haematologica
Journal of the Ferrata Storti Foundation

Germline *EZH2* mutation cooperates with somatic *KIT* D816V to drive early pediatric advanced systemic mastocytosis

by Clotilde Devin, Lucas Khellaf, Julien Rossignol, Nathalie Droin, Philippe Drabent, Sylvie Fraitag, Marine Madrange, Christine Leaute-Labreze, Andreas E. Kulozik, Stephane Ducassou, Eric Solary, Olivier Hermine, Christine Bodemer and Laura Polivka

Received: October 15, 2025.
Accepted: July 23, 2026.

Citation: Clotilde Devin, Lucas Khellaf, Julien Rossignol, Nathalie Droin, Philippe Drabent, Sylvie Fraitag, Marine Madrange, Christine Leaute-Labreze, Andreas E. Kulozik, Stephane Ducassou, Eric Solary, Olivier Hermine, Christine Bodemer and Laura Polivka. Germline EZH2 mutation cooperates with somatic KIT D816V to drive early pediatric advanced systemic mastocytosis. Haematologica. 2026 July 30. doi: 10.3324/haematol.2025.300049 [Epub ahead of print]

Germline *EZH2* mutation cooperates with somatic *KIT* D816V to drive early pediatric advanced systemic mastocytosis

Clotilde Devin[1], Lucas Khellaf[1], Julien Rossignol[1,2], Nathalie Droin[3], Philippe Drabent[4], Sylvie Fraitag[4], Marine Madrange[1], Christine Leaute-Labreze[5], Andreas E Kulozik[6], Stephane Ducassou[7], Eric Solary[3], Olivier Hermine[1,2]*, Christine Bodemer[1,8]*, Laura Polivka[1,8]

*Equal contribution

1. French reference center on mastocytosis and mast related disorders (CEREMAST), Imagine Institute, INSERM U1163, AP-HP, Necker Children's Hospital, Paris Centre University, Paris, France
2. Department of Hematology, Necker-Cochin Children's Hospital, AP-HP, Paris Centre University, Paris, France
3. INSERM UMR 1287, Gustave Roussy Cancer Center, Paris-Saclay University, Villejuif, France.
4. Department of Pathology, Necker Children's Hospital, AP-HP, Paris Centre University, Paris, France
5. Department of Pediatric Dermatology, Pellegrin Children's Hospital Group, Bordeaux University Hospital (CHU de Bordeaux), Bordeaux, France
6. Department of Pediatric Oncology, Hematology, and Immunology, Heidelberg University Hospital, Heidelberg, Germany
7. Department of Pediatric Hematology–Oncology, Pellegrin Children's Hospital Group, Bordeaux University Hospital (CHU de Bordeaux), Bordeaux, France
8. Department of Dermatology, Reference Center for Rare Skin Disorders in Children, AP-HP, Necker Children's Hospital, Paris Centre University, Paris, France

Corresponding author
Laura Polivka, MD, PhD
149 rue de Sèvres, F-75015 Paris, France
Phone: +33-144-494-627
Fax: +33-1-71-39-65-27
laura.polivka@institutimagine.org

Conflict of interest
No conflicts of interest to disclose.

Contributions
CD, LK, JR, MM, OH, CB and LP, contributed to the concept and design of this study; PD and SF performed histological analyses of bone marrow and skin biopsies; ND and ES performed transcriptomic analyses of the adult mastocytosis cohort on skin biopsies; CLL and SD provided clinical care for the patient during hospitalization at Bordeaux University Hospital; AEK was responsible for the initial clinical management at the Heidelberg University Hospital. All authors critically reviewed and approved the final version of the manuscript for publication.

Acknowledgments
The authors thank the patient and his family for their participation, and Pr. Yves Perel for providing clinical care.

Funding
This research did not receive any specific funding from agencies or organizations in the public, commercial, or not-for-profit sectors. LP was funded by the Bettencourt Schueller Foundation.

Data-sharing statement
The data that support the findings of this study are available from the corresponding author upon reasonable request

Key words: Pediatric mastocytosis, *KIT* mutation, aggressive systemic mastocytosis, diffuse cutaneous mastocytosis, midostaurin, EZH2.

Abbreviations

Adv-SM: advanced SM

ASM: aggressive systemic mastocytosis

CM: cutaneous mastocytosis

DCM: diffuse cutaneous mastocytosis

EP-Adv-SM: early pediatric advanced SM

ISM: indolent systemic mastocytosis

JMML: juvenile myelomonocytic leukemia

MC: mast cell

PT: prothrombin time

SM: systemic mastocytosis

SM-AHN: SM with associated hematologic neoplasm

TKI: tyrosine kinase inhibitor

VAF: variant allele frequency

WT: wild type

Mastocytosis encompasses heterogeneous disorders characterized by abnormal mast cell (MC) accumulation in various organs.(1) Cutaneous mastocytosis (CM) is limited to the skin, whereas systemic mastocytosis (SM) involves extra-cutaneous organs and is classified into non-advanced and advanced SM (Adv-SM). Adv-SM includes aggressive SM (ASM), defined by organ dysfunction (C-finding), MC leukemia, and SM with associated hematologic neoplasm (SM-AHN).(2) While non-Adv-SM is associated with a favorable prognosis, Adv-SM carries a poor prognosis, and its underlying pathophysiology remains incompletely understood. A *KIT* D816V mutation, causing constitutive KIT activation, is found in over 85% of adult cases.(1) In Adv-SM, additional somatic mutations are commonly observed, primarily in epigenetic and splicing regulators associated with clonal hematopoiesis and myeloid malignancies.(3) Early pediatric Adv-SM (EP-Adv-SM) is extremely rare, as children typically present with CM. Here, we report a case of EP-Adv-SM harboring both a somatic *KIT* D816V mutation and a novel germline *EZH2* G181R variant. Through functional studies in MC lines and transcriptomic analysis of adult patient samples, we demonstrate that EZH2 loss of function selectively cooperates with *KIT* D816V to confer a proliferative advantage, supporting a double-hit model of disease pathogenesis in EP-Adv-SM.

A full-term male infant presented at birth with generalized infiltrated erythematous skin and episodes of bullae formation (Figure 1). Skin biopsy revealed MC infiltration. By one month of age, he developed recurrent diarrhea, failure to thrive, and hepatosplenomegaly. Baseline serum tryptase was 193 ng/mL, with a normal *TPSAB1* genotype ($\alpha\beta/\beta\beta$). Bone marrow biopsy revealed 25% MC infiltration with aberrant CD25 and CD30 co-expression (Supplemental Figure 1). The *KIT* D816V mutation was detected with a variant allele frequency (VAF) of 40%, consistent with early embryonic acquisition. According to the WHO criteria, malabsorption with weight loss (manifesting as failure to thrive), constituted a C-finding, establishing the diagnosis of ASM. (4,5) Marked monocytosis (8×10^9/L), despite the absence of additional *JAK2*, *KRAS*, *NRAS*, *PTPN11* or *c-CBL* mutations and the lack of bone marrow dysplasia, prompted consideration of SM-AHN with a juvenile myelomonocytic leukemia (JMML)-like component. Whole-exome sequencing identified a heterozygous germline missense mutation in *EZH2* (c.541G>C, p.G181R), located in the SANT domain (histone-binding region) and inherited from his asymptomatic mother. No other pathogenic mutations were detected (Figure 2A). Initial treatment with three cycles of cytarabine and 6-mercaptopurine showed no significant response in either MC infiltration or monocytosis. Midostaurin (10 mg/kg) was initiated at 6 months, leading to a partial therapeutic response, with normalized growth, resolution of diarrheal episodes, normalization of monocyte count, and good tolerability. Specifically, the patient achieved incomplete remission per Valent criteria (2007), a major response per Mayo Clinic criteria

(2010), and clinical improvement per IWG-MRT-ECNM consensus criteria.(6) While still receiving midostaurin, the patient died at the age of 5 from an accidental cause unrelated to the disease. The case report protocol was approved by the local institutional review board (CPPRB, Groupe Hospitalier de Paris Necker, France; reference 03-01-05; approved 2003-07-07; amended 2021-02). Written informed consent was obtained from the patient's parents.

A systematic literature review identified 12 additional cases of EP-Adv-SM (Table 1 and supplemental Table 1). Overall, EP-Adv-SM showed a marked male predominance (sex ratio 3.33), a diffuse cutaneous presentation mimicking diffuse cutaneous mastocytosis (DCM) (n=12/13 patients), consistent hepatosplenomegaly, and elevated baseline serum tryptase (median (range): 190 ng/mL (64–306)). The most common C-findings were cytopenias (69%), hypersplenism (62%), and hepatomegaly with liver dysfunction (38%). A diagnosis of AHN was not considered, despite the presence of leukocytosis, often including neutrophilia, in 40% of patients. Six patients (46%) received tyrosine kinase inhibitors (TKI). Imatinib given to a *KIT* D816V–positive patient provoked disease flares and severe cutaneous toxicity, prompting a switch to dasatinib, which unexpectedly achieved partial disease control.(7) In contrast, another patient received dasatinib in combination with cladribine and vincristine without improvement, and died at 10 weeks due to the aggressiveness of MC infiltration and multiple infections.(8) Among three patients treated with midostaurin, two of them showed clinical improvement and tryptase reduction, though hepatosplenomegaly persisted.(9–11) In addition, one *KIT* D816V^{+} patient treated with sirolimus, resulting in clinical improvement.(12) Overall mortality rate was 62% (median (range) survival: 91 days (2-335)) but with targeted therapies it decreased to 29% (2/7). Among the patients who underwent *KIT* sequencing (n=9/13), all harbored either the D816V mutation or a functionally equivalent variant. Whole-exome sequencing was performed in three additional patients identified in the literature review. Germline mutations in two other tumor suppressor genes were identified: *GLI3* (a major repressor of Hedgehog signaling) and *SETD2* (involved in gene regulation and histone modification *via* H3K36 trimethylation, H3K36me3), each found in one patient.

To investigate whether these pathways are involved in adult ASM, we analyzed *EZH2* and *SETD2* mRNA expression, along with their downstream targets in lesional skin biopsies from ~~mastocytosis lesions of~~ adult ASM (n=3) and indolent SM (ISM, n=11) patients. *EZH2* expression was 33% lower in ASM than in ISM (p=.018), with six of its 27 target genes significantly upregulated in ASM (Figure 2B). *SETD2* expression was also lower in ASM, although not significantly, while *ERG* expression was significantly increased

(p=.0088). These findings indicate that EZH2 deficiency is not restricted to cases harboring *EZH2* mutations but extends more broadly across ASM. To directly assess the oncogenic potential of *EZH2* G181R and its interaction with *KIT* D816V, we performed lentiviral transduction of *EZH2* WT or *EZH2* G181R in ROSA MC lines expressing either *KIT* WT or *KIT* D816V.(13) In ROSA *KIT* D816V cells, *EZH2* G181R conferred a significant proliferative advantage compared with both the Mock and *EZH2* WT conditions (Figure 2C). To further evaluate the impact of EZH2 loss of function, we treated both cell lines with increasing concentrations of the EZH2 inhibitor tazemetostat (EPZ-6438). A dose-dependent increase in proliferation was observed exclusively in ROSA *KIT* D816V cells, with no significant effect in *KIT* WT cells (Figure 2D). Western blot analysis confirmed effective EZH2 inhibition, as evidenced by reduced H3K27me3 levels (Figure 2E).

EP-Adv-SM is a rare, severe SM variant that overlaps clinically with pediatric DCM and adult Adv-SM, presenting with diffuse cutaneous MC infiltration, hepatosplenomegaly, C-findings, and life-threatening symptoms. A key hallmark of EP-Adv-SM is its early onset, congenital in most cases, and the consistent presence of *KIT* D816V mutations, which are found in only ~40% of pediatric mastocytosis cases overall.(14) Any case of diffuse CM presentation associated with hepatosplenomegaly, C-findings (including failure to thrive in children), and a *KIT* D816V mutation should raise strong suspicion for EP-Adv-SM.(15) We propose two hypotheses to explain the pathogenesis of EP-Adv-SM (supplemental Figure 2). The first hypothesis suggests that the occurrence of *KIT* D816V mutations early during embryonic development, supported by reports of *in utero* disease onset in several cases and by the high VAF observed in our patient, may account for the aggressiveness of the disease. The second hypothesis posits a double-hit model: Adv-SM develops when a somatic *KIT* D816V mutation arises in the context of reduced EZH2 function or other cooperating abnormalities. This is supported by observations that, in adult patients, *KIT* D816V alone is insufficient to initiate disease. Only a subset of adult Adv-SM patients harbors somatic *EZH2* mutations (~5%), without clustering in canonical hotspot domains, in contrast to B-cell lymphomas.(16) In the present study, we extend these observations by showing that even in the absence of *EZH2* mutations, primary MCs from adult patients with aggressive disease exhibit reduced *EZH2* expression together with upregulation of EZH2 target genes, consistent with a loss-of-function phenotype. Similar epigenetic dysregulation has been reported for other chromatin modifiers such as SETD2, whose expression is reduced in approximately 50% of adult ASM. (17) We also previously reported Hedgehog pathway activation in primary MCs from adults with Adv-SM in the absence of *GLI3* mutations.(12) These observations are consistent with the double-hit model. A key mechanistic insight from our functional studies is the context dependency of EZH2 loss of function.

Specifically, oncogenic cooperation between *KIT* D816V and *EZH2* was demonstrated by the enhanced proliferation observed exclusively in *KIT* D816V cells expressing *EZH2* G181R, whereas *EZH2* overexpression or *EZH2* G181R expression was toxic in ROSA KIT WT cells. Furthermore, pharmacological inhibition of EZH2 with tazemetostat (EPZ-6438) induced a dose-dependent increase in proliferation exclusively in *KIT* D816V cells. Together, these findings support a model in which epigenetic deregulation, whether genetic or functional, synergizes with constitutive KIT activation to drive disease aggressiveness in Adv-SM.

The emergence of an AHN may depend on the underlying germline event and can phenocopy the somatic co-mutation patterns seen in adult Adv-SM. For example, monocytosis in adult SM often fulfills diagnostic criteria for chronic myelomonocytic leukemia. In our patient, a JMML component was considered despite the absence of classically associated mutations (*NRAS*, *KRAS*, *PTPN11* or *c-CBL*) due to marked monocytosis. Interestingly, although monocytes do not typically express KIT, their counts often decline with avapritinib, a selective KIT D816V inhibitor. A paracrine mechanism of monocyte proliferation, likely mediated by MC–derived cytokines such as TNF-α, has recently been suggested.(18) The germline *EZH2* mutations detected in the reported patient may have increased the monocytic lineage responsiveness to MC–derived cytokines, leading to marked monocytosis despite the absence of JMML-defining mutations. Unfortunately, plasma cytokine levels were not measured in this patient, which would have been of particular interest to better understand the mechanisms underlying the monocytosis.

Together, our findings support a model in which a germline mutation in a tumor suppressor and/or epigenetic regulator (e.g., GLI3, EZH2, SETD2) predisposes to EP-Adv-SM that emerges after acquisition of a somatic *KIT* D816V mutation in hematopoietic cells. Recognizing this pattern supports early blood testing for *KIT* D816V and targeted germline evaluation in infants with diffuse cutaneous disease and systemic involvement.

References

1. Valent P, Akin C, Sperr WR, et al. New Insights into the pathogenesis of mastocytosis: emerging concepts in diagnosis and therapy. Annu Rev Pathol. 2023;18(1):361-386.
2. Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization Classification of haematolymphoid tumours: myeloid and histiocytic/dendritic neoplasms. Leukemia. 2022;36(7):1703-1719.
3. Schwaab J, Schnittger S, Sotlar K, et al. Comprehensive mutational profiling in advanced systemic mastocytosis. Blood. 2013;122(14):2460-2466.
4. Valent P, Akin C, Hartmann K, et al. Updated diagnostic criteria and classification of mast cell disorders: a consensus proposal. Hemasphere. 2021;5(11):e646.
5. Valent P, Horny HP, Escribano L, et al. Diagnostic criteria and classification of mastocytosis: a consensus proposal. Leuk Res. 2001;25(7):603-625.
6. Shomali W, Gotlib J. Response criteria in advanced systemic mastocytosis: evolution in the era of KIT inhibitors. Int J Mol Sci. 2021;22(6):2983.
7. Larouche V, Paré MF, Grenier PO, et al. A review of the clinical features and management of systemic congenital mastocytosis through the presentation of an unusual prenatal-onset case. Curr Oncol. 2023;30(10):8992-9003.
8. Huang A, Fiadorchanka N, Brar K, Balderacchi JL, Glick SA. In utero presentation of aggressive systemic mastocytosis in a neonate. Br J Dermatol. 2017;177(5):1439-1441.
9. Krase I, Tiano R, Varghese M, et al. Infantile aggressive systemic mastocytosis treated with oral midostaurin. Ann Allergy Asthma Immunol. 2022;129:5(Supplement):S91.
10. Voelker D, Bednarski JJ, Nieman E, Carter MC, Polk B. Hematopoietic KIT D816Y mutation presenting as in utero aggressive systemic mastocytosis with response to midostaurin. J Allergy Clin Immunol Pract. 2023;11(4):1323-1325.e1.
11. Kiwit AT Julian; Blohm, Martin; Apostolidou, Sofia; Lehmberg, Kai; Singer, Dominique. Germline p.D816V ckit mutation in connatal systemic mastocytosis. Z Für Geburtshilfe Neonatol. 2023;227(03):e81.
12. Polivka L, Parietti V, Bruneau J, et al. The association of Greig syndrome and mastocytosis reveals the involvement of the hedgehog pathway in advanced mastocytosis. Blood. 2021;138(23):2396-2407.
13. Arock M, Wedeh G, Hoermann G, et al. Preclinical human models and emerging therapeutics for advanced systemic mastocytosis. Haematologica. 2018;103(11):1760-1771.
14. Bodemer C, Hermine O, Palmérini F, et al. Pediatric Mastocytosis Is a Clonal Disease Associated with D816V and Other Activating c-KIT Mutations. J Invest Dermatol. 2010;130(3):804-815.
15. Pernea P, Méni C, Rossignol J, et al. Characteristics and Therapeutic Strategies for Diffuse Cutaneous Mastocytosis. JAMA Dermatol. 2025;161(8):855-862.
16. González-López O, Muñoz-González JI, Orfao A, Álvarez-Twose I, García-Montero AC. Comprehensive analysis of acquired genetic variants and their prognostic impact in systemic mastocytosis. Cancers. 2022;14(10):2487.
17. Martinelli G, Mancini M, De Benedittis C, et al. SETD2 and histone H3 lysine 36 methylation deficiency in advanced systemic mastocytosis. Leukemia. 2018;32(1):139-148.
18. Greiner G, Witzeneder N, Klein K, et al. Tumor necrosis factor α promotes clonal dominance of KIT D816V+ cells in mastocytosis: role of survivin and impact on prognosis. Blood. 2024;143(11):1006-1017.

Table 1. Clinical and laboratory characteristics of patients from the literature review and from our case report.

	Global cohort n=13	Our case
Demographic characteristics		
Sex		
Female, n (%)	3 (23%)	
Male, n (%)	10 (77%)	Male
Sex ratio (M/F))	3,33	
Familial mastocytosis	n=0 (0%)	
Clinical characteristics		
Age at onset		
- Median age of diagnosis (range)	0 month [0-3]	At birth
- *In utero*	4 (31%)	
- At birth	7 (54%)	
- After 1month of age	2 (15%)	
Symptoms related to MC infiltration*, n (%)*	13 (100%)	
Cutaneous manifestations		
- Type of cutaneous presentation		
o Diffuse infiltrate	12 (92%)	Diffuse infiltrate
o Maculopapular lesions	1 (8%)	
Organomegaly		
- Hepatomegaly	13 (100%)	Hepatomegaly
- Splenomegaly	13 (100%)	Splenomegaly
- Lymphadenopathy	5 (38%)	
Gastrointestinal manifestations		
- Failure to thrive	4 (31%)	Failure to thrive
- Diarrhea with blood and mucus	1 (8%)	
Hepatic manifestations		
- Signs of portal hypertension	3 (23%)	
- Ascites	3 (23%)	
Pulmonary manifestations		
- Pulmonary hypertension	3 (23%)	
Cardiac manifestations		
- Cardiomegaly	1 (8%)	
Symptoms related to MC activation*, n (%)*	12 (92%)	
Cutaneous manifestations		
- Positive Darier's sign (reported)	4 (31%)	Positive Darier's sign
- Blistering lesions	11 (85%)	Blistering lesions
Gastrointestinal manifestations		
- Nausea/vomiting	3 (23%)	Nausea/vomiting
- Diarrhea w/o blood and mucus	6 (46%)	Diarrhea
Pulmonary manifestations		
- Dyspnea	4 (31%)	
- Bronchial Hyperreactivity	1 (8%)	
Allergic reaction		
- Anaphylaxis	1 (8%)	
- Food allergy	2 (15%)	
Laboratory variables		
Cytopenias*, n (%)*	9 (69%)	
- Thrombopenia	8 (62%)	
o Median (range)	104 x10^9/L (24 -133)	
- Anemia	6 (46%)	Anemia, 10.3g/dL
o Median (range)	9.5g/dL (6 -10,5)	
- Leukopenia	0 (0%)	
Other Complete Blood Count abnormalities		
- Leukocytosis	5 (38 %)	Leukocytosis, 52 x10^9/L
o Median (range)	26.2 x10^9/L (20 - 92)	
- Monocytosis	1 (20%)	Monocytosis, 8 x10^9/L
- Neutrophilia	3 (60%)	
- Not specified	1 (20%)	
- Thrombocytosis	0 (0%)	No, 202 x10^9/L
Hepatic abnormalities*, n (%)*		
- Moderate cytolysis (3-5xULN)	4 (31%)	
- Prolonged prothrombin time (PT)	2 (15%)	

- Albumin (NR: 35-49 g/L) - Alkaline phosphatase (NR : 104 – 518 UI/L)	3 (23%)	34.5 902
Basal serum tryptase Median (range)	190ng/mL (64-306)	193ng/mL
C-findings		
- Cytopenias due to bone marrow dysfunction	13 (100%) 9 (69%)	
- Hepatomegaly with functional impairment	5 (38%)	
- Skeletal involvement	0 (0%)	
- Malabsorption with weight loss	6 (46%)	Malabsorption with weight loss
- Splenomegaly with hypersplenism	8 (62%)	
Molecular variables		
Molecular sequencing of the KIT gene*, n (%)*	9 (69%)	
- Codon 816 mutation		
o D816V	8 (62%)	*KIT* D816V (VAF=40%)
o D816Y	1 (8%)	
- Other KIT mutation	0 (0%)	
- WT KIT	0 (0%)	
Treatments		
Non-systemic treatments*, n (%)*	11 (85%)	
- Long-term corticosteroid therapy	7 (54%)	
- Short-term corticosteroids (<1month)	1 (8%)	
- Histamine antagonist	11 (85%)	H1 and H2 antagonists,
- Sodium cromoglycate	7 (54%)	
- Antileukotriene	2 (15%)	
Systemic treatments*, n (%)*	8 (62%)	
- Chemotherapy	3 (23%)	
o Hydroxyurea	1 (8%)	
o Vincristine, cladribine	1 (8%)	
o Cytarabine, 6-mercaptopurine	1 (8%)	Cytarabine, 6 mercaptopurine, corticosteroids (3 cycles)
- TKI	6 (46%)	
o Imatinib (dosage not specified)	1 (8%)	
o Dasatinib (dosage not specified)	2 (15%)	
o Midostaurin (dosage range: 4.8-10 mg/kg)	4 (31%)	Midostaurin (10mg/kg)
- mTOR inhibitor		
o Sirolimus (target trough: 8 ng/mL)	1(8%)	
- Interferon α	1 (8%)	

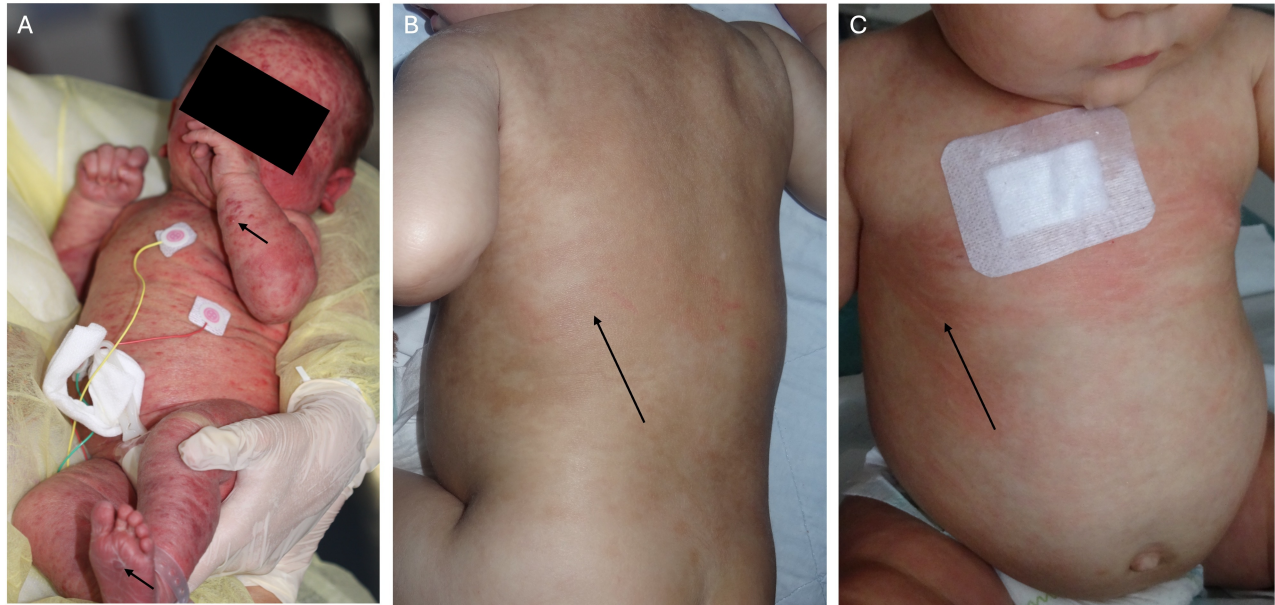
Descriptive statistics, including the mean (range) for continuous variables and the frequency (proportions) for categorical variables, were used to summarize the baseline characteristics of the study population.

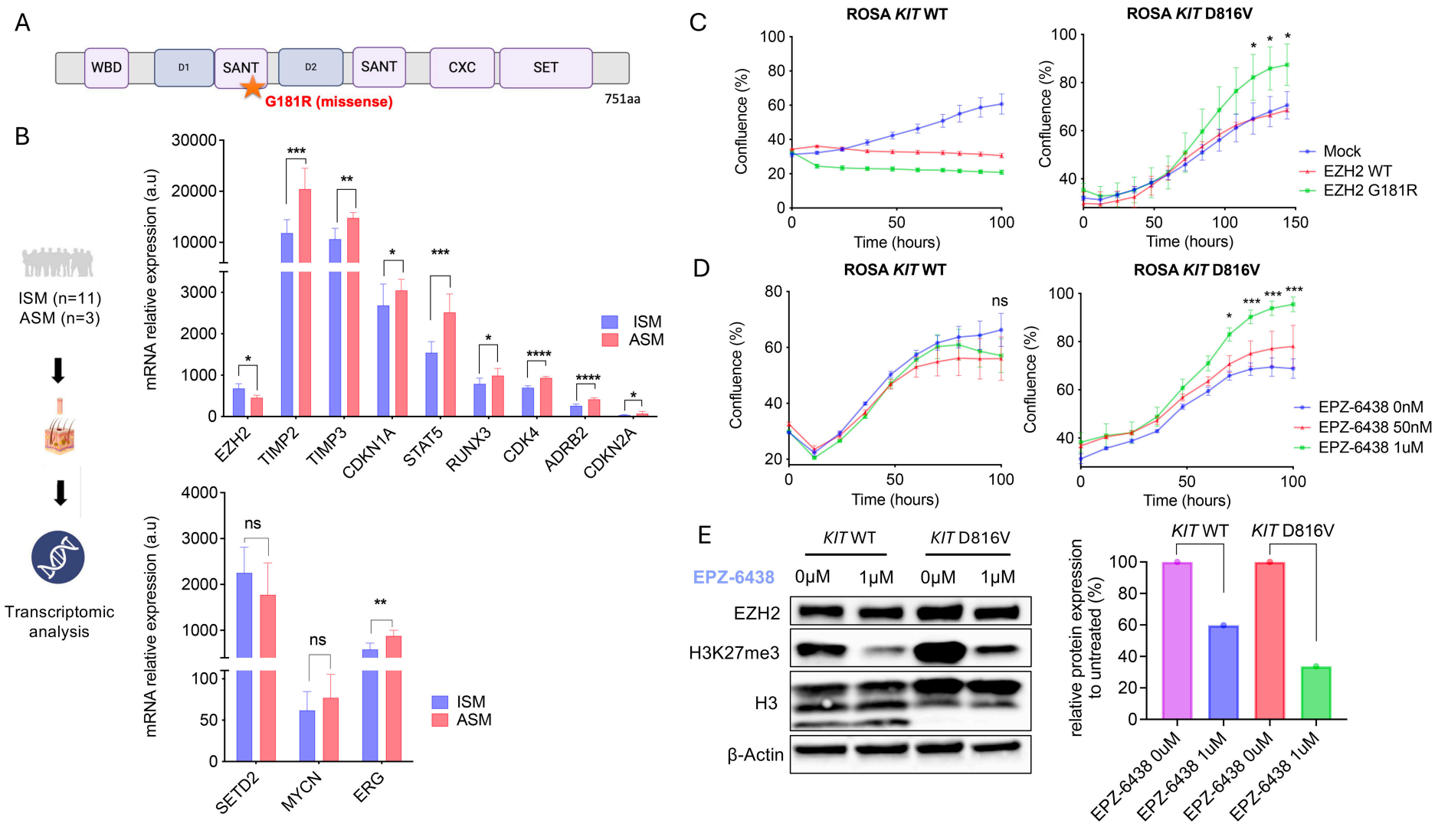
DCM = diffuse cutaneous mastocytosis; NR = normal range; MC = mast cell; MPCM = maculopapular cutaneous mastocytosis; TKI = tyrosine kinase inhibitor; ULN: upper limit of normal; VAF = variant allele frequency.

Figure Legends

Figure 1. Clinical pictures of the patient at birth (A) and at 30 months of age (B, C). (A) Diffuse erythematous cutaneous infiltration with hemorrhagic bullae (arrows). (B, C) Clinical response to midostaurin with improvement in cutaneous infiltration, resolution of diffuse erythema with persistence of a subtle "peau d'orange" appearance (B) and Darier sign–like urtication mimicking dermatographism (C).

Figure 2. EZH2 loss-of-function cooperates with *KIT* D816V to drive mast cell proliferation. (A) Schematic representation of the EZH2 protein domain structure (751 aa). The G181R missense mutation (orange star) is in the first SANT domain (histone-binding region). Black dots indicate previously reported somatic mutations. WBD: WD40-binding domain; SANT: SWI3-ADA2-NCoR-TFIIIB domain; CXC: cysteine-rich domain; SET: catalytic domain. (B) mRNA expression of *EZH2*, its target genes (*TIMP2*, *TIMP3*, *STAT5*, *CDK4*, *ADRB2*, *CDKN2A*), and *SETD2*, its target genes (*MYCN*, *ERG*) in lesional skin biopsies from patients with indolent systemic mastocytosis (ISM, n=11, blue) and aggressive systemic mastocytosis (ASM, n=3, red). Data are expressed as relative expression (a.u.). Statistical comparisons were performed using Mann-Whitney test (*p<0.05, **p<0.01). (C) Proliferation curves (confluence %) of ROSA *KIT* WT (left) and ROSA *KIT* D816V (right) MC lines transduced with lentiviral vectors encoding Mock (empty vector, blue), *EZH2* WT (red), or *EZH2* G181R (green). Confluence was monitored over time (hours) by live-cell imaging. Statistical significance was assessed at indicated time points (*p<0.05). (D) Proliferation curves of ROSA *KIT* WT (left) and ROSA *KIT* D816V (right) cell lines treated with increasing concentrations of EPZ-6438 (tazemetostat): 0 nM (blue), 50 nM (red), or 1 µM (green). ns: not significant; *p<0.05, ***p<0.001. (E) Western blot analysis of EZH2, H3K27me3, total H3, and β-Actin in ROSA *KIT* WT and ROSA *KIT* D816V cell lines treated with EPZ-6438 at 0 µM or 1 µM. The bar graph shows H3K27me3 levels normalized to total H3.


A
B
C


A
WBD
D1
SANT
D2
SANT
CXC
SET
G181R (missense)
751aa
B
ISM (n=11)
ASM (n=3)
Transcriptomic analysis
mRNA relative expression (a.u)
EZH2
TIMP2
TIMP3
CDKN1A
STAT5
RUNX3
CDK4
ADRB2
CDKN2A
ISM
ASM
ns
SETD2
MYCN
ERG
C
ROSA KIT WT
ROSA KIT D816V
Confluence (%)
Time (hours)
Mock
EZH2 WT
EZH2 G181R
D
EPZ-6438 0nM
EPZ-6438 50nM
EPZ-6438 1uM
E
KIT WT
KIT D816V
EPZ-6438
0μM
1μM
EZH2
H3K27me3
H3
β-Actin
relative protein expression to untreated (%)
EPZ-6438 0uM
EPZ-6438 1uM

Supplementary

References	Clinical description at birth	Evolution	Treatment	Molecular abnormalities
Shah (1998)[1] *Patient 1*	DCM and HSM since birth	4 months: failure to thrive and pneumonia Death at 5 months from bacterial endocarditis, pneumonia and ascites Post-mortem findings: persistent myeloproliferative disorder, portal hypertension, liver fibrosis and anatomic pulmonary vascular remodeling.	H1 and H2 antagonists, Oral sodium cromoglycate Corticosteroids	*KIT* D816V
Murphy (1999)[2] *Patient 2*	MPCM since birth HSM by 6 months Generalized lymphadenopathy by 12 months	Massive hypotension secondary to mast cell degranulation at 17 months (unknown factor) Post-mortem findings: MC in liver, spleen, LN from collapse, attributed to massive hypotension	H1 and H2 antagonists, sodium cromoglycate	Not performed
Kuint (1999)[3] *Patient 3*	*In utero* presentation with HSM and elevated alpha-fetoprotein. DCM since birth	Malabsorption, liver dysfunction, severe infection leading to death at 4 months for sepsis, DIC Death at 4 months from sepsis, DIC and respiratory failure, severe ascites	Corticosteroids	*KIT* D816V
Angus (2008) [4] *Patient 4*	*In utero* presentation with cardiomegaly, HSM and progressive ascites. DCM since birth (34 weeks)	Clinical deterioration, marked by increasing abdominal distension, bloody diarrhea, and a need for ventilatory support. Death at 1 month after the first dose of vincristine, from chronic lung disease and hepatic fibrosis complicating ASM Post-mortem findings: mast cell infiltration and fibrosis in multiple organs	Chlorpheniramine, fresh-frozen plasma with cryoprecipitate. Vincristine from day 29	Not performed
Huang (2017)[5] *Patient 5*	*In utero* presentation with massive HSM and ascites. DCM since birth (37 weeks)	Pulmonary hypertension, Multiple infections (bacteremia), Refractory ascites and sepsis. **Under dasatinib and chemotherapy – No response:** Death at 10 weeks from cardiorespiratory failure due to the aggressiveness of mast cell infiltration and multiple infections	H1 and H2 antagonists, Oral and topical sodium cromoglycate, Methylprednisolone Cytoreductive agents (vincristine, cladribine and dasatinib) with no clinical improvement	*KIT* D816V

		Post-mortem findings: MC in liver, spleen, pancreas, LN, BM, fibrotic liver and spleen		
Hussain (2019)[6] *Patient 6*	Presenting at birth with DCM and respiratory distress	Rapid severe systemic deterioration (hypotension, respiratory distress, PH, coagulopathy) Death at 2 days of life of multi-organ failure after morphine administration Post-mortem findings: MC in liver, spleen, BM and LN	Labile blood product Morphine	Not performed
Sinha (2020) [7] *Patient 7*	DCM and HSM since birth	Death on the 7th day after birth due to massive mast cell degranulation causing cardiovascular collapse complicated by infection-induced sepsis	H1 and H2 antagonists Dexamethasone	Not performed
Krase (2022)[8] *Patient 8*	DCM and HSM by 3 months	**Under midostaurin treatment - Partial remission:** - Reduction in hepatosplenomegaly - Near-complete resolution of head and neck lesions - 45% decrease in serum tryptase levels (from 234 to 129 ng/mL)	H1 and H2 antagonists Oral sodium cromoglycate Midostaurin (30mg/m^2 every 12 hours)	*KIT* D816V
Polivka (2023)[9] *Patient 9*	DCM since birth HSM by 3 months	At 3 months: hepatic, splenic, and digestive infiltration Macrocrania and associated Greig syndrome Cognitive and developmental delay Associated eosinophilic inflammatory pancolitis Liver cirrhosis **Under sirolimus treatment – Partial remission (6 years of follow-up):** - Resolution of anemia (from 7 g/dL to 13 g/dL) - Reduction in hepatomegaly - Disappearance of bone marrow infiltration - Catch-up growth (from −2 SD to −0.3 SD) - 43% decrease in serum tryptase levels (from 68 to 39 ng/mL) Accidental death unrelated to EP-ASM at 9 years	H1 and H2 antagonists Oral sodium cromoglycate Montelukast Corticosteroids Sirolimus (8ng/mL)	*KIT* D816V *GLI3* G1559WfsX6

Larouche (2023)[10] *Patient 10*	DCM and HSM at birth. Dysmorphic features, including microtia	At 3 months: portal hypertension and malabsorption. At 4 years: Spinal Germinoma with strong c-KIT expression. Epilepsy, hypotonia, cognitive and developmental delay, moderate bilateral hearing loss and cirrhosis. At 12 years: 2 autoimmune hepatitis. **Under Dasatinib treatment – Stable disease (12-year follow-up):** - Progression of liver fibrosis secondary to type 2 autoimmune hepatitis - Stabilization of cutaneous lesions and mast cell infiltration - 130% increase of serum tryptase levels (from 85 to 195ng/mL)	H1 and H2 antagonists Sodium cromoglycate Prednisone (discontinued) Imatinib (discontinued) Interferon alpha(discontinued) Radiation therapy (SG) Dasatinib Topiramate (epilepsy) Corticosteroids and azathioprine (autoimmune hepatitis)	*KIT* D816V
Voelker (2023)[11] *Patient 11*	Intrauterine growth restriction DCM, HSM, enlarged LN since birth (38 weeks)	**Under midostaurin treatment – Partial remission (4-years follow-up):** - Resolution of thrombopenia - Normal statural and weight growth - Reduction in hepatosplenomegaly with stable fibrosis - 82% decrease of serum tryptase levels (from 306 to 54.5ng/mL)	H1 and H2 antagonists sodium cromoglycate Hydroxyurea Midostaurin (4.8mg/kg)	*KIT* D816Y *SETD2* K31E
Kiwit[12] (2023) *Patient 12*	*In utero* presentation with undetectable ductus venosus, organomegaly and ascites. DCM since birth (35+1weeks)	**Under midostaurin treatment – No response:** - Initiated as a bridge to hematopoietic stem cell transplantation - Improvement of clinical signs (notably skin and blood pressure fluctuations) and serum markers - Patient remained ventilator-dependent and died at 12 weeks due to progressive multi-organ failure	H1 and H2 antagonists, Montelukast Prednisone Midostaurin	*KIT* D816V
Our patient	DCM at birth. HSM by 1 month	**Under midostaurin treatment – Partial remission:** - Normalization of monocytosis (WBC from 52 to 9.6 $x10^9$/L, monocytes from 8 to 0.4 $x10^9$/L) - Reduction in hepatosplenomegaly - Improvement in cutaneous infiltration with resolution of bullae - Normal statural and weight growth	H1 and H2 antagonists, Sodium cromoglycate Chemotherapy (cytarabine, 6-mercaptopurine, corticosteroids, 3 cycles) Midostaurin (10mg/kg)	*KIT* D816V *EZH2* G181R

		- 65% decrease of serum tryptase levels (from 193 to 68ng/mL)		

BM = bone marrow; DCM = diffuse cutaneous mastocytosis; DIC = disseminated intravascular coagulation; PH = pulmonary hypertension; EP-ASM = early pediatric aggressive systemic mastocytosis; LN = lymph nodes; MC = mast cells; MPCM = maculopapular cutaneous mastocytosis; SG = spinal germinoma

Supplemental Table 1. Summary of the EP-Adv-SM patient cohort reported in the literature. A comprehensive literature review on EP-Adv-SM was conducted using Embase and Medline, *via* the Embase Elsevier platform. The search used the following Emtree terms: "mastocytosis", or "mast cell" or "KIT mutation" or "mastocytosis/exp" AND "neonatal" or "newborn" or "pediatric" or "infant" or "congenital" AND "observational study" or "longitudinal study" or "register" or "registries" or "case reports" or "case study series". Case reports were included if they described Adv-SM in infants with symptom onset within the first 24 months of life. Only cases meeting WHO diagnostic criteria for Adv-SM were included. Articles were included only if they were freely accessible or available through university library subscriptions; articles that could not be accessed due to subscription restrictions were excluded. All the following information was recorded: sex, family history, diagnostic criteria, results of skin and bone marrow biopsies, *KIT* mutation status, other molecular findings (NGS, WGS, WES, FISH, karyotype), clinical and biological features, organomegaly, systemic symptoms, treatment and outcome.

1. Shah PY, Sharma V, Worobec AS, Metcalfe DD, Zwick DC. Congenital bullous mastocytosis with myeloproliferative disorder and c-kit mutation. *J Am Acad Dermatol*. 1998;39(1):119-121. doi:10.1016/s0190-9622(98)70413-x

2. Murphy M, Walsh D, Drumm B, Watson R. Bullous Mastocytosis: A Fatal Outcome. *Pediatr Dermatol*. 1999;16(6):452-455. doi:10.1046/j.1525-1470.1999.00116.x

3. Kuint J, Bielorai B, Gilat D, Birenbaum E, Amariglio N, Rechavi G. c-kit activating mutation in a neonate with in-utero presentation of systemic mastocytosis associated with myeloproliferative disorder. *Br J Haematol*. 1999;106(3):838-839. doi:10.1046/j.1365-2141.1999.01683.x

4. Angus J, Leach IH, Grant J, Ravenscroft JC. Systemic mastocytosis with diffuse cutaneous involvement and haematological disease presenting in utero treated unsuccessfully with vincristine. *Clin Exp Dermatol*. 2008;33(1):36-39. doi:10.1111/j.1365-2230.2007.02555.x

5. Huang A, Fiadorchanka N, Brar K, Balderacchi JL, Glick SA. *In utero* presentation of aggressive systemic mastocytosis in a neonate. *Br J Dermatol*. 2017;177(5):1439-1441. doi:10.1111/bjd.15506

6. Hussain A, Sanders M, Riotte C, Hussain N. Full-term newborn infant with blistering skin lesions—Caution regarding use of pain medications. *Pediatr Dermatol*. 2019;36(3):362-364. doi:10.1111/pde.13794

7. Sinha A, Vasudevan B, Sharma N, Verma R, Aneesh KP. A rare case of aggressive systemic mastocytosis manifesting with bullous lesions in a neonate. *Indian J Dermatol Venereol Leprol*. 2020;86:706. doi:10.4103/ijdvl.IJDVL_1035_19

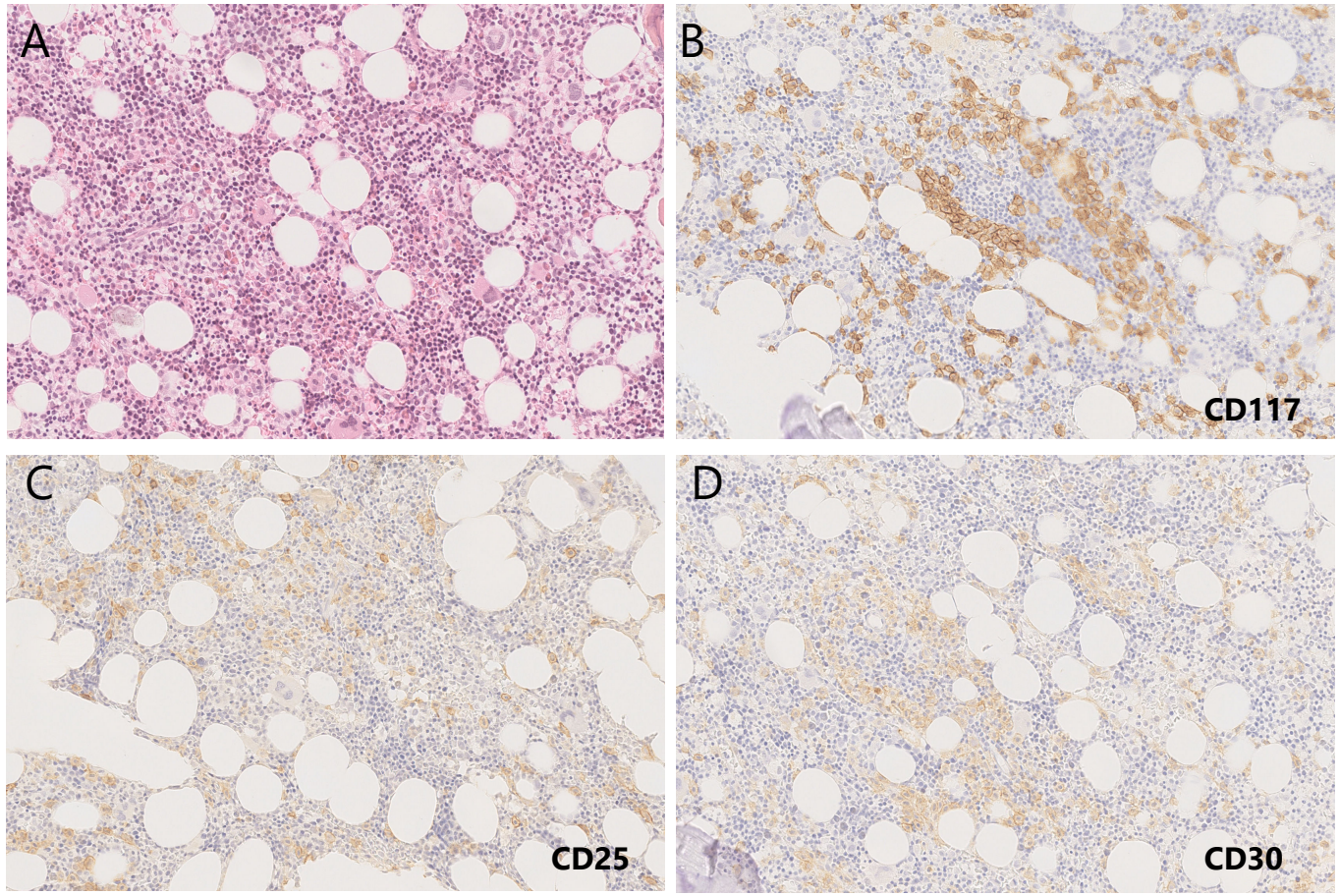
8. Krase I, Tiano R, Varghese M, et al. Infantile aggressive systemic mastocytosis treated with oral midostaurin. *Ann Allergy Asthma Immunol*. 2022;129(5, Supplement):S91. doi:10.1016/j.anai.2022.08.757

9. Polivka L, Parietti V, Bruneau J, et al. The association of Greig syndrome and mastocytosis reveals the involvement of the hedgehog pathway in advanced mastocytosis. *Blood*. 2021;138(23):2396-2407. doi:10.1182/blood.2020010207

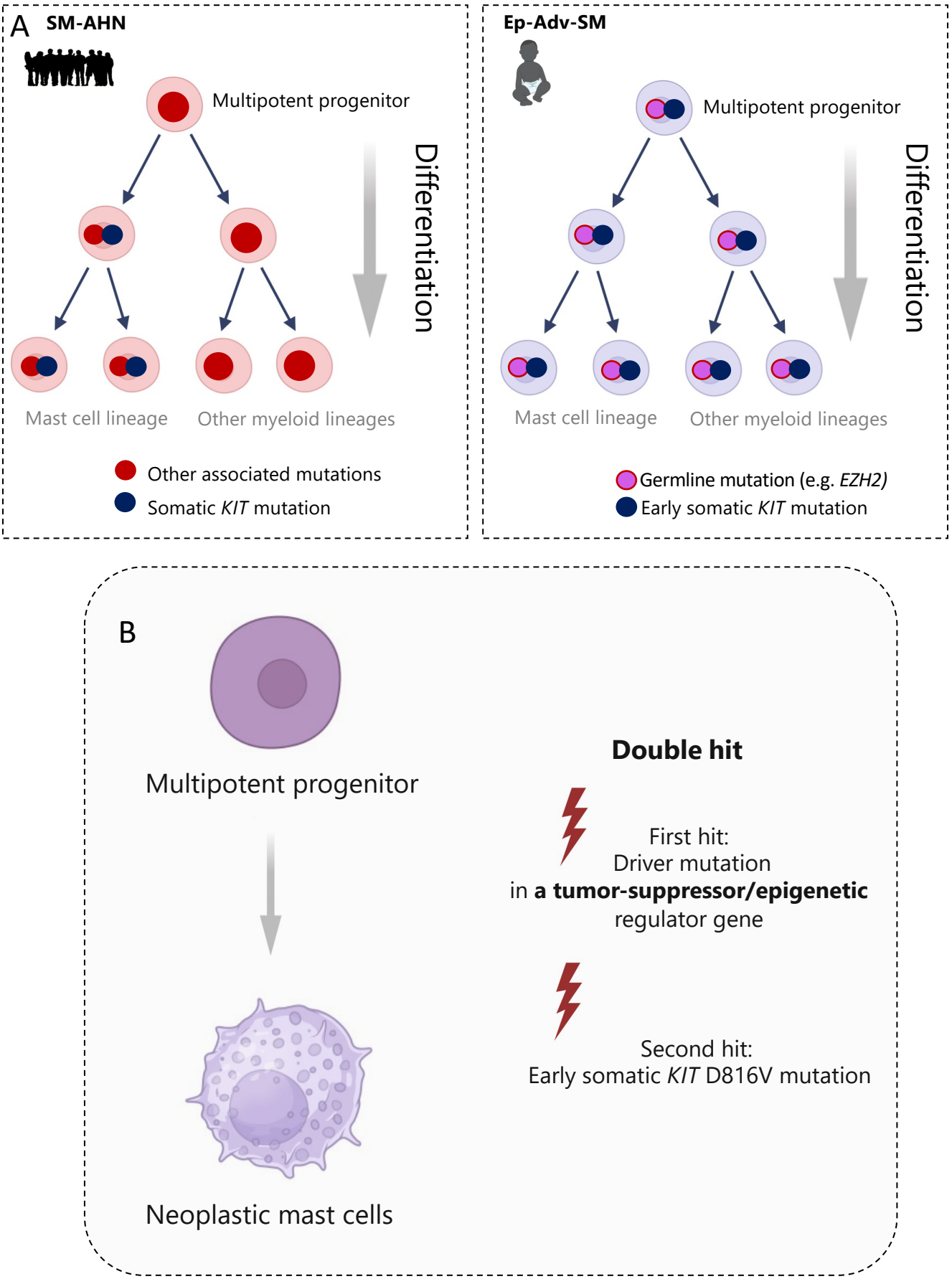
10. Larouche V, Paré MF, Grenier PO, et al. A Review of the Clinical Features and Management of Systemic Congenital Mastocytosis through the Presentation of An Unusual Prenatal-Onset Case. *Curr Oncol*. 2023;30(10):8992-9003. doi:10.3390/curroncol30100649

11. Voelker D, Bednarski JJ, Nieman E, Carter MC, Polk B. Hematopoietic KIT D816Y mutation presenting as in utero aggressive systemic mastocytosis with response to midostaurin. *J Allergy Clin Immunol Pract*. 2023;11(4):1323-1325.e1. doi:10.1016/j.jaip.2022.12.016

12. Kiwit ATJ; Blohm M; Apostolidou S; Lehmberg K; Singer D. Germline p.D816V ckit mutation in connatal systemic mastocytosis. *Z Für Geburtshilfe Neonatol*. 2023;227(03):P08-08. doi:10.1055/s-0043-1769335



Supplemental Figure 1. Bone marrow histology of the patient. Cellular infiltrates (A) show positive staining for CD117 (B), with aberrant expression of CD25 (C) and CD30 (D). Original magnification: ×2.


A
SM-AHN
Multipotent progenitor
Differentiation
Mast cell lineage
Other myeloid lineages
Other associated mutations
Somatic KIT mutation
Ep-Adv-SM
Multipotent progenitor
Differentiation
Mast cell lineage
Other myeloid lineages
Germline mutation (e.g. EZH2)
Early somatic KIT mutation
B
Multipotent progenitor
Double hit
First hit:
Driver mutation
in a tumor-suppressor/epigenetic
regulator gene
Second hit:
Early somatic KIT D816V mutation
Neoplastic mast cells

Supplemental Figure 2. Hypothetical clonal architecture (A) and double-hit model (B) in EP-Adv-SM. Schematic representation of the hypothesized clonal architecture in EP-Adv-SM *versus* SM-AHN (A). In EP-Adv-SM, a germline mutation in tumor suppressor or epigenetic regulator (e.g., *EZH2*) and an early somatic *KIT* D816V mutation likely occurs earlier in hematopoietic stem or progenitor cells, driving dominant clonal expansion and MCs proliferation. In contrast, in SM-AHN, *KIT* D816V typically emerges later and is often accompanied by additional somatic mutations. The development of EP-Adv-SM may be explained by the "double-hit" hypothesis (B), which suggests that two major genetic alterations are required for EP-Adv-SM initiation and progression.