-, aggregation-, and migration-associated receptors, sorted CD2 + and CD2 − HMC-1.2 subsets were examined by gene array studies and RT-PCR. For gene array studies, RNA isolated from CD2 − or CD2 + HMC-1.2 cells was labeled and hybridized to human genome-wide U133 2.0 plus arrays. A total of 83 genes were expressed at higher levels in CD2 + HMC-1.2 cells compared to CD2 − HMC-1.2 cells (Table S4). Table 3 shows a summary of the 12 most upregulated mRNA species, including CD2. An obvious discriminating feature of CD2 + HMC-1.2 cells was the overexpression of several gene products known to be either directly or indirectly associated with adhesion, migration or extracellular matrix interaction, such as galectin-3 (LGALS3), tensin-1, cadherin 11 (osteoblast-cadherin), cerebral endothelial cell adhesion molecule (CERCAM), and neuronal cell adhesion molecule (NRCAM) (Table 3, Table S4). However, apart from CD2, no other major cell-cell aggregation-related receptor was up- or downregulated at the mRNA level in CD2 + HMC-1.2 cells compared to CD2 − HMC-1.2 cells, suggesting that the loss of aggregation of CD2 − HMC-1.2 cells, described in our previous studies [36], is attributable primarily, if not entirely, to disrupted CD2-CD58 interaction. A summary of mRNA products specific for surface antigens relevant to adhesion or aggregation expressed in CD2 − and CD2 + HMC-1.2 cells is shown in Tables S3B (RT-PCR data) and S4 (gene array data).

Lack of CD2 in HMC-1.2 cells is associated with reduced survival of pfp/rag-2 mice

All pfp/rag2 mice injected with HMC-1.2 cells developed intraperitoneal mastocytoma lesions as well as mastocytoma lesions in the GI tract and/or lungs. Based on termination criteria, mice had to be sacrificed between days 63 and 91. The time to termination and thus survival of pfp/rag2 mice injected with CD2 − HMC-1.2 cells, was significantly shorter compared to mice injected with CD2 + HMC-1.2 cells. In particular, median survival in mice receiving CD2 − HMC-1.2 cells was 69 days, whereas survival in mice receiving CD2 + HMC-1.2 cells was 85 days ($p < 0.005$ by log-rank test) (Fig. 1A). Total weight of intraperitoneal MC tumors was not significantly different in the two groups of mice, although there was a clear tendency towards larger MC tumor masses in the group injected with CD2 − HMC-1.2 cells (CD2 + HMC-1.2: 3.133 ± 0.58 gram versus CD2 − HMC-1.2: 4.244 ± 0.60 gram; $p = 0.2022$, by unpaired t-test) (Fig. 1B). Figure 1C shows the weight of the total excised MC tumor lesions per mouse in the two animal groups (injected with CD2 − or CD2 + HMC-1.2 cells) in relation to their survival. In most animals, larger

tumor lesions were associated with shorter survival and most of these mice had been injected with CD2 − HMC-1.2 cells (Fig. 1C).

The infiltration pattern in the lung depends on expression of CD2 in HMC-1.2 cells

To study the infiltration patterns of HMC-1.2 cells during invasion of secondary target organs, we examined lung histology patterns in affected animals. The degree of lung infiltration varied among animals (Table S5; Figure S4). In all but two pfp/rag2 mice injected with CD2 − HMC-1.2 cells, diffuse infiltration was observed in CD44-stained lung tissue sections, ranging from a few cells (graded as "+") to dense confluent infiltrates (+++) (Table S5). In most mice injected with CD2 + HMC-1 cells, staining of lung sections with a CD44 antibody revealed not only diffuse infiltration but also several compact mastocytoma nodules scattered in the connective tissues of the lungs (Table S5, Figure S5A). Overall, there was a clear tendency towards fewer nodules and a more diffuse spread of MC in mice injected with CD2 − HMC-1.2 cells (Figure S5B). However, this difference did not reach statistical significance based on the highly variable number of nodules in mice injected with CD2 + cells ($p > 0.05$).

Detection of the Hermes invasion receptor CD44 on HMC-1.2 cells infiltrating the lungs and peritoneal cavity of pfp/rag-2 mice

The Hermes receptor CD44 is an important invasion receptor and prognostic marker in SM.[37] We stained mouse tissue sections with an antibody against CD44. Staining of solid nodules of MC invading the intraperitoneal organs, including pancreas, revealed that HMC-1.2 cells in the center of these nodules expressed low levels of CD44 while the cells at the invasion margin and isolated invading HMC-1.2 cells stained highly positive for CD44 (Fig. 2). Corresponding results were found in the lungs (Figure S5A, Table S5). Particularly high levels of CD44 were found in HMC-1.2 cells invading intraperitoneal organs including pancreas (Fig. 2A). There was no difference in CD44 expression in the invading HMC-1.2 cells when comparing CD2 − and CD2 + cells (Table S5).

Role of E- and P-selectins in HMC-1.2 cell invasion in scid mice

To further study the mechanisms underlying HMC-1.2 cell expansion and organ invasion, we conducted xenograft experiment with E- and P-selectin deficient scid mice (scid-select) and control (selectin-competent) scid mice. In a first step, we confirmed that CD2 + and CD2 − HMC-1.2 cells engraft in these mice in the same way as in B6 pfp/rag2 mice. Control scid mice (expressing E- and P-selectins) injected with HMC-1.2 cells reached termination criteria after 63 to 83 days. As expected, the survival of control scid mice was significantly shorter when these mice were injected with CD2 − HMC-1.2 cells compared to mice receiving CD2 + HMC-1.2 cells (median survival of mice injected with CD2 − HMC-1.2 cells: 65 days vs CD2 + HMC-1.2 cells: 76 days; $p < 0.05$ by log-rank test) (Fig. 3A). One scid mouse of the CD2 +

HMC-1.2 group died a few days after injection without any visible MC lesions. In the scid-select mice injected with HMC-1.2 cells experiments had to be terminated at day 121. Interestingly, the difference in survival between selectin-deficient mice receiving CD2 − or CD2 + HMC-1.2 cells was not significant ($p = 0.247$) (Fig. 3B). Figure 3C and 3D show the weight of the excised intraperitoneal MC tumors in scid mice and scid select mice, respectively, in the two animal groups (injected with CD2 − or CD2 + HMC-1.2) in relation to survival. The differences in survival between scid and scid-select mice was significant, both for animals receiving CD2 + HMC-1.2 cells and those receiving CD2 − HMC-1.2 (median survival in mice injected with CD2 + cells: scid, 76 days vs scid-select, 98.5 days, $p < 0.0001$; median survival in mice injected with CD2 − cells: scid, 65 days vs scid-select, 80 days; $p = 0.0053$; log rank tests) (Fig. 3E and 3F). The longer survival of scid-select mice was associated with fewer and smaller MC tumor lesions in the lungs (Table 4). While in all scid mice, the lungs displayed large HMC-1.2 infiltrates, such larger lesions were only found in 5 of 17 scid-select mice (Table 4).

Prognostic impact of expression of CD2 on MC in patients with SM

Recent data suggest that CD2 expression on MC is of prognostic significance in SM.[21] We found that CD2 is expressed more frequently on MC in patients with non-advanced SM compared to patients with advanced SM ($p < 0.05$) (Fig. 4A). Moreover, patients with CD2 + SM were found to have a better OS compared to patients with CD2 − SM (Fig. 4B) ($p < 0.05$).

Discussion

In advanced SM, aggressive multi-organ invasion by MC and their leukemic expansion are major prognostic variables.[6,8,18] However, little is known about mechanisms and molecules contributing to organ invasion and the diffuse leukemic spread of MC in advanced SM. We employed HMC-1.2 cells and two xenotransplantation models to study the role of adhesion receptors in organ invasion and leukemic expansion of neoplastic MC. Our data show that E- and P-selectins mediate organ invasion of HMC-1.2 cells in pfp/rag2 and scid mice, and that CD2 + HMC-1.2 cells produce a more focal infiltration-pattern, whereas CD2 − HMC-1.2 cells produced rapid diffuse infiltration, and a more fatal disease.

Selectins and selectin-ligands are known to mediate tethering and rolling of circulating cells on endothelial surfaces, thereby preparing these cells for subsequent adhesion and transmigration. This concept holds true for normal myeloid cells, circulating cancer cells, and myeloid leukemias.[44,46,50,51] Normal and neoplastic MC also express various adhesion and homing receptors, including integrins and selectin-ligands.[32–39,50,52] In the present study, we confirmed that HMC-1.2 cells express multiple adhesion and homing antigens, such as PECAM-1 (CD31), Hermes (CD44), ICAM-1 (CD54), and the selectin ligand PSGL-1 (CD162). Furthermore, we found that homing of HMC-1.2 cells to certain target organs in pfp/rag2 and scid mice depends on the presence of E- and P-selectins. In fact, organ invasion by HMC-1.2 cells was prominent in control scid mice whereas in scid-select mice, no or only minimal organ invasion was seen. In addition, the survival of scid mice injected with HMC-1.2 cells was

significantly shorter compared to that of scid-select mice. These results strongly suggest that MC homing in various organs is selectin-dependent. Our data also confirm earlier studies in lung and colon carcinomas as well as in myeloid and eosinophilic leukemia.[43,44,51] In addition, these data are in line with results suggesting that E- and P-selectins are essential for the attachment of immature murine MC under flow conditions.[53] In the present study, we also analyzed adhesion under shear stress-conditions, and found that CD2 − HMC-1.2 cells produce significantly more adhesion-events on endothelial cells compared to CD2 + HMC-1.2 cells. Interestingly, CD2 − HMC-1.2 cells also expressed higher levels of ICAM-1 (CD54), a known endothelial-leukocyte adhesion receptor. Overall, these data suggest that CD2 − MC are prepared and behave like circulating leukemic cells that can invade in various target organs by their capacity to bind to vascular endothelial cells.

An interesting aspect is that HMC-1.2 cell homing and expansion was seen in pfp/rag2 mice not only in the GI tract, a well-known target organ in SM, but also in the lungs of these mice. In fact, although it has been reported that neoplastic MC can also be detected in the lungs of patients with SM, progressive lung infiltration is uncommon even in advanced SM.[54,55] One explanation for this discrepancy may be that infiltration and expansion of HMC-1.2 cells in the lungs was facilitated by the lack of intact NK cells and thus a proper immune system in pfp/rag2 mice, which would also explain why the lungs were not affected in the same way in scid mice exhibiting normal numbers of NK cells. Another explanation could be that during long-term culture, HMC-1.2 cells acquired additional oncogenic pathway that facilitated their expansion not only in the GI tract but also in the lungs of pfp/rag2 mice. Finally, we cannot exclude that some of the HMC-1.2 cells injected into the peritoneal cavities were able to rapidly migrate or transit via a vascular route into lungs of our pfp/rag2 mice.

CD2, also known as LFA-2, binds to its counter-receptor CD58 (LFA-3) with substantial affinity, resulting in aggregate- and cluster formation of neoplastic MC.[36] In addition, lack or loss of CD2 has been implicated with progression of SM in individual case reports.[38] Recently, this hypothesis was confirmed in a larger series of patients.[21] In the present study, we applied CD2 + and CD2 − HMC-1.2 cells in a scid mouse model and found that the injected CD2 + HMC-1.2 cells produce a more focal infiltration pattern in the GI tract and lungs, whereas CD2 − HMC-1.2 cells produced a more diffuse infiltration-pattern typically seen in advanced SM. The hypothesis that loss of CD2 is a critical event contributing to disease progression was supported by the fact that the survival of mice injected with CD2 + HMC-1.2 cells was significantly longer compared to mice injected with CD2 − HMC-1.2 cells regardless of the type of recipient mice.

To confirm that loss of CD2 plays a predominant role in disease progression, we asked whether additional aggregation receptors are differentially expressed in CD2 − and CD2 + HMC-1.2 cells. However, although CD2 + HMC-1.2 cells expressed higher levels of various adhesion molecules in our gene array studies and lower surface levels of CD54 (ICAM-1) compared to CD2 − HMC-1.2 cells, no additional major aggregation receptors (apart from CD2) that could explain loss of aggregation of MC, were found to be differentially expressed in CD2 − versus CD2 + HMC-1.2 cells. These data confirm the hypothesis

that CD2 is a major cell-cell adhesion receptor promoting MC aggregation in indolent SM, and that the loss of CD2 contributes to dis-aggregation and a more diffuse leukemic spread of MC.

The notion that absence of CD2 on MC is associated with a poor survival is in line with recently published results obtained in a larger series of SM patients collected in the ECNM registry.[21] In the present study, we confirmed the prognostic impact of absence of CD2 on MC in our patients with SM. In particular, we confirmed that lack of CD2 in MC correlates with advanced SM whereas in most patients with indolent SM, neoplastic MC displayed CD2. Moreover, we found that overall survival of our patients with CD2 − SM is shorter compared to patients with CD2 + SM. However, absence of CD2 in neoplastic MC is not always associated with a poor survival even if advanced SM is diagnosed. For example, in almost all patients with well differentiated SM (WDSM), including advanced forms, MC lack CD2.[56]

The prognostic impact of absence of CD2 on MC prompted us to ask whether CD2 + and CD2 − HMC-1.2 cells are characterized by specific molecular features or by a loss or gain of additional antigens relevant to homing or invasion of MC. However, CD2 + and CD2 − HMC-1.2 cells exhibited an almost identical pattern of classical adhesion antigens, including CD44 and CD58. Next, we performed gene array studies and found that CD2 + HMC-1.2 cells express a large number of adhesion-relevant and matrix-relevant antigens in excess over CD2 − HMC-1.2 cells, including galectin-3, alpha-1 tubulin, and cadherin 11. Cluster- and pathway analyses confirmed that CD2 + HMC-1.2 cells display multiple homing- and adhesion-receptors. This observation suggest that CD2-positivity may be a strong intracellular signal for adhesion and homing, preparing these cells not only for aggregation but also for interacting with the surrounding microenvironment (niche) and matrix-structures. Indeed, MC in the BM of patients with SM are often surrounding blood vessels, fat cells, or endosteal cells, and MC aggregates often contain other leukocytes, such as lymphocytes or eosinophils.[2,26–29,59–61]

The Hermes-receptor CD44 is a surface adhesion molecule that mediates homing of stem cells and more mature cells in various organs in physiologic states and various disease models, including myeloid neoplasms. In patients with SM, CD44 has been described to be expressed abundantly on MC and neoplastic stem cells.[37,57,58] In addition, MC express higher levels of CD44 compared to normal MC, and the highest levels of CD44 on MC were found in MCL.[37] Corresponding results were obtained in the current study. In particular, we found that CD44 is expressed abundantly in HMC-1.2 cells, and that this antigen is overexpressed especially at the cell-invasion front after injection into scid mice. These observations confirm the functional impact of CD44 as an invasion receptor and prognostic antigen in SM.[37]

Together, our data suggest that E- and P-selectins mediate MC-homing to various organs in scid mice, that CD44-expression is associated with HMC-1.2 invasion, and that loss of CD2 leads to a more diffuse, leukemic expansion of MC in scid mice. These observations may have clinical implications and suggest that increasing CD44 expression and loss of CD2 contribute to disease progression in SM.

Acknowledegments

We like to thank Gabriele Stefanzl, Nadine Witzeneder, Katharina Fröhlich, Alex Kilbertus, David Fuchs, and Christian Milosits for their skillful technical assistance.

Authors´ Contributions

Peter Valent, Udo Schumacher and Daniel Wicklein provided the scientific concept and the study design. Emir Hadzijusufovic and Gerit-Holger Schernthaner established and distributed CD2 + and CD2 – HMC-1.2 subclones. Daniela Berger and Isma Ishaq-Parveen performed immunocytochemical staining experiments and qPCR experiments on HMC-1.2 cells. Nina Nehmann, Hanna Maar and Daniel Wicklein performed xenotransplantation experiments with HMC-1.2 cells in pfp/rag2 mice and scid mice, including survival data and measurement of organ invasion. Daniel Wicklein and Hans-Peter Horny examined immunohistochemical staining experiments in mouse tissue sections. Irena Angelova-Fischer, Alex Stefan, Johannes Nikolaus Woltsche, Karoline V. Gleixner, and Wolfgang R. Sperr provided data from patients with mastocytosis. Wolfgang R. Sperr provided statistical analyses and calculated the probability of survival in patients with mastocytosis. Martin Bilban provided whole genome array profiling studies. All authors contributed substantially by writing and reviewing parts of the manuscript. All authors, except Udo Schumacher, who passed away before this article was finalized, approved the final version of the document.

Abbreviations

ASM Aggressive systemic mastocytosis

AHN Associated hematologic neoplasm

BM Bone marrow

BMM Bone marrow mastocytosis

CERCAM Cerebral endothelial cell adhesion molecule

FCS Fetal calf serum

GI tract Gastrointestinal tract

HUVEC Human cultured umbilical vein endothelial cells (HUVEC)

ICC Immunocytochemistry

ICAM-1 Intercellular adhesion molecules-1

i.p. Intraperitoneally

ISM Indolent SM

LFA-2 Lymphocyte function-associated antigen-2

LFA-3 Lymphocyte function-associated antigen-3

mAb Monoclonal antibody

MC Mast cell(s)

MCL Mast cell leukemia

NRCAM Neuronal cell adhesion molecule

OS Overall survival

pfp/rag2 Pore forming gene/recombinase activating gene 2

PFS Progression-free survival

SCF Stem cell factor

scid Severe combined immunodeficient

SM Systemic mastocytosis

SM-AHN Systemic mastocytosis with concomitant AHN

VEGF Vascular endothelial growth factor

WHO World Health Organization

Declarations

Acknowledegments

We like to thank Gabriele Stefanzl, Nadine Witzeneder, Katharina Fröhlich, Alex Kilbertus, David Fuchs, and Christian Milosits for their skillful technical assistance.

Authors´ Contributions

Peter Valent, Udo Schumacher and Daniel Wicklein provided the scientific concept and the study design. Emir Hadzijusufovic and Gerit-Holger Schernthaner established and distributed CD2+ and CD2− HMC-1.2 subclones. Daniela Berger and Isma Ishaq-Parveen performed immunocytochemical staining experiments and qPCR experiments on HMC-1.2 cells. Nina Nehmann, Hanna Maar and Daniel Wicklein performed xenotransplantation experiments with HMC-1.2 cells in pfp/rag2 mice and scid mice, including survival data and measurement of organ invasion. Daniel Wicklein and Hans-Peter Horny examined immunohistochemical staining experiments in mouse tissue sections. Irena Angelova-Fischer,

Alex Stefan, Johannes Nikolaus Woltsche, Karoline V. Gleixner, and Wolfgang R. Sperr provided data from patients with mastocytosis. Wolfgang R. Sperr provided statistical analyses and calculated the probability of survival in patients with mastocytosis. Martin Bilban provided whole genome array profiling studies. All authors contributed substantially by writing and reviewing parts of the manuscript. All authors, except Udo Schumacher, who passed away before this article was finalized, approved the final version of the document.

Availability of data and materials

All source data and material will be made available upon reasonable request. The gene array data obtained in HMC-1.2 cells are available at Expression Omnibus under: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE33504.

For original data and relevant material, please contact:

1. Peter Valent (peter.valent@meduniwien.ac.at) and

2. Daniel Wicklein (daniel.wicklein@medicalschool-hamburg.de)

Competing interests – conflict of interest (COI) statement

The authors report no study-related COI.

The authors report the following study-unrelated COI:

Peter Valent: 1. Research grant: AOP Orphan, 2. Consultancy honoraria: Novartis, Blueprint Medicines, Cogent, Pfizer, AOP Orphan, Stemline, Abbvie, Delbert, Pentabase, Daiichi-Sankyo, Amgen;

Emir Hadzijusufovic: no COI;

Nina Nehmann: no COI;

Hanna Maar: no COI;

Gerit-Holger Schernthaner: no COI;

Daniela Berger: no COI;

Irena Angelova-Fischer: no COI;

Alex Stefan: no COI;

Isma Ishaq-Parveen: no COI;

Johannes Woltsche: no COI;

Karoline V. Gleixner: Honoraria: Jazz pharmaceuticals, AbbVie, Novartis, Servier, Blueprint Medicines, Incyte, Celgene Corporation, Astellas, Daiichi Sankyo.

Martin Bilban: no COI;

Wolfgang R. Sperr: Consultancy honoraria, scientific grants or travel support: AbbVie, Blueprint-Medicines, BMS-Celgene, Incyte, Jazz, Novartis, Otsuka, Pfizer, Servier, StemLine, Thermo Fisher;

Hans-Peter Horny: Consultancy honoraria: Novartis, Blueprint;

Udo Schumacher: -*;

Daniel Wicklein: no COI.

The authors declare that they have no other conflicts of interest to disclose.

Funding Statement

The authors declare that they did not get grant support for this project.

Ethics Approvals and Consent to Participate

The study was performed in accordance with the declaration of Helsinki and approved by the local ethics committee (EC) of the Medical University of Vienna. All patients provided written informed consent to participate. All experiments in mice were approved by the local licensing authority: Behörde für Soziales, Gesundheit, Familie, Verbraucherschutz; Amt für Gesundheit und Verbraucherschutz, Hamburg, Germany.

List of E-Mails of the Co-Authors*:

Peter Valent peter.valent@meduniwien.ac.at

Emir Hadzijusufovic emir.hadzijusufovic@meduniwien.ac.at

Nina Nehmann ninanehmann@web.de

Hanna Maar hanna.maar@uni-luebeck.de

Gerit-Holger Schernthaner gerit.schernthaner@meduniwien.ac.at

Daniela Berger daniela.berger@meduniwien.ac.at

Irena Angelova-Fischer Irena.Angelova-Fischer@kepleruniklinikum.at

Alex Stefan alex.stefan@kepleruniklinikum.at

Isma Ishaq-Parveen isma.ishaqparveen@meduniwien.ac.at

Johannes Nikolaus Woltsche johannes.woltsche@medunigraz.at

Karoline V. Gleixner karoline.gleixner@meduniwien.ac.at

Martin Bilban martin.bilban@meduniwien.ac.at

Wolfgang R. Sperr wolfgang.r.sperr@meduniwien.ac.at

Hans-Peter Horny Hans-Peter.Horny@med.uni-muenchen.de

Udo Schumacher -*

Daniel Wicklein daniel.wicklein@medicalschool-hamburg.de

*We dedicate this publication to our good friend, Udo Schumacher, who contributed essentially in this project for many years but unfortunately passed away before we finalized the article.

References

1. Akin C, Metcalfe DD. Systemic mastocytosis. Annu Rev Med. 2004;55:419–32.
2. Horny HP, Sotlar K, Valent P. Mastocytosis: state of the art. Pathobiology. 2007;74:121–32.
3. Theoharides TC, Valent P, Akin C. Mast cells, mastocytosis and related disorders. N Engl J Med. 2015;373:163–72.
4. Valent P, Akin C, Sperr WR, Horny HP, Arock M, Metcalfe DD, et al. New insights into the pathogenesis of mastocytosis: emerging concepts in diagnosis and therapy. Annu Rev Pathol. 2023;18:361–86.
5. Hartmann K, Escribano L, Grattan C, Brockow K, Carter MC, Alvarez-Twose I, et al. Cutaneous manifestations in patients with mastocytosis: Consensus report of the European Competence Network on Mastocytosis; the American Academy of Allergy, Asthma & Immunology; and the European Academy of Allergology and Clinical Immunology. J Allergy Clin Immunol. 2016;137:35–45.
6. Valent P, Akin C, Sperr WR, Escribano L, Arock M, Horny HP, et al. Aggressive systemic mastocytosis and related mast cell disorders: current treatment options and proposed response criteria. Leuk Res. 2003;27:635–41.
7. Reiter A, George TI, Gotlib J. New developments in diagnosis, prognostication, and treatment of advanced systemic mastocytosis. Blood. 2020;135:1365–76.
8. Akin C, Arock M, Carter MC, George TI, Valent P: Mastocytosis. Nat Rev Dis Primers. 2025;11:30.
9. Nagata H, Worobec AS, Oh CK, Chowdhury BA, Tannenbaum S, Suzuki Y, et al. Identification of a point mutation in the catalytic domain of the protooncogene c-kit in peripheral blood mononuclear cells of patients who have mastocytosis with an associated hematologic disorder. Proc Natl Acad Sci (USA). 1995;92:10560–64.

10. Longley BJ, Tyrrell L, Lu SZ, Ma YS, Langley K, Ding TG, et al. Somatic c-kit activating mutation in urticaria pigmentosa and aggressive mastocytosis: establishment of clonality in a human mast cell neoplasm. Nat Genet. 1996;12:312–14.
11. Hoermann G, Gleixner KV, Dinu GE, Kundi M, Greiner G, Wimazal F, et al. The KIT D816V allele burden predicts survival in patients with mastocytosis and correlates with the WHO type of the disease. Allergy. 2014;69:810–13.
12. Arock M, Sotlar K, Akin C, Broesby-Olsen S, Hoermann G, Escribano L, et al. KIT mutation analysis in mast cell neoplasms: recommendations of the European Competence Network on Mastocytosis. Leukemia. 2015;29:1223–32.
13. Muñoz-González JI, Álvarez-Twose I, Jara-Acevedo M, Henriques A, Viñas E, Prieto C, et al. Frequency and prognostic impact of KIT and other genetic variants in indolent systemic mastocytosis. Blood. 2019;134:456–68.
14. Kristensen T, Vestergaard H, Bindslev-Jensen C, Møller MB, Broesby-Olsen S. Clinical validation of a new commercial highly sensitive KIT D816V mutation analysis in mastocytosis. Allergy. 2020;75:1489–91.
15. Navarro-Navarro P, Álvarez-Twose I, Pérez-Pons A, Henriques A, Mayado A, García-Montero AC, et al. KITD816V mutation in blood for the diagnostic screening of systemic mastocytosis and mast cell activation syndromes. Allergy. 2023;78:1347–59.
16. Damaj G, Joris M, Chandesris O, Hanssens K, Soucie E, Canioni D, et al. ASXL1 but not TET2 mutations adversely impact overall survival of patients suffering systemic mastocytosis with associated clonal hematologic non-mast-cell diseases. PLoS One. 2014;9:e85362.
17. Jawhar M, Schwaab J, Schnittger S, Meggendorfer M, Pfirrmann M, Sotlar K, et al. Additional mutations in SRSF2, ASXL1 and/or RUNX1 identify a high-risk group of patients with KIT D816V(+) advanced systemic mastocytosis. Leukemia. 2016;30:136–43.
18. Jawhar M, Schwaab J, Hausmann D, Clemens J, Naumann N, Henzler T, et al. Splenomegaly, elevated alkaline phosphatase and mutations in the SRSF2/ASXL1/RUNX1 gene panel are strong adverse prognostic markers in patients with systemic mastocytosis. Leukemia. 2016;30:2342–50.
19. Kluin-Nelemans HC, Jawhar M, Reiter A, van Anrooij B, Gotlib J, Hartmann K, et al. Cytogenetic and molecular aberrations and worse outcome for male patients in systemic mastocytosis. Theranostics. 2021;11:292–303.
20. Lübke J, Schmid A, Christen D, Oude Elberink HNG, Span LFR, Niedoszytko M, et al. Serum chemistry profiling and prognostication in systemic mastocytosis: a registry-based study of the ECNM and GREM. Blood Adv. 2024;8:2890–2900.
21. Rüfer A, Nilius H, Hermine O, Niedoszytko M, Oude Elberink JNG, Bonadonna P, et al. Prognostic impact of expression of CD2, CD25, and/or CD30 in/on mast cells in systemic mastocytosis: a registry study of the European Competence Network on Mastocytosis. Leukemia. 2025;39:675–83.
22. Valent P, Horny HP, Escribano L, Longley BJ, Li CY, Schwartz LB, et al. Diagnostic criteria and classification of mastocytosis: a consensus proposal. Leuk Res. 2001;25:603–25.

23. Valent P, Akin C, Metcalfe DD. Mastocytosis. 2016 updated WHO classification and novel emerging treatment concepts. Blood. 2017;129:1420–27.
24. Valent P, Akin C, Hartmann K, Alvarez-Twose I, Brockow K, Hermine O, et al. Updated diagnostic criteria and classification of mast cell disorders: a consensus proposal. Hemasphere. 2021;5:e646.
25. Valent P, Sotlar K, Horny HP, Arock M, Akin C. World Health Organization classification and diagnosis of mastocytosis: update 2023 and future perspectives. Immunol Allergy Clin North Am. 2023;43:627–49.
26. Horny HP, Valent P. Diagnosis of mastocytosis: general histopathological aspects, morphological criteria, and immunohistochemical findings. Leuk Res. 2001;25:543–51.
27. Horny HP, Sotlar K, Sperr WR, Valent P. Systemic mastocytosis with associated clonal haematological non-mast cell lineage diseases: a histopathological challenge. J Clin Pathol. 2004;57:604–608.
28. Horny HP, Sotlar K, Stellmacher F, Krokowski M, Agis H, Schwartz LB, et al. The tryptase positive compact round cell infiltrate of the bone marrow (TROCI-BM): a novel histopathological finding requiring the application of lineage specific markers. J Clin Pathol. 2006;59:298–302.
29. Travis WD, Li CY, Hoagland HC, Travis LB, Banks PM. Mast cell leukemia: report of a case and review of literature. Mayo Clin Proc. 1986;61:957 – 66.
30. Georgin-Lavialle S, Lhermitte L, Dubreuil P, Chandesris MO, Hermine O, Damaj G. Mast cell leukemia. Blood. 2013;121:1285–95.
31. Valent P, Sotlar K, Sperr WR, Escribano L, Yavuz S, Reiter A, et al. Refined diagnostic criteria and classification of mast cell leukemia (MCL) and myelomastocytic leukemia (MML): a consensus proposal. Ann Oncol. 2014;25:1691–1700.
32. Valent P, Bevec D, Maurer D, Besemer J, Di Padova F, Butterfield JH, et al. Interleukin 4 promotes expression of mast cell ICAM-1 antigen. Proc Natl Acad Sci (USA). 1991;88:3339–42.
33. Valent P, Bettelheim P. Cell surface structures on human basophils and mast cells: biochemical and functional characterization. Adv Immunol. 1992;52:333–423.
34. Sperr WR, Agis H, Czerwenka K, Klepetko W, Kubista E, Boltz-Nitulescu G, et al. Differential expression of cell surface integrins on human mast cells and human basophils. Ann Hematol. 1992;65:10–16.
35. Escribano L, Orfao A, Díaz-Agustin B, Villarrubia J, Cerveró C, López A, et al. Indolent systemic mast cell disease in adults: immunophenotypic characterization of bone marrow mast cells and its diagnostic implications. Blood. 1998;91:2731–36.
36. Schernthaner GH, Jordan JH, Ghannadan M, Agis H, Bevec D, Nuñez R, et al. Expression, epitope analysis, and functional role of the LFA-2 antigen detectable on neoplastic mast cells. Blood. 2001;98:3784–92.
37. Mueller N, Wicklein D, Eisenwort G, Jawhar M, Berger D, Stefanzl G, et al. CD44 is a RAS/STAT5-regulated invasion receptor that triggers disease expansion in advanced mastocytosis. Blood. 2018;132:1936–50.

38. Escribano L, Orfao A, Villarrubia J, Martín F, Madruga JI, Cuevas M, et al. Sequential immunophenotypic analysis of mast cells in a case of systemic mast cell disease evolving to a mast cell leukemia. Cytometry. 1997;30:98–102.
39. Escribano L, Díaz-Agustín B, Bellas C, Navalón R, Nuñez R, Sperr WR, Schernthaner GH, et al. Utility of flow cytometric analysis of mast cells in the diagnosis and classification of adult mastocytosis. Leuk Res. 2001;25:563–70.
40. Teodosio C, García-Montero AC, Jara-Acevedo M, Sánchez-Muñoz L, Alvarez-Twose I, Núñez R, et al. Mast cells from different molecular and prognostic subtypes of systemic mastocytosis display distinct immunophenotypes. J Allergy Clin Immunol. 2010;125:719–26.
41. Butterfield JH, Weiler D, Dewald G, Gleich GJ. Establishment of an immature mast cell line from a patient with mast cell leukemia. Leuk Res. 1988;12:345–55.
42. Bilban M, Haslinger P, Prast J, Klinglmüller F, Woelfel T, Haider S, et al. Identification of novel trophoblast invasion-related genes: heme oxygenase-1 controls motility via peroxisome proliferator-activated receptor gamma. Endocrinology. 2009;150:1000–1013.
43. Richter U, Schröder C, Wicklein D, Lange T, Geleff S, Dippel V, et al. Adhesion of small cell lung cancer cells to E- and P-Selectin under physiological flow conditions: implications for metastasis formation. Histochem Cell Biol. 2011;135:499–512.
44. Köhler S, Ullrich S, Richter U, Schumacher U. E-/P-selectins and colon carcinoma metastasis: first in vivo evidence for their crucial role in a clinically relevant model of spontaneous metastasis formation in the lung. Br J Cancer. 2010;102:602–9.
45. Sodeur S, Ullrich S, Gustke H, Zangemeister-Wittke U, Schumacher U. Increased numbers of spontaneous SCLC metastasis in absence of NK cells after subcutaneous inoculation of different SCLC cell lines into pfp/rag2 double knock out mice. Cancer Lett. 2009;282:146–51.
46. Jojovic M, Schumacher U. Quantitative assessment of spontaneous lung metastases of human HT29 colon cancer cells transplanted into SCID mice. Cancer Lett. 2000;152:151–56.
47. Everest-Dass A, Nersisyan S, Maar H, Novosad V, Schröder-Schwarz J, Freytag V, et al. Spontaneous metastasis xenograft models link CD44 isoform 4 to angiogenesis, hypoxia, EMT and mitochondria-related pathways in colorectal cancer. Mol Oncol. 2024;18:62–90.
48. Sperr WR, Escribano L, Jordan JH, Schernthaner GH, Kundi M, Horny HP, Valent P. Morphologic properties of neoplastic mast cells: delineation of stages of maturation and implication for cytological grading of mastocytosis. Leuk Res. 2001;25:529–36.
49. Sotlar K, George TI, Kluin P, Reiter A, Schwaab J, Panse J, et al. Standards of Pathology in the Diagnosis of Systemic Mastocytosis: Recommendations of the EU-US Cooperative Group. J Allergy Clin Immunol Pract. 2022;10:1986-98.e2.
50. Valent P, Sadovnik I, Eisenwort G, Herrmann H, Bauer K, Mueller N, et al. Redistribution, homing and organ-invasion of neoplastic stem cells in myeloid neoplasms. Semin Cancer Biol. 2020;60:191–201.

51. Wicklein D, Schmidt A, Labitzky V, Ullrich S, Valent P, Schumacher U. E- and p-selectins are essential for repopulation of chronic myelogenous and chronic eosinophilic leukemias in a scid mouse xenograft model. PLoS One. 2013;8:e70139.
52. Wimazal F, Ghannadan M, Müller MR, End A, Willheim M, Meidlinger P, et al. Expression of homing receptors and related molecules on human mast cells and basophils: a comparative analysis using multi-color flow cytometry and toluidine blue / immuno-fluorescence staining technique. Tissue Antigens. 1999;54:499–507.
53. Dudeck A, Leist M, Rubant S, Zimmermann A, Dudeck J, Boehncke WH, Maurer M. Immature mast cells exhibit rolling and adhesion to endothelial cells and subsequent diapedesis triggered by E- and P-selectin, VCAM-1 and PECAM-1. Exp Dermatol. 2010;19:424–34.
54. Hermans MA, Broijl A, van Daele PL. A unique presentation of pulmonary disease in advanced systemic mastocytosis, proven by the presence of mast cells in bronchoalveolar lavage: a case report. J Med Case Rep. 2016;10:283.
55. Zaremba-Pataj E, Patkowska E, Krzywdzińska A, Szumera-Ciećkiewicz A, Chlebowska-Tuz J. et al. Acute mast cell leukemia without KIT D816V mutation and lack of CD2 and CD25-a case report of rare entity. J Hematop. 2023;16:39–47.
56. Alvarez-Twose I, Jara-Acevedo M, Morgado JM, et al. Clinical, immunophenotypic, and molecular characteristics of well-differentiated systemic mastocytosis. J Allergy Clin Immunol. 2016;137:168–178.e1.
57. Horny HP, Menke DM, Kaiserling E. Neoplastic human tissue mast cells express the adhesion molecule CD44/HCAM. Virchows Arch. 1996;429:91–94.
58. Eisenwort G, Sadovnik I, Schwaab J, Jawhar M, Keller A, Stefanzl G, et al. Identification of a leukemia-initiating stem cell in human mast cell leukemia. Leukemia. 2019;33:2673–84.
59. Horny HP, Kaiserling E. Lymphoid cells and tissue mast cells of bone marrow lesions in systemic mastocytosis: a histological and immunohistological study. Br J Haematol. 1988;69:449–55.
60. Horny HP, Lange K, Sotlar K, Valent P. Increase of bone marrow lymphocytes in systemic mastocytosis: reactive lymphocytosis or malignant lymphoma? Immunohistochemical and molecular findings on routinely processed bone marrow biopsy specimens. J Clin Pathol. 2003;56:575–78.
61. Horny HP, Sotlar K, Valent P. Differential diagnoses of systemic mastocytosis in routinely processed bone marrow biopsy specimens: a review. Pathobiology. 2010;77:169–80.

Tables

Tables 1 to 4 are available in the Supplementary Files section.

Figures

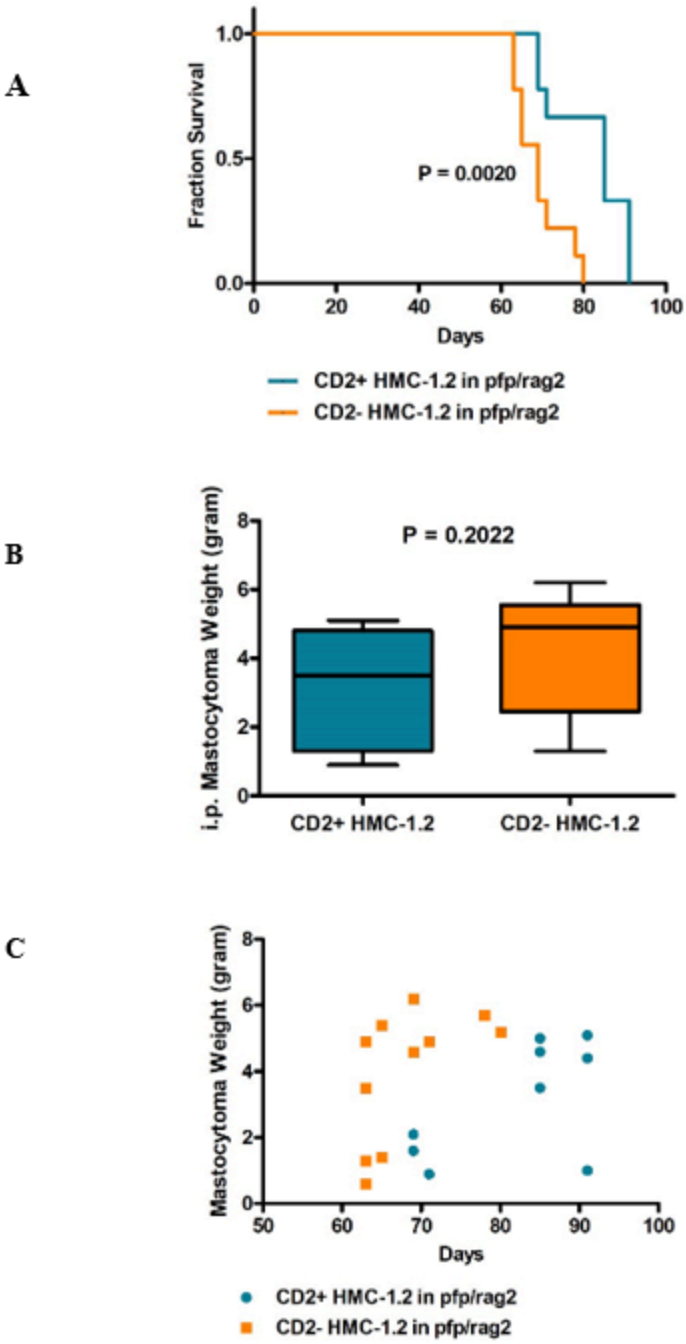
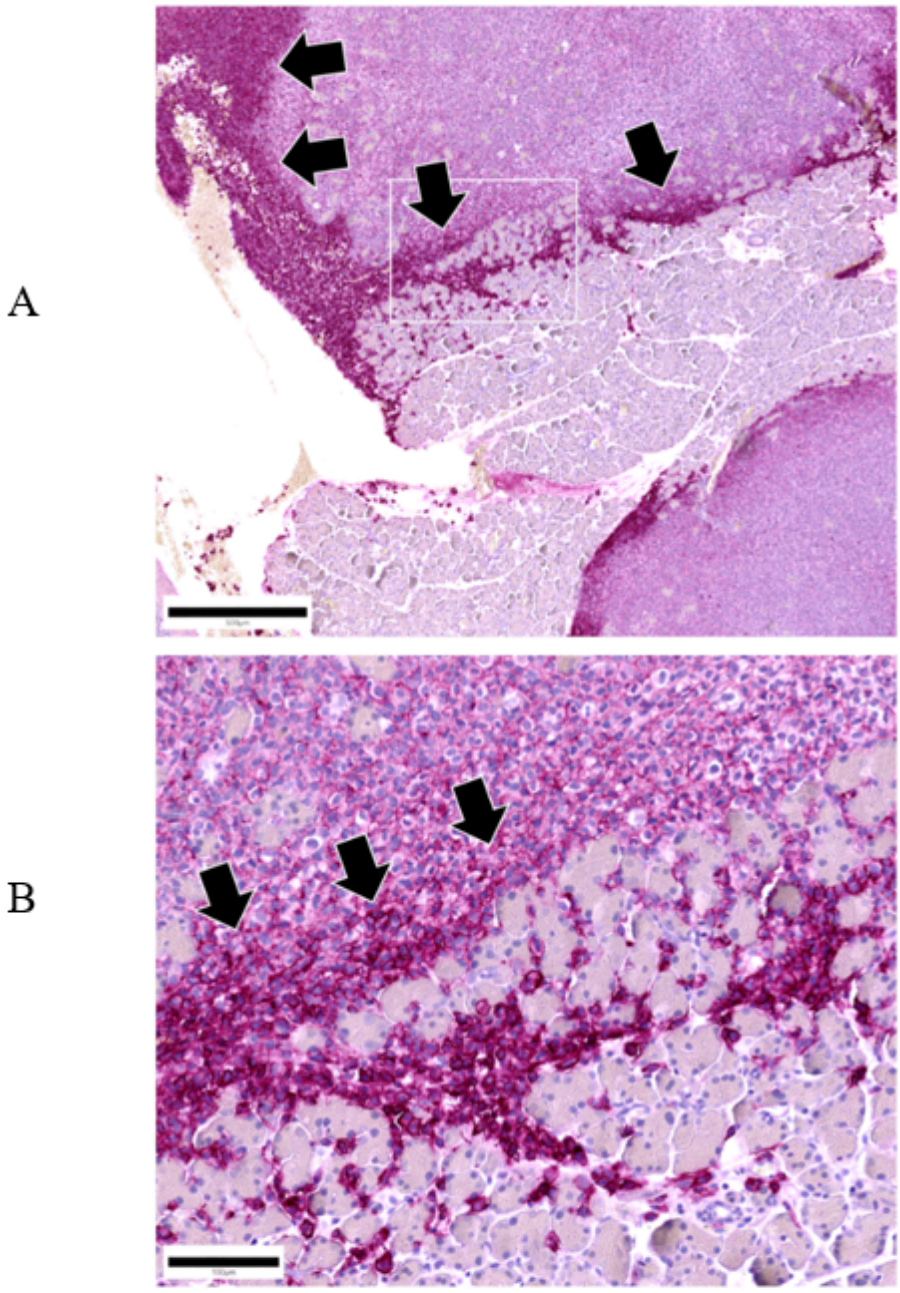


Figure 1

Impact of CD2 expression on HMC-1.2 cells transplanted into pfp/rag2 mice on survival and mast cell expansion (tumor weight)

Before injection, CD2+ HMC-1.2 cells or CD2– HMC-1.2 cells were suspended in 1 ml serum-free medium. A total volume of 200 µl containing 1 x 10^6 HMC-1.2 cells (per mouse) were injected intraperitoneally (i.p.) into pfp/rag2 mice (10 mice per group). After injection, mice were inspected daily.

Animals were sacrificed when tumors started to ulcerate or when the tumor weight exceeded 20% of their initial body weight. Survival was defined as time between injection and scarification or unexpected death. A: pfp/rag2 mice injected i.p. with CD2+ HMC-1 cells (n=9, blue graph) showed a significantly longer survival compared with mice injected with CD2− HMC-1.2 cells (n=9, yellow graph) (p=0.002 by log rank test). B: Box plot analysis of i.p. mastocytoma weight in pfp/rag2 mice at the time of death (CD2+ HMC-1.2 cells, blue box plot: CD2− HMC-1.2 cells, yellow box plot). The total mass of i.p. mastocytomas did not significantly differ between the 2 groups (p=0.2022, by unpaired t test). C: Correlation between i.p. mastocytoma weight (in g) and survival (d, day) in each mouse (dot) in the two cohorts: CD2+ HMC-1.2 cells (blue dots) and CD2− HMC-1.2 cells (yellow dots). Apparently, mastocytomas in mice injected with CD2− HMC-1.2 cells reached masses critical for the animals' overall condition (survival) earlier than CD2+ HMC-1.2 mastocytomas.



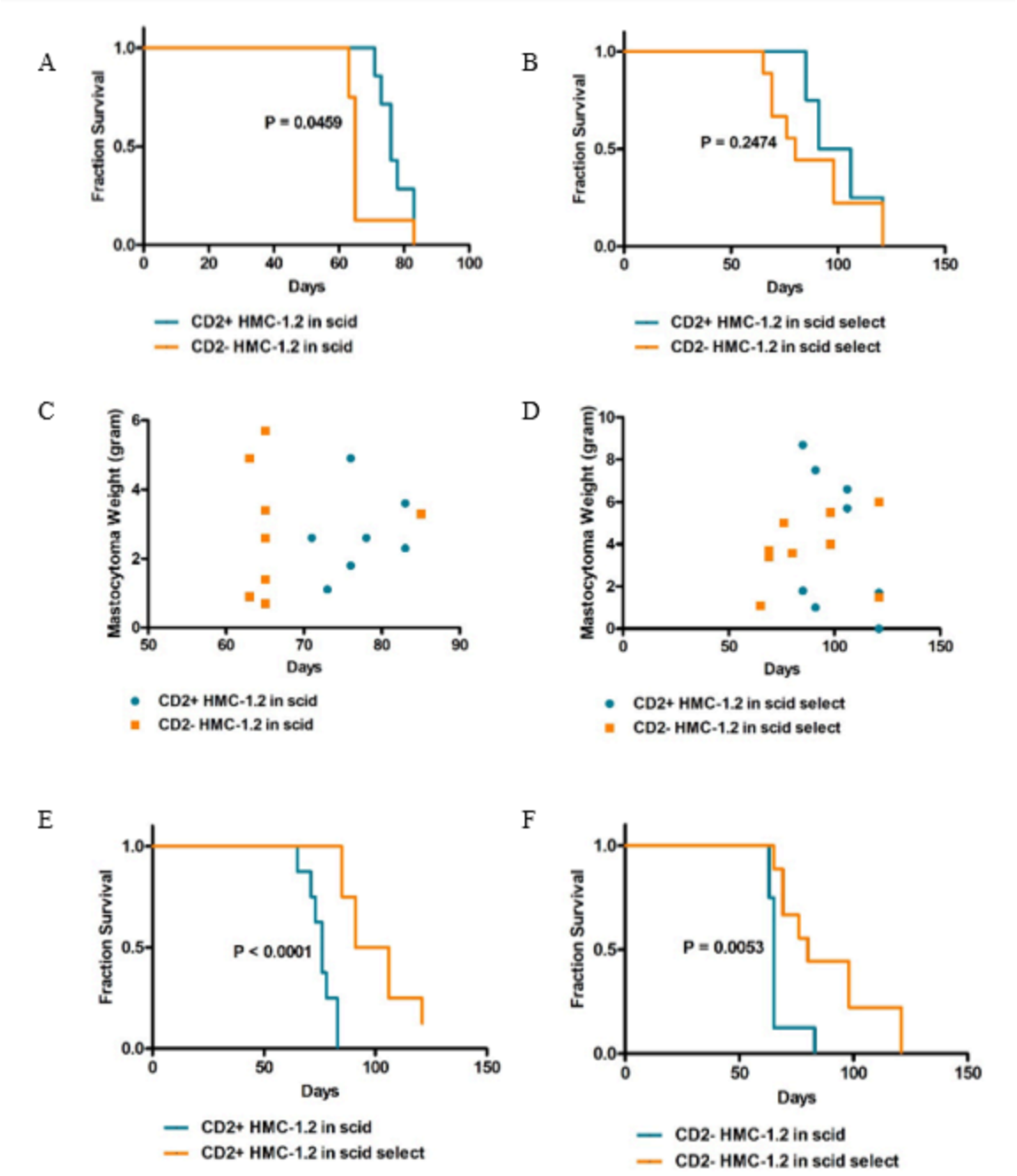
Valent et al, Figure 2

Figure 2

Detection of CD44 at the invasion front of infiltrating HMC-1.2 cells

After intraperitoneal injection, CD2+ and CD2− HMC-1.2 cells formed compact infiltrates and diffuse invasive infiltration in various gastrointestinal organs. Figure 2 shows the pattern of infiltration of CD2− HMC-1.2 cells in the pancreas of a pfp/rag2 mouse. Paraffin-embedded tissue sections were stained

with anti-CD44 antibody as described in the supplement. Murine cells were visualized by eosin-hematoxylin staining. A: CD44+ HMC-1.2 cells formed compact nodules in pancreatic tissue. In addition, a diffuse infiltration of HMC-1.2 cells was seen. Bar: 500 µm. B: Higher magnification of a region in ´A´ (indicated as insert in ´A´). Note that the HMC-1.2 cells in the center of the nodule are not stained intensively by the CD44 antibody while HMC-1.2 cells in the invasion front (black arrows) and isolated HMC-1.2 cells in the diffuse infiltrate portion express substantial amounts of CD44. Bar: 100 µm.

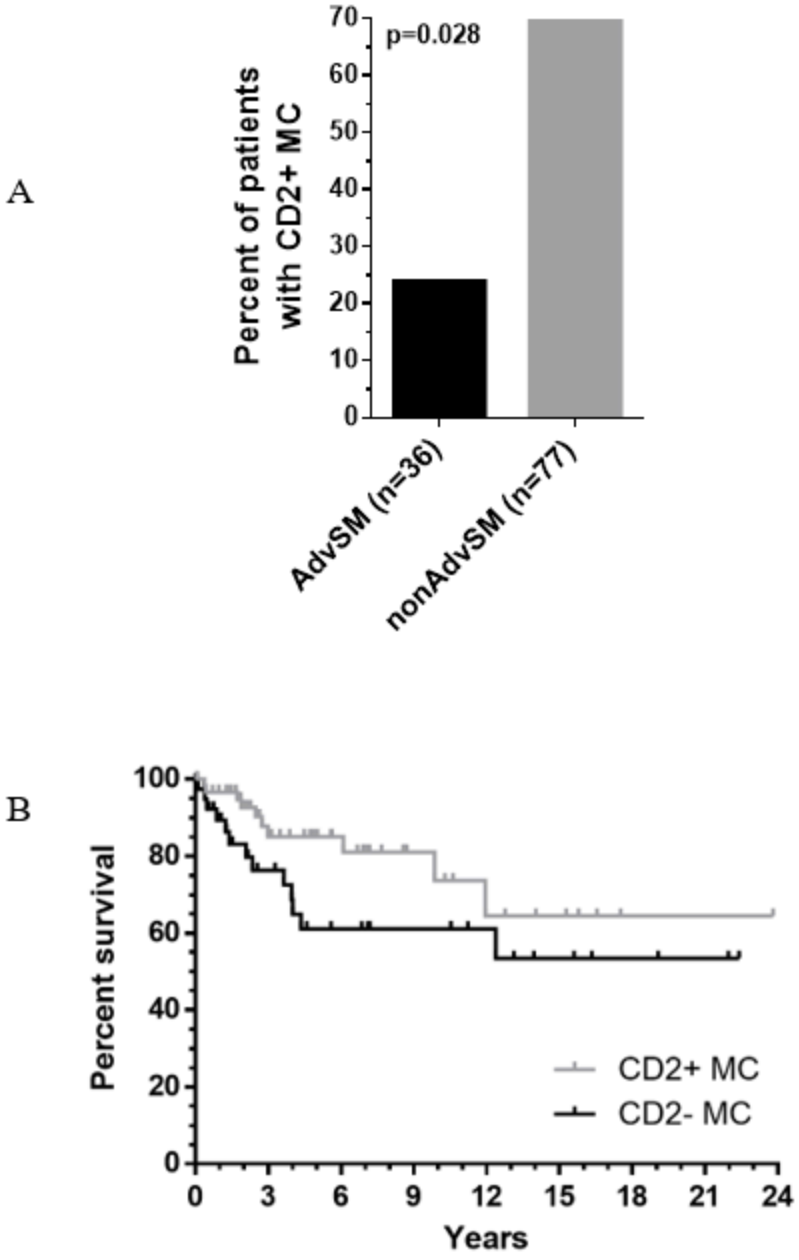


Valent et al, Figure 3

Figure 3

Survival scid and scid-select mice injected with CD2+ and CD2– HMC-1.2 cells and correlation with the infiltration pattern

Scid and scid select mice were injected with HMC-1.2 cell subsets and sacrificed according to termination criteria as described in the text. A: scid mice injected with CD2+ HMC-1.2 cells (n=7, blue graph) showed a significantly longer survival compared to mice injected with CD2– cells (n=8, yellow graph; p=0.0459; log rank test). B: scid select mice injected with CD2+ HMC-1 cells (n=8, blue graph) did not display a significantly longer survival compared to scid select mice injected with CD2– cells (n=9, yellow graph; p=0.2674, log rank test). C: Total intraperitoneal mastocytoma weight (in gram, g) versus survival time (in days, d) for each scid select mouse (E- and P-selectin-deficient) of the group injected with CD2+ HMC-1.2 cells (teal symbols) and CD2– HMC-1.2 cells (orange symbols). Apparently, tumors produced by CD2– HMC-1.2 cells did not reach masses critical for the animals' condition at an earlier time point compared to mice injected with CD2+ HMC-1.2 cells. D: By contrast, in scid mice, mastocytomas produced by CD2– HMC-1 cells reached masses critical for the animals' overall condition earlier than scid mice injected with CD2+ HMC-1.2 cells, which is in line with results obtained in pfp/rag2 mice exhibiting normal selectin expression. E: Comparison of survival of scid mice (blue graph, n=7) and scid select mice (yellow graph, n=8) injected with CD2+ HMC-1.2 cells. Scid select mice survived longer compared to scid mice ($p<0.0001$ by log rank test). F: Similarly, scid select mice (yellow graph; n=9) injected with CD2– HMC-1.2 cells had a longer survival compared to scid mice injected with CD2– HMC-1.2 cells (blue graph, n=8) ($p<0.01$ by log rank test).



Valent et al, Figure 4

Figure 4

Survival of patients with SM: comparison between CD2+ SM and CD2− SM

A total of 113 patients with SM were diagnosed and followed in regular routine visits between 2015 and 2024. Median age was 52 years (range: 20-90 years). A: Patients with non-advanced SM and advanced SM were analyzed for expression of CD2 in mast cells (MC). As visible, the prevalence of cases with CD2+ MC was higher in the group with non-advanced SM (nonAdvSM, n=77) compared to those with

advanced SM (AdvSM, n=36) ($p<0.05$, Chi-Square test). B: Overall survival (OS) of patients with CD2+ and CD2− SM. The probability of OS (Kaplan & Meier) was higher in patients with CD2+ SM compared to those with CD2− SM ($p<0.05$, by Gehan-Breslow-Wilcoxon test).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ValentetalSupplement.docx
- Tables.docx