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Preclinical human models and emerging therapeutics for advanced systemic mastocytosis

Michel Arock,^{1,2} Ghaith Wedeh,¹ Gregor Hoermann,^{3,4} Siham Bibi,¹ Cem Akin,⁵ Barbara Peter,^{4,6} Karoline V. Gleixner,^{4,6} Karin Hartmann,⁷ Joseph H. Butterfield,⁸ Dean D. Metcalfe⁹ and Peter Valent^{4,6}

¹LBPA CNRS UMR8113, Ecole Normale Supérieure Paris-Saclay, Cachan, France;

²Laboratory of Hematology, Pitié-Salpêtrière Hospital, Paris, France; ³Department of Laboratory Medicine, Medical University of Vienna, Austria; ⁴Ludwig Boltzmann Cluster Oncology, Medical University of Vienna, Austria; ⁵Michigan Medicine Allergy Clinic, University of Michigan, Ann Arbor, MI, USA; ⁶Department of Medicine I, Division of Hematology and Hemostaseology, Medical University of Vienna, Austria; ⁷Department of Dermatology, University of Luebeck, Germany; ⁸Mayo Clinic, Division of Allergic Diseases, Rochester, MN, USA and ⁹Laboratory of Allergic Diseases, NIAID, NIH, Bethesda, MD, USA

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ABSTRACT

Mastocytosis is a term used to denote a group of rare diseases characterized by an abnormal accumulation of neoplastic mast cells in various tissues and organs. In most patients with systemic mastocytosis, the neoplastic cells carry activating mutations in *KIT*. Progress in mastocytosis research has long been hindered by the lack of suitable *in vitro* models, such as permanent human mast cell lines. In fact, only a few human mast cell lines are available to date: HMC-1, LAD1/2, LUVA, ROSA and MCPV-1. The HMC-1 and LAD1/2 cell lines were derived from patients with mast cell leukemia. By contrast, the more recently established LUVA, ROSA and MCPV-1 cell lines were derived from CD34⁺ cells of non-mastocytosis donors. While some of these cell lines (LAD1/2, LUVA, ROSA^{KIT^{WT}} and MCPV-1) do not harbor *KIT* mutations, HMC-1 and ROSA^{KIT^{D816V}} cells exhibit activating *KIT* mutations found in mastocytosis and have thus been used to study disease pathogenesis. In addition, these cell lines are increasingly employed to validate new therapeutic targets and to screen for effects of new targeted drugs. Recently, the ROSA^{KIT^{D816V}} subclone has been successfully used to generate a unique *in vivo* model of advanced mastocytosis by injection into immunocompromised mice. Such a model may allow *in vivo* validation of data obtained *in vitro* with targeted drugs directed against mastocytosis. In this review, we discuss the major characteristics of all available human mast cell lines, with particular emphasis on the use of HMC-1 and ROSA^{KIT^{D816V}} cells in preclinical therapeutic research in mastocytosis.

Correspondence:

arock@ens-cachan.fr

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Introduction

Mast cells (MC) are tissue-fixed cells found in all vascularized organs. These cells are involved in a number of physiological processes, such as adaptive and innate immune responses.¹ Moreover, MC play a central role in many pathological conditions, including allergic reactions and mastocytosis.² MC develop from bone marrow CD34⁺/CD117⁺ progenitor cells,³ which enter the circulation and migrate into tissues, where they mature into MC in response to their major growth factor, stem cell factor (SCF), the ligand of KIT, also known as CD117. KIT is a transmembrane receptor with intrinsic tyrosine kinase activity (Figure 1).⁴ Besides, mature tissue MC express the high affinity receptor for IgE (FcεRI) and can be activated through this receptor during allergic reactions.⁵

Mastocytosis designates a group of rare disorders characterized by a pathological accumulation of MC in one or more organs.⁶ Clinical presentations of mastocytosis range from skin-limited disease (cutaneous mastocytosis) occurring mainly in childhood and often regressing spontaneously, to systemic disease categories (systemic mastocytosis; SM), mostly seen in adults. SM variants usually involve the bone marrow and sometimes other internal organs, such as the spleen, liver, and/or gastrointestinal tract.

According to the World Health Organization (WHO), mastocytosis can be classified into three major categories: cutaneous mastocytosis, the most common variant, followed by SM, and MC sarcoma, a rare localized MC tumor (*Online Supplementary Table S1*).⁷ SM is subdivided into five distinct categories: indolent SM (ISM), smoldering SM (SSM), SM with an associated hematologic neoplasm (SM-AHN), aggressive SM (ASM) and MC leukemia (MCL) (*Online Supplementary Table S1*).⁷ While patients with ISM have a normal or near-normal life expectancy, patients with SM-AHN, ASM or MCL, collectively termed advanced SM, share a poor prognosis.⁸ The diagnosis of SM is based on WHO criteria and is established when one major criterion and one minor criterion or at least three minor criteria are present (*Online Supplementary Table S2*).⁹ Once the diagnosis of SM has been established, patients are further graded according to the presence of B-findings reflecting a high MC burden, and of C-findings reflecting organ damage related to MC infiltration (*Online Supplementary Table S3*).¹⁰ The pathophysiology of mastocytosis is complex and if acquired activating mutations in *KIT* (mostly *KIT* D816V: NM_000222.2(*KIT*):c.2447A>T, p.Asp816Val) seem to be major drivers of disease in ISM, the same cannot be said for advanced SM in which, in addition to *KIT* mutants, *KIT*-independent signaling pathways are activated and additional genetic defects are frequently found.

Given the complex pathophysiology of mastocytosis, *in vitro* models mimicking neoplastic MC found in SM patients could be useful for developing new therapeutic approaches. To date only a few human MC lines have been described, namely HMC-1¹¹ and its subclones (HMC-1.1 and HMC-1.2),¹² LAD (subclones 1 through 5),¹³ LUVA,¹⁴ ROSA^{KIT WT} and its subclone ROSA^{KIT D816V},¹⁵ and MCPV-1.1 through MCPV-1.4.¹⁶ While LAD, LUVA and ROSA^{KIT WT} cells express *KIT* wild-type (WT), HMC-1.1, HMC-1.2 and ROSA^{KIT D816V} cells harbor *KIT* activating mutations,^{15,17} and MCPV-1 are *RAS*-mutated cells.¹⁶ Although these molecular aberrancies do not recapitulate all the characteristics of neoplastic MC found in advanced SM, these last four cell lines are currently the best available models for identifying molecular targets and defining the effects of several interventional (targeted) drugs currently used to treat advanced SM.

Pathophysiology of mastocytosis

The pathophysiology of mastocytosis is governed by the presence of *KIT* activating mutations in neoplastic MC.¹⁸ Indeed, various *KIT* activating mutations have been described, initially in patients with SM,¹⁹ then in children with cutaneous mastocytosis.²⁰ In adult SM patients, *KIT* mutations affect primarily exon 17 encoding for the phosphotransferase domain, usually D816V (>80% of all patients) (Figure 1).²¹ Other less frequent mutations affect exons 2, 8 and 9 encoding for the extracellular domain or

exons 13 and 14 encoding for kinase domain 1.²¹ By contrast, in children, *KIT* mutations are found in nearly 75% of biopsies of skin lesions, but the *KIT* D816V mutation is found in only 30% of all cases.²⁰ Indeed, a significant percentage of children present with *KIT* mutants located in the extracellular domain (codons 8 and 9) (Figure 1).²⁰

In *KIT* D816V⁺ SM patients, the development of neoplastic MC is principally governed by the PI3K/AKT and JAK/STAT5 signaling pathways activated downstream of *KIT*.^{22,23} Indeed, AKT and STAT5 are constitutively acti-

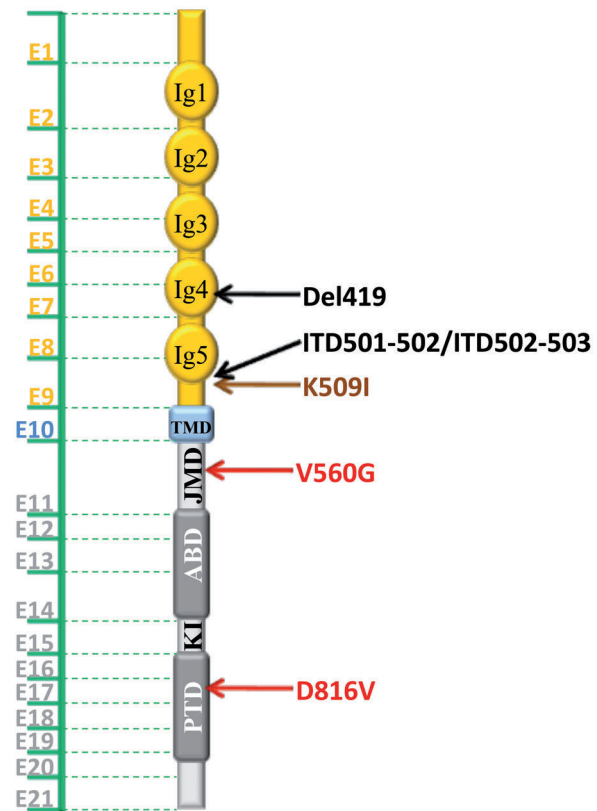


Figure 1. Normal structure of the *KIT* receptor and *KIT* mutations described in human mast cell leukemia-like cell lines and in patients with mastocytosis. In humans, *KIT*, located on chromosome 4q12, contains 21 exons transcribed/translated into a transmembrane receptor with tyrosine kinase activity (145 kDa and 976 amino acids). *KIT* is presented in its monomeric form, but dimerizes as a result of stem cell factor (SCF) ligation. The extracellular domain, in yellow, comprises five immunoglobulin (Ig)-like subunits where the ligand binding site (SCF for *KIT*) and the dimerization site are located. The cytoplasmic region contains a transmembrane domain (TMD) made by a single helix, in blue. The intracellular portion of *KIT*, in gray, contains an auto-inhibitory juxtamembrane domain (JMD) and a kinase domain (in dark gray) which is split into two parts: an ATP-binding domain (ABD) and a phosphotransferase domain (PTD) linked by a large kinase insert (KI) domain of ~ 60–100 residues. For the sake of clarity and readability, only the most frequent mutations found in patients and/or in human MC lines are represented. In red, mutants found in adult patients and in cell lines (*KIT* D816V found in >80% of adult patients with systemic mastocytosis and 30% of children with cutaneous mastocytosis, as well as in HMC-1.2 and ROSA^{KIT D816V} MCL-like cell lines, and *KIT* V560G found in the MCL-like cell lines HMC-1.1 and HMC-1.2, but only in a very few adult patients). In black, three of the *KIT* defects most frequently found in pediatric patients: *KIT* Del419 (NM_000222.2(*KIT*):c.1255_1257del, p.Asp419del), *KIT* ITD501-502 (NM_000222.2(*KIT*):c.1500_1505dup, p.Ser501_Ala502dup) and *KIT* ITD502-503 (NM_000222.2(*KIT*):c.1503_1508dup, p.Ala502_Tyr503dup) and in brown, the *KIT* K509I mutant found in several familial cases of the disease. For a complete overview of the various *KIT* mutations found in pediatric and adult mastocytosis patients, see Valent *et al.* Del, deletion; ITD, internal tandem duplication.

vated in neoplastic MC in such patients and in *KIT*-mutated MCL-like cell lines, and inhibition of these pathways induces growth arrest in such cells.^{22,24} Other intracellular pathways and molecules, such as the Feline sarcoma oncoprotein,²⁵ or the mechanistic target of rapamycin (mTOR) complex,²⁶ are also potential triggers of oncogenesis. In addition, the *KIT* mutant activates ERK independently of SRC, in contrast to *KIT* WT.²⁷ Finally, LYN and BTK are found activated in neoplastic MC in a *KIT*-independent manner.²⁸

Because *KIT*-activating mutations are found in most SM patients, several *KIT*-targeted tyrosine kinase inhibitors (*KIT*-TKI) have been developed. However, the nature of the mutation influences the sensitivity of the mutant to these TKI. For instance, the *KIT* D816V mutant is insensitive to imatinib.²⁹ By contrast, patients presenting with *KIT* WT, or *KIT* mutant outside exon 17, may potentially respond to imatinib.³⁰ While in ISM the *KIT* D816V mutant seems to be the unique molecular abnormality found, additional and recurrent somatic mutations of myeloid malignancy-related genes have been reported in advanced SM. The genes most frequently affected are *TET2*, *SRSF2*, *ASXL1*, *RUNX1*, *JAK2*, *N/KRAS* and *CBL*,³¹⁻³⁵ while *EZH2*, *IDH2*, *ETV6*, *U2AF* or *SF3B1* are less often affected.³⁶ All these mutations may be co-expressed with *KIT* D816V in the same cells or may be expressed in other myeloid cells but not in MC, especially in (A)SM-AHN with *TET2*, *SRSF2* and *ASXL1* mutants, in which acquisition of *KIT* D816V is often a late event conferring a mastocytosis phenotype on a pre-existing clonal condition.³⁷ These defects, and particularly the *SRSF2*, *ASXL1* and *RUNX1* (S/A/R) mutations, have a negative impact on the disease prognosis.^{31-34,35} Thus, targets other than *KIT* and drug combinations might be considered in order to develop more effective therapies for advanced SM. The potential of new targets and of new targeted drugs or drug combinations is currently investigated using the available human MCL-like MC lines, which will be described hereafter.

Major characteristics of available human mast cell leukemia-like mast cell lines

It is beyond the scope of this review to develop all the applications of the available human MC lines. We will only provide a detailed description of the cell lines that qualify as MCL-like, namely HMC-1, ROSA and MCPV-1. Indeed, these cell models have been and are still used *in vitro* and/or *in vivo* to evaluate the potential effects of drugs and drug combinations to treat mastocytosis. Table 1 shows a summary of the major characteristics of all available human MC lines, while Tables 2 and 3 provide detailed phenotypic information.

The HMC-1 cell line and subclones (HMC-1.1 and HMC-1.2)

The origin, major characteristics and phenotype of HMC-1 cells are presented in Tables 1 through 4. HMC-1 cells are metachromatic cells containing histamine and tryptase.¹¹ The original cell line presented with a complex karyotype (Table 1), which might have potentially played a role in the cells' immortalization. However, despite this fact, HMC-1 cells remained sensitive to *KIT* inhibitors, in favor of a critical role of the *KIT* mutants in their maintenance. Indeed, in HMC-1 cells, *KIT* is constitutively phosphorylated on tyrosine residues in the absence of SCF.¹⁷ Sequencing of the coding region of *KIT* cDNA revealed that *KIT* in HMC-1 cells is composed of a normal WT

allele and a mutant allele with two point mutations, *KIT* V560G (NM_000222.2(*KIT*):c.1679T>G, p.Val560Gly) and *KIT* D816V.³⁸

Seven years later, two HMC-1 subclones, namely HMC-1.1 and HMC-1.2, were described.¹² Both subclones have a heterozygous *KIT* V560G mutation,¹² but only HMC-1.2 cells display the *KIT* D816V mutation.¹² In both subclones, *KIT* was found constitutively phosphorylated in the absence of SCF, although the presence of the *KIT* D816V mutation seemed to confer a slight growth advantage to HMC-1.2 cells over HMC-1.1 cells.¹²

HMC-1 cells have been extensively used to study *KIT* mutant-related and *KIT* mutant-independent signaling pathways and to evaluate anti-neoplastic effects of cytoreductive/targeted drugs developed to treat advanced SM.

The ROSA^{KIT WT} and ROSA^{KIT D816V} subclones

The SCF-dependent ROSA^{KIT WT} cell line was established from a CD34⁺ fraction of normal umbilical cord blood cells.¹⁵ CD34⁺ cord blood cells were cultured in the presence of human SCF and, after an 8-week culture period, cells continued to proliferate, with virtually all cells being MC. The doubling time was relatively short (48-72 h) in the presence of SCF.¹⁵ ROSA^{KIT WT} cells are round cells with a relatively high nuclear-to-cytoplasm ratio and metachromatic cytoplasmic granules.¹⁵ ROSA^{KIT WT} cells stain strongly positive for tryptase and *KIT*, but express only little if any chymase.¹⁵

ROSA^{KIT WT} cells were found to express FcεRI, *KIT* (CD117), CD33, CD4, CD9, CD203c, and CD300a, consistent with a MC phenotype, while they did not express CD2 or CD25 (Tables 2 and 3).¹⁵ Moreover, similar to primary cord blood-derived MC, incubation of ROSA cells with interleukin-4 and IgE for 4-5 days enhanced surface expression of FcεRI. In addition, ROSA cells sensitized with interleukin-4 and IgE were fully activated by anti-IgE.¹⁵ However, over long periods of continuous culture, expression of FcεRI tends to fade on the cells, which become less sensitive to FcεRI cross-linking (*unpublished observation*).

ROSA^{KIT WT} cells have a normal *KIT* structure, but harbor a complex karyotype, with a derivative chromosome 1 [der(1)inv(1)(p31q21)del(1)(q24q32)]. In fact, the cell line consists of two subclones: one minor subclone carrying a complete trisomy 5, and the other predominant subclone carrying a partial trisomy 5 [+del(5)(q14q34)].¹⁵ In addition, molecular studies revealed that both ROSA^{KIT WT} subclones have a *P53* deletion and a hot spot K700E mutation in *SF3B1* (*unpublished observation*). We assume that these alterations contributed to the immortalization of ROSA^{KIT WT} cells and provide a premalignant (permissive) cellular background sufficient to trigger proliferation when a driver, such as *KIT* D816V, is introduced.

Indeed, when ROSA^{KIT WT} cells were further transfected with a lentivirus encoding for GFP + *KIT* D816V, the resulting subclone proliferated independently of SCF. This subclone, termed ROSA^{KIT D816V}, has the same doubling time as ROSA^{KIT WT} cells cultured in SCF.¹⁵ Similar to their parental cells, ROSA^{KIT D816V} cells have a rather mature morphology with numerous cytoplasmic granules.¹⁵

ROSA^{KIT D816V} cells exhibit the same complex karyotype and the same *SF3B1* K700E mutation as ROSA^{KIT WT} cells.¹⁵ Moreover, the phenotype of ROSA^{KIT D816V} cells is similar to that of the parental cell line, including expression of the FcεRI and negativity for CD2 and CD25 (detailed in

Tables 1-4), although, in contrast to ROSA^{KIT WT} cells, repeated attempts to activate ROSA^{KIT D816V} cells by cross-linking FcεRI failed in our hands.¹⁵ Interestingly, KIT (CD117) is expressed at higher levels in ROSA^{KIT D816V} cells than in ROSA^{KIT WT} cells.¹⁵ While KIT phosphorylation in ROSA^{KIT WT} cells needs the presence of SCF, KIT is constitutively phosphorylated in ROSA^{KIT D816V} cells.¹⁵ In addition, STAT5 and AKT are constitutively phosphorylated in ROSA^{KIT D816V} cells, as in primary neoplastic MC.^{22,24,39} Interestingly, inhibition of AKT or STAT5 decreases ROSA^{KIT D816V} cell proliferation.¹⁵ As expected, ROSA^{KIT WT} cells responded to imatinib, while ROSA^{KIT D816V} cells were resistant to imatinib, but sensitive to dasatinib or midostaurin (PKC412),¹⁵ making these couple of cell lines a convenient tool for determining the relative selectivity of TKI towards the two forms of KIT (WT *versus* D816V).

Of note, ROSA^{KIT D816V} cells were reported to engraft NOD/SCID IL-2Rγ^{-/-} (NSG) mice efficiently, giving rise to an ASM/MCL-like disease *in vivo*,¹⁵ described later in this manuscript. Thus, the ROSA^{KIT D816V} cell line is a unique model of human KIT D816V⁺ ASM/MCL useful for *in vitro* and *in vivo* studies.

Finally, ROSA cells also appear well suited to investigating the transforming potential of KIT mutants found in other categories of mastocytosis. For example, starting from ROSA^{KIT WT}, we created ROSA subclones stably expressing the mutant *KIT* Del417-419insY (NM_000222.2(KIT):c.1249_1255delinsT, p.Thr417_Asp419delinsTyr), or the mutant *KIT* K509I (NM_000222.2(KIT):c.1526A>T, p.Lys509Ile), both found in pediatric patients.²⁰ In each case, the cells became SCF-independent, and KIT was found constitu-

Table 1. Major characteristics of the available human mast cell lines.

Cell line	Date of first description	Origin	Doubling time	KIT status	SCF-dependence	FcεRI expression	Authentication by DNA fingerprinting	Karyotype	Presence of non-KIT somatic mutation(s)
HMC-1	1988	PB of a patient with MCL	Short (48-72 h)	<i>KIT</i> V560G and <i>KIT</i> D816V	No	No	No	Complex (46 < 2n > XX, ins(10;16)(q25;q22q12), add(13)(q33))	u.k.
Subclones HMC-1.1 HMC-1.2	<i>KIT</i> V560G								
	<i>KIT</i> V560G and <i>KIT</i> D816V								
LAD subclones (1-5)*	2003	BM cells of a patient with <i>KIT</i> D816G ⁺ MCL	Long (2 weeks)	<i>KIT</i> WT	Yes	Yes	Yes	Complex (41-72,XXY, -[7],+2[11], +4[2],+5[6],+7[2], +16[3], +18[2], -21[14] cp15) [§]	u.k.
LUVA	2011	PB of a patient with allergic disease	Short (48-72 h)	<i>KIT</i> WT	No	Yes	No	u.k.	u.k.
ROSA ^{KIT WT}	2014	Normal CB-derived CD34 ⁺ cells	Short (48-72 h)	<i>KIT</i> WT	Yes	Yes	No	Complex (see description in the text)	<i>SF3B1</i> K700E
ROSA ^{KIT D816V}		ROSA ^{KIT WT} cells transduced with a <i>KIT</i> D816V construct		<i>KIT</i> D816V	No				
MCPV-1 subclones (1.1-1.4)	2014	Normal CB-derived MC progenitors transduced with <i>HRAS</i> G12V	Short (48-72 h)	<i>KIT</i> WT	No	No	No	u.k.	<i>HRAS</i> G12V

BM: bone marrow; CB: cord blood; MC: mast cell; MCL: mast cell leukemia; PB: peripheral blood; SCF: stem cell factor; u.k.: unknown. *Only the LAD2 subclone has been widely distributed since its description. [§]Karyotype of the LAD2 subclone.

Table 2. Expression of activation-induced antigens, cytokine receptors and molecular targets[†] by the human mastocytosis-like mast cell lines.

CD	Name	HMC-1	ROSA ^{KIT D816V}	MCPV-1.1
CD16	FcγRIII	–	–	–
CD23	FcεRII	–	–	–
CD26	DPP4	–	n.t.	–
CD27	TNFRSF7	–	n.t.	–
CD32	FcγRII	+	+	+
CD59	MIRL	+	n.t.	+
CD63	LAMP-3	+	+	+
CD64	FcγRI	–	+/-	–
CD66a	BGP-1	+	+	–
CD69	AIM	+/-	+	+
CD71	TR1	+	+	+
CD95	FAS	+	–	+
CD105	ENDOGLIN	–	–	–
CD114	G-CSFR	–	–	+/-
CD115	M-CSFR	–	–	–
CD116	GM-CSFRα	–	–	–
CD127	IL-7R	–	–	–
CD129	IL-9R	–	n.t.	+
CD135	FLT3	–	n.t.	+/-
CD138	SYND1	–	–	–
CD164	MGC-24	+	+/-	+
CD184	CXCR4	+/-	n.t.	–
CD203c	ENPP3	–	+	+
CD213a1	IL-13Rα1	–	–	–
CD218a	IL-18Rα	–	–	–
CD243	MDR-1	–	n.t.	–
CD304	NRP1	–	n.t.	+
CD309	VEGFR2	–	–	–

[†]some of the related antigens are also listed in Table 4. n.t.: not tested; +: strong expression; +/-: weak expression; -: no expression.

tively phosphorylated. In addition, both subclones remained, as expected, sensitive to the growth inhibitory effects of imatinib (*unpublished observations*). These additional data demonstrate that ROSA cells are reasonable tools for investigating the oncogenic potential of newly discovered KIT mutants as well as for screening for their sensitivity to TKI.

The MCPV-1 subclones

The human MCPV-1 subclones (MCPV-1.1 through -1.4) were generated from cord blood-derived CD34⁺ progenitors by culturing these cells with SCF and interleukin-6 for 8 weeks and then stably transducing *HRAS* G12V, SV40 TAG and *TERT*.¹⁶ Single-cell clones were then isolated and cultured for more than 2 years to demonstrate immortalization. Light microscopy of Wright-Giemsa-stained MCPV-1.1 cells reveals large, immature cells with bi-, tri-, or multi-lobed (often cloverleaf-like-shaped) nuclei characteristic of MC precursors.¹⁶ MCPV-1 cells contain a basophilic cytoplasm, cytoplasmic protrusions and sparse granulation. Moreover, MCPV-1.1 cells exhibit an immunophenotype consistent with MC progenitors (Tables 2 and 3).¹⁶ MCPV-1 cells express tryptase but lack

Table 3. Expression of lineage-related markers and adhesion molecules[†] by the human mastocytosis-like mast cell lines.

CD	Name	HMC-1	ROSA ^{KIT D816V}	MCPV-1.1
CD2	LFA2	+	n.t.	–
CD3	TCR	–	–	–
CD4	T4	–	+	–
CD5	T1	–	n.t.	–
CD8	T8	–	+	–
CD9	MRP-1	+	+	–
CD10	CALLA	–	+	–
CD14	LPSR	–	n.t.	–
CD15	LeX	–	+	–
CD17	LacCer	–	n.t.	–
CD19	B4	–	n.t.	–
CD20	B1	–	–	–
CD22	SIGLEC-2	–	–	–
CD24	BA-1	+	–	–
CD31	PECAM-1	+	n.t.	+
CD38	T10	–	–	–
CD45	LCA	+	+	–
CD48	BLAST1	+	+	+
CD50	ICAM-3	+	+	+
CD54	ICAM-1	+	+	+
CD56	NCAM	–	+	–
CD58	LFA-3	+	n.t.	+
CD90	THY1	–	+	–
CD96	TACTILE	–	–	+
CD133	AC133	–	–	–
CD134	OX-40	–	–	–
CD144	VE-CADHERIN	–	–	–
CD146	MUC18	–	–	–
CD150	SLAM	–	–	–
CD153	CD30L	–	–	–
CD166	ALCAM	+	–	+
CD326	EPCAM	–	–	–

[†]some of the related antigens are also listed in Table 4. n.t.: not tested; +: strong expression; +/-: weak expression; -: no expression.

surface FcεRI.¹⁶ MCPV-1 cells grow independently of SCF and produce a MCL-like disease in NSG mice.

Human mast cell leukemia-like cell lines as models for *in vitro* testing of growth-inhibiting drugs

Treatment of ISM mainly aims at symptomatic relief of MC mediator symptoms.⁴⁰ By contrast, treatment of advanced SM is challenging and relies principally on non-targeted and/or targeted cytoreductive therapy.⁴¹ In unusual cases (rare *KIT*-mutant forms or WT *KIT*) the disease may respond to imatinib or masitinib.^{30,42,43} In a subgroup of patients with slowly progressing ASM, low-dose prednisolone and interferon-α may be efficacious.⁴⁴ In addition, low-dose methylprednisolone and cyclosporine A may show some (usually minor) effects in ASM patients.⁴⁵ Cladribine (2CdA) is often recommended as first-line therapy in patients with advanced SM with multi-organ involvement and slow progression.^{46,47} A

forthcoming new standard of therapy in advanced SM is midostaurin (PKC412).^{48,49} This drug was approved for treatment of advanced SM by the American Food and Drug Administration and the European Medicines Agency in 2017. For ASM/MCL patients with rapid progression and those resistant to 2CdA or midostaurin, poly-chemotherapy is usually recommended, followed, when possible, by allogeneic hematopoietic stem cell transplantation.⁵⁰ Almost all drug-based cytoreductive therapies have been validated preclinically *in vitro* using MCL-like MC lines. The most widely used cells for this purpose have been (and still remain) the two HMC-1 subclones. However, the newly emerging MCL-like human MC lines, ROSA and MCPV-1, have also been used repeatedly in such drug-testing studies. A summary of drug-testing approaches and of results obtained with these cell lines is provided in the following paragraphs.

HMC-1 cell lines and their responses to targeted and non-targeted cytoreductive drugs

Numerous antineoplastic drugs have been tested for their effects on HMC-1 cells.⁵¹ Among conventional anti-neoplastic drugs, doxorubicin and cytosine arabinoside were the most active agents.⁵¹ Other effective agents were vinblastine, etoposide and mitomycin.⁵¹ The potent effects of these chemotherapy-type drugs, otherwise used to treat acute myeloid leukemia, formed the basis to suggest treatment of patients with rapidly progressing ASM and MCL as well as patients with SM-acute myeloid leukemia with standard induction chemotherapy, often as preparation for allogeneic stem cell transplantation.

The effects of interferon(s) on the growth of HMC-1 cells have also been analyzed.⁵² HMC-1 cell numbers decreased in the presence of interferon- γ but were unaffected by interferon- α ,⁵² contrasting with the activity of interferon- α in a subset of patients with advanced SM.⁴⁴ This example highlights the fact that not all drug effects observed *in vitro* can be translated into clinical practice and that in each case, drugs and drug combinations need to be tested in additional disease models and finally in interventional clinical trials.

Studies of the *in vitro* anti-proliferative activity of 2CdA on HMC-1 cells were published after this drug was used *in vivo* to treat patients with advanced SM. Indeed, the first reports on the *in vivo* effects of 2CdA in patients were published between 2001 and 2004,⁵³⁻⁵⁵ but it was not until 2006 that the *in vitro* effects of 2CdA on HMC-1 cells were described.⁵⁶ While 2CdA alone produced growth-inhibitory effects on HMC-1 cells, the drug was also found to cooperate with midostaurin.⁵⁶ The observation that midostaurin can induce apoptosis and growth inhibition in HMC-1 cells and that efficacy was identical in HMC-1.1 and HMC-1.2 cells prompted further investigations and led to the initiation of clinical trials.^{48,49}

Human mast cell leukemia-like cell lines as models of drug resistance

Because most patients with SM harbor an activating point mutation in *KIT* (mostly *KIT* D816V) which is associated with disease pathology, considerable efforts have been made to identify drugs capable of inhibiting the kinase activity of the *KIT* mutant. The effects of imatinib, a drug targeting *KIT* WT, on cell lines harboring various *KIT* mutations, were investigated soon after the drug was

Table 4. Expression by the human mastocytosis-like mast cell lines of antigens aberrantly expressed or overexpressed on malignant mast cells and/or their neoplastic progenitors in patients with systemic mastocytosis.

CD	Name	HMC-1	ROSA ^{KIT D816V}	MCPV-1
CD13	APN	+	+	+
CD25	IL-2R α	-	-	-
CD30	KI-1	-	-	-
CD33	SIGLEC-3	+	+	+
CD34	HPCA-1	-	-	-
CD44	PGP-1	+	+	+
CD52	CAMPATH-1	+/-	+	+
CD87	UPAR	+	+	+
CD117	KIT	+	+	+
CD123	IL-3R α	-	+/-	-

+: strong expression; +/-: weak expression; -: no expression.

found to block growth of leukemic cells in Philadelphia chromosome-positive chronic myeloid leukemia. In 2000, Ma *et al.* reported that imatinib inhibited *KIT* WT at low concentrations, without significant effects on the *KIT* D816V mutant.⁵⁷ In 2003, these findings were confirmed using HMC-1.2 cells and patient-derived *KIT* D816V⁺ MC.^{29,58} More recently, it was also confirmed that ROSA^{KIT D816V} cells are insensitive to imatinib.¹⁵ Masitinib, another TKI active on *KIT* WT, although devoid of activity on *KIT* D816V *in vitro*,⁵⁹ was administered in a randomized, double-blind, placebo-controlled, phase 3 study in a cohort of severely symptomatic ISM or SSM patients resistant to classical anti-mediator therapy.⁶⁰ Interestingly, masitinib improved mediator-related symptoms in a subset of patients as compared to placebo-treated patients, regardless of *KIT* mutational status.⁶⁰ This clinical activity was linked to the *in vitro* inhibitory effects of masitinib on two molecules involved in MC activation, namely LYN and FYN.⁵⁹

Given the inefficacy of imatinib on the *KIT* D816V mutant, several other TKI have been evaluated *in vitro* (and for a few of them *in vivo*) for their potential activity in the SM context. Dasatinib is a multikinase inhibitor highly active on BCR-ABL1, *KIT* and PDGFR α .^{61,62} The potential activity of this drug against *KIT* D816V was investigated *in vitro* in HMC-1 cells, SM patient-derived *KIT* D816V⁺ cells and ROSA cells.^{15,63} In each instance, dasatinib exerted *in vitro* cytotoxic effects at relatively low half maximal inhibitory concentrations (IC₅₀), although the IC₅₀ for dasatinib was higher in *KIT* D816V⁺ cells than in *KIT* D816V⁻ cells.^{15,63} However, when evaluated *in vivo* in clinical trials or in individual SM patients, dasatinib unexpectedly demonstrated only marginal activity.⁶⁴⁻⁶⁶ While the *in vivo* effects of dasatinib have been disappointing, midostaurin, a potent multikinase inhibitor, has proven to be highly promising. Indeed, midostaurin decreased the proliferation of *KIT* D816V⁺ cell lines at pharmacological concentrations.^{15,56,63,67} In addition, the drug abrogated *KIT* phosphorylation in MCL-like cell lines harboring *KIT* D816V and induced their apoptosis.^{15,56,68} Moreover, midostaurin suppressed the growth of primary human *KIT* D816V⁺ neoplastic MC.⁶⁸ Finally, midostaurin was found to block IgE-dependent histamine release from MC and basophils.^{67,69,70} Based on these data,

clinical trials have been conducted to determine the efficacy of midostaurin in patients with advanced SM, with promising results.^{48,49,71} An overview of the effects of midostaurin and of several other TKI on the growth of HMC-1 and ROSA cells is presented in Figure 2 and Online Supplementary Table S4.

Several other TKI with different mechanisms of action were also found to exert antineoplastic effects *in vitro* on *KIT* D816V⁺ neoplastic MC, including HMC-1 cells. These drugs include 17-AAG (17-allylamino-17-demethoxygeldanamycin),⁷² EXEL-0862 (a TKI active against fibroblast growth factor receptors, vascular endothelial growth factor receptors, platelet-derived growth factor receptors, FLT3 and *KIT*),⁷³ triptolide (a diterpenoid),⁷⁴ ponatinib (a multi-kinase blocker),⁶⁸ and bosutinib (a LYN/BTK-inhibiting TKI),²⁸ which was administered to a patient with advanced SM, with no benefit.⁷⁵ Nilotinib, which showed some effects *in vitro* on mutant *KIT*,⁷⁶ was recently administered to 61 SM patients, with transient activity in some patients.⁷⁷

More recently, several new *KIT*-TKI with inhibitory activity *in vitro* on several *KIT* mutants, including *KIT* D816V, have been developed. DCC-2618 (Deciphera Inc.) is a switch control type II *KIT* inhibitor, which arrests *KIT* in an inactive state, regardless of activating mutations, such as *KIT* D816V.⁷⁸ In a recent study, it was found that DCC-2618 inhibits proliferation and survival of HMC-1.1, HMC-1.2 and ROSA^{KIT D816V} cells at IC₅₀ < 1 μM.⁷⁹ BLU-285 is a TKI developed by Blueprint Medicines. At low concentrations, BLU-285 selectively inhibited *KIT* D816V enzymatic activity (IC₅₀ = 0.27 nM).⁸⁰ The cellular activity of BLU-285 on this mutant was also measured by autophosphorylation in HMC-1.2 cells with an IC₅₀ of 4.0 nM.⁸⁰ Finally, BLU-285 inhibited *in vitro* the proliferation

of *KIT* D816V⁺ HMC-1.2 cells with an IC₅₀ of 125 nM, while being less active on *KIT* D816V⁻ HMC-1.1 cells (IC₅₀ = 344 nM).⁸¹

Human mastocytosis-like mast cell lines and drugs targeting *KIT*-dependent or *KIT*-independent signaling pathways

Since *KIT* D816V is equally present in ISM and advanced SM patients, who have different life expectancies,⁸² the current assumption is that additional, *KIT*-independent pathways and pro-oncogenic hits and lesions are responsible for disease progression in advanced SM. Such pathways and pro-oncogenic molecules include LYN, BTK, STAT5, PI3-K, mTOR and members of the BCL-2 family.

For instance, LYN and BTK are phosphorylated in HMC-1.1 and HMC-1.2 subclones independently of *KIT*, and short interfering RNA against LYN and BTK decreased the survival of both subclones.²⁸ In the same set of experiments, dasatinib blocked not only the kinase activity of *KIT*, but also LYN and BTK activation in neoplastic MC, while bosutinib inhibited LYN and BTK activation without decreasing *KIT* kinase activity.²⁸

Another molecule, STAT5, seems critical for *KIT* D816V-driven proliferation in MCL-like MC lines as well as in neoplastic MC in SM patients.^{24,83} Chaix *et al.* reported that the *KIT* D816V mutant can directly phosphorylate STAT5 *in vitro*.⁸³ Interestingly, STAT5 is transcriptionally active in the HMC-1 cell line and in ROSA^{KIT D816V} cells,^{15,24} and drugs targeting STAT5 are effective in decreasing the growth rate of these cells.^{84,85} Figure 3 shows representative curves of dose-dependent inhibition of the viability of MC lines by STAT5 inhibitors.

Gabillot-Carre *et al.* reported constitutive activation of the mTOR signaling pathway in both HMC-1 sub-

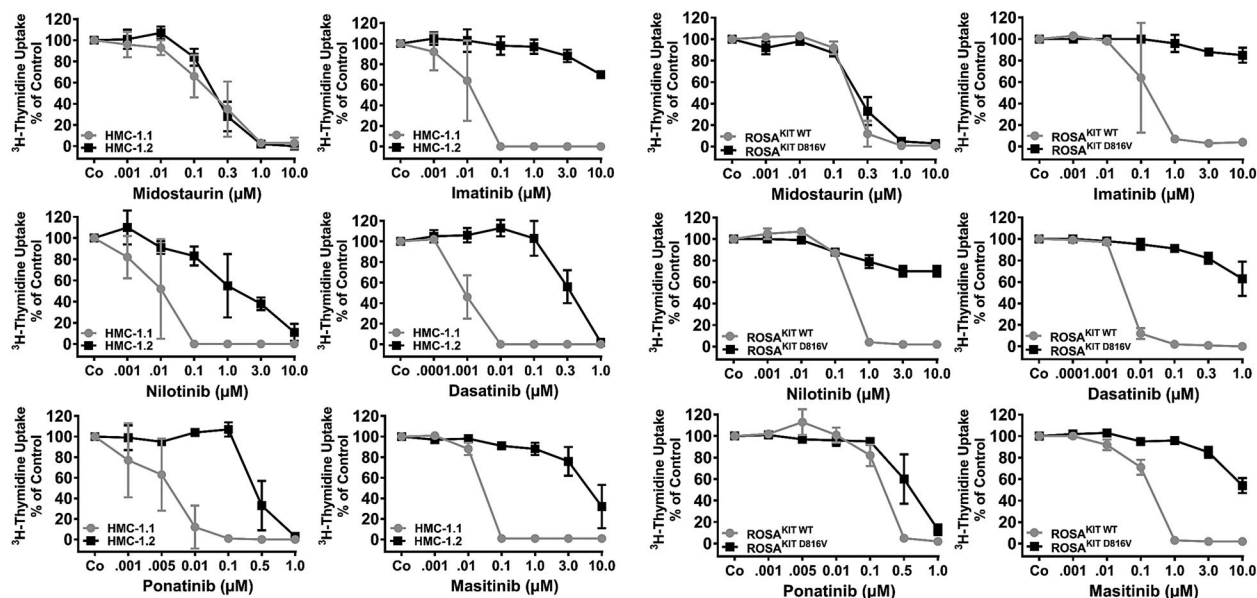


Figure 2. Dose-dependent inhibition of the proliferation of wild-type and mutant *KIT* human mast cell lines by various tyrosine kinase inhibitors *in vitro*. Human mast cell lines expressing *KIT* D816V (HMC-1.2 or ROSA^{KIT D816V}; black lines) or lacking *KIT* D816V (HMC-1.1 or ROSA^{KIT WT}; gray lines) were incubated in control medium (Co) or in medium containing various concentrations of tyrosine kinase inhibitors (as indicated) at 37 °C for 48 h. Thereafter, ³H-thymidine uptake was measured. Results are expressed as percent of ³H-thymidine uptake compared to the control and represent the mean ± standard deviation of three different experiments.

clones.⁸⁶ However, the mTOR inhibitor rapamycin induced apoptosis only in HMC-1.2 cells.⁸⁶ To support this unexpected selectivity, the authors demonstrated that rapamycin inhibited the phosphorylation of 4E-BP1, a downstream substrate of the mTOR pathway, only in HMC-1.2 cells.⁸⁶ More recently, it was reported that the dual PI3-kinase/mTOR blocker NVP-BEZ235 has similar growth inhibitory effects in HMC-1.1 and HMC-1.2 cells.⁸⁷ However, despite these encouraging data, no objective response was observed in a study in which everolimus, an oral mTOR inhibitor, was given at a dose of 10 mg daily to ten SM patients.⁸⁸

Finally, aberrant accumulation of neoplastic MC in SM might result from deregulation of apoptosis pathways.⁸⁹ Indeed, the anti-apoptotic molecules BCL-2, BCL-xL and MCL-1 are overexpressed in *KIT* D816V⁺ neoplastic MC in SM patients,^{90,92} while the expression of the pro-apoptotic molecule BIM is suppressed in these cells.⁹³ It has also been reported that MCL-1 is detectable in HMC-1.1 and HMC-1.2 cells.⁹³ Moreover, exposure of these cells to MCL-1-specific antisense oligonucleotides or to MCL-1-specific short interfering RNA resulted in reduced cell survival and increased apoptosis.⁹³ In further studies, evidence was provided that the pan-BCL-2 family blocker obatoclax inhibited the proliferation of HMC-1 cells, together with increased expression of *PUMA*, *NOXA*, and *BIM* mRNA, and apoptosis.⁹⁴

Human mastocytosis-like mast cell lines and drugs targeting surface antigens or epigenetic regulators

Although drugs targeting *KIT* D816V have demonstrated activity on MC *in vitro* and *in vivo*, these agents do not cure patients with advanced SM.^{48,49,71} Apart from several different mechanisms of resistance, neoplastic cells in these patients may exhibit a complex pattern of genetic alterations together with, or even often preceding the appearance of, the *KIT* mutant, as it is the case for *TET2*, *SRSF2* and *ASXL1* mutants,³⁷ which could explain resistance to TKI. For this reason, attention has been focused recently on alternative targets which could help to overcome such resistance, namely surface antigens specifically or aberrantly expressed by neoplastic MC and epige-

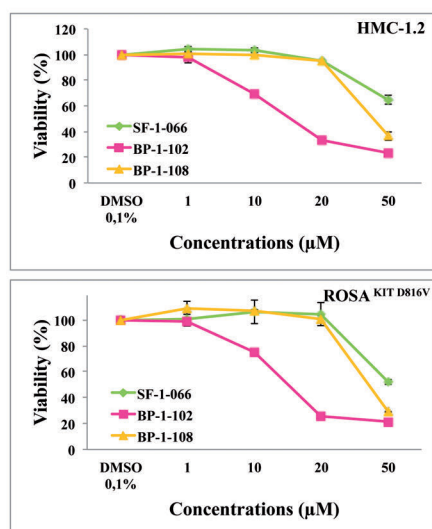
netic targets. Antibodies or drugs directed against these targets may also be able to overcome intrinsic neoplastic stem cell resistance, often associated with quiescence and altered drug influx or rapid drug efflux. Antibody-based drugs may exert antineoplastic effects independently of such mechanisms of resistance.

Targeting surface antigens

Several antigens are aberrantly expressed or overexpressed on neoplastic MC and on their progenitors in SM, including CD13, CD25, CD30, CD33, CD44, CD52, CD117 and CD123,^{95,97} and might, therefore, be considered as potential therapeutic targets. Table 4 provides an overview of cell surface targets expressed on human MCL-like cell lines.

CD30 is aberrantly expressed by neoplastic MC in a subset of patients with SM, but not by normal/reactive MC.⁹⁸ In a recent study, it was observed that the MCPV-1.1 subclone expressed high levels of CD30, while HMC-1.1 cells expressed low CD30 levels, and HMC-1.2 cells did not express CD30.⁹⁹ The CD30-targeting antibody-conjugate brentuximab-vedotin inhibited the *in vitro* proliferation of neoplastic MC, with lower IC₅₀ values obtained for MCPV-1.1 cells (10 µg/mL) than for HMC-1.2 cells (>50 µg/mL).⁹⁹ In addition, brentuximab-vedotin produced apoptosis in primary CD30⁺ neoplastic MC.⁹⁹ However, overall, the effects of brentuximab-vedotin on neoplastic MC are relatively weak. Correspondingly, no major clinical activity has been reported in clinical trials to date. In addition, neoplastic stem cells in advanced SM usually lack CD30 (*personal information*, PV).

In contrast to normal MC and MC from ISM patients, CD52 is abundantly expressed on neoplastic MC in most patients with advanced SM.¹⁶ Recently, it was reported that the CD52-targeting antibody alemtuzumab counteracts growth of MCPV-1.1 cells.¹⁶ Additionally, MCPV-1.1 cells were injected into NSG mice which were then treated with alemtuzumab or control vehicle. The alemtuzumab-treated mice had increased survival compared to controls, and reduced organ infiltration by neoplastic MC.¹⁶ Given that neoplastic (leukemic) stem cells identified in advanced SM may also express CD52,¹⁰⁰ it can be



	IC ₅₀ (HMC-1.2)
SF-1-066	/
BP-1-108	34 µM*
BP-1-102	11 µM*

	IC ₅₀ (ROSA ^{KIT} D816V)
SF-1-066	/
BP-1-108	22 µM*
BP-1-102	11 µM*

*calculated IC₅₀ values (µM) of inhibitors (GraphPad Prism)

Figure 3. Dose-dependent inhibition of the proliferation of *KIT* D816V⁺ human mast cell lines by STAT5 inhibitors *in vitro*. ROSA^{KIT} D816V and HMC-1.2 cells were cultured in 96-well plates for 72 h in control medium containing 0.1% dimethylsulfoxide (DMSO) or with increasing concentrations (between 1.0 and 50.0 µM) of SF-1066, a very weak STAT5 inhibitor (K_i >25 µM on STAT5), and of more specific and potent STAT5 inhibitors BP-1-102 and BP-1-108 (K_i >10 µM on STAT5).⁷⁴ Viability was calculated in each condition by the MTT method. Results are expressed as percent of control and represent the mean ± standard deviation of triplicate experiments. The half maximal inhibitory concentration (IC₅₀) at day 3 of each compound for each cell line was calculated using Prism GraphPad 4.0 software after plotting log concentration versus response. As expected, while the IC₅₀ for SF-1066 was >50 µM, IC₅₀ values for the more STAT5-specific compounds were lower: 11 µM for BP-1-102 on both *KIT* D816V⁺ neoplastic human MC lines and 34 and 22 µM for BP-1-108 for, respectively, HMC-1.2 and ROSA^{KIT} D816V cells. Although these values are still irrelevant at the pharmacological level, they open hopes that drug optimization might lead in a near future to the design of more potent small molecules inhibiting STAT5 activity.

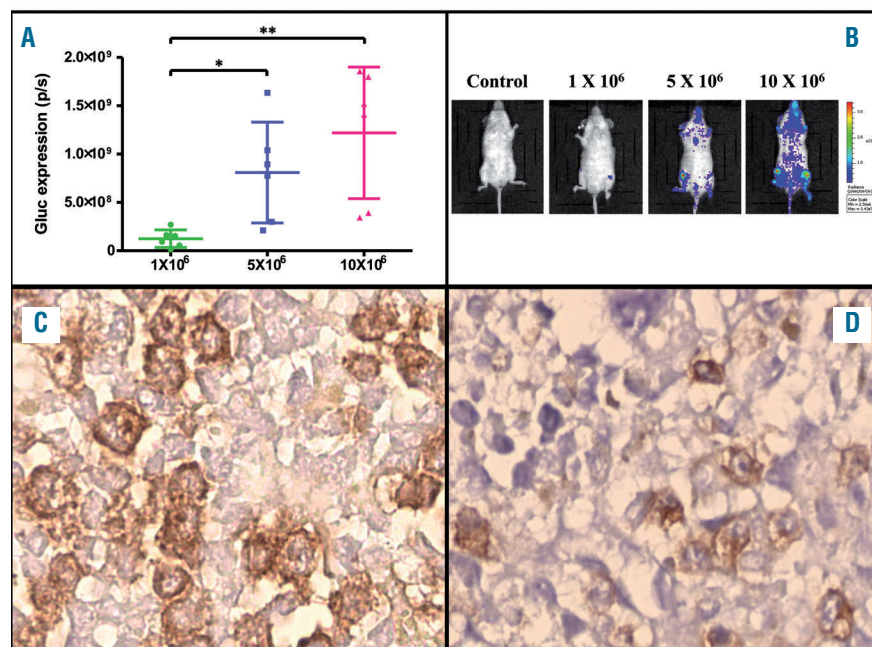


Figure 4. Engraftment of ROSA^{KIT D816V-Gluc} cells in NSG mice. Increasing numbers of ROSA^{KIT D816V-Gluc} cells (1x10⁶, 5x10⁶ or 10x10⁶) were injected into the tail vein of NOD-SCID IL-2Rγ^{-/-} (NSG) mice (3 groups; 6 mice per group) 24 h after irradiation at 2.5 Gy from a cesium-137 source.¹¹³ After 10 weeks, engraftment was assessed using (A) quantitative measurements of Gluc activity in plasma and (B) *in vivo* bioluminescence imaging (IVIS) on engrafted mice. Ten weeks after engraftment, mice were sacrificed and (C) bone marrow and (D) spleen sections from the three groups of mice were stained by immunohistochemistry with an anti-human tryptase antibody. Staining was visualized by a Histomouse Kit, showing human MC with a brownish stain, which massively invaded the bone marrow and to a lesser extent the spleen. (C) and (D) are from one representative mouse from the group injected with 10x10⁶ ROSA^{KIT D816V-Gluc} cells. Original magnification, x100.

hypothesized that a combination of a KIT-TKI and a monoclonal antibody against CD52 might help to achieve major antineoplastic effects in advanced SM.

Another potential surface target is CD33. In fact, CD33 is invariably expressed on neoplastic MC and their stem cells in patients with advanced SM.⁹⁵ In addition, it has been described that the CD33-targeted antibody-construct gemtuzumab-ozogamicin is able to suppress growth and survival of neoplastic MC.¹⁰¹ In the light of the revival of gemtuzumab-ozogamicin, its clinical efficacy in patients with acute myeloid leukemia and its effects on neoplastic MC,¹⁰² it might be reasonable to propose clinical trials testing the effects of gemtuzumab-ozogamicin alone or in combination with other antineoplastic drugs or stem cell transplantation in advanced SM.

Targeting epigenetic regulators

The epigenetic reader bromodomain-containing 4 protein (BRD4), a member of the BET family proteins, has recently been identified as a promising target in acute myeloid leukemia.^{103,104} In addition, highly selective BET bromodomain inhibitors, including JQ1,¹⁰⁵ I-BET151,^{106,107} and I-BET762,^{106,108} have demonstrated *in vitro* and *in vivo* activity against several hematopoietic malignancies. It has also been evaluated whether BRD4 might be a target in advanced SM. Indeed, BRD4 was found to be expressed in HMC-1.1, HMC-1.2, ROSA^{KIT WT} and ROSA^{KIT D816V} cells as well as in primary neoplastic MC.¹⁰⁹ Independently of the grade or variant of disease, neoplastic MC exhibit nuclear BRD4.¹⁰⁹ However, in ASM and MCL, neoplastic MC also express substantial amounts of cytoplasmic BRD4.¹⁰⁹ In line with this observation, HMC-1 and ROSA cells express cytoplasmic and nuclear BRD4 as well.¹⁰⁹ The KIT-TKI midostaurin and dasatinib suppressed the expression of BRD4 in all MC lines.¹⁰⁹ BRD4-specific short hairpin RNA and JQ1 decreased the proliferation of HMC-1 and ROSA cells.¹⁰⁹ Based on these data,

BRD4 is a promising target in advanced SM, although this needs to be confirmed in forthcoming clinical studies.

Human mast cell leukemia-like cell lines as tools to develop *in vivo* models

In vivo models have been developed in order to understand the pathophysiology of SM better. In addition to transgenic mouse models,^{110,111} another approach is to create a SM-like disease *in vivo* by transplanting human neoplastic MC into immunodeficient mice.

The HMC-1 cell line engrafts immunodeficient mice after intravenous or subcutaneous injection, giving rise to subcutaneous tumors after 2 to 5 months.^{11,112} The reason why intravenous injection does not give rise to a MCL-like disease is unknown, but limits the usefulness of HMC-1 cells to establish an *in vivo* model of advanced SM. More recently, an *in vivo* model of advanced SM was established using ROSA cells. Indeed, we engineered a ROSA subclone, termed ROSA^{KIT D816V-Gluc}, which naturally secretes *Gaussia princeps* luciferase (Gluc), used as a reporter.¹¹³ In this study, intravenous injection of NSG mice with ROSA^{KIT D816V-Gluc} cells led to an advanced SM phenotype, with neoplastic MC invading the bone marrow, spleen and liver, as testified by the quantification of engrafting cells by measuring Gluc reporter activity in peripheral blood and by an *in vivo* imaging system (IVIS).¹¹³ The detailed characteristics of this *in vivo* model are presented in Figure 4. All in all, this *in vivo* model of advanced SM is potentially the best available to date for *in vivo* testing of drugs previously identified as active *in vitro* on neoplastic MC.

Summary and future perspectives

Despite decades of intensive research, only a few human MC lines have been established to date: HMC-1, LAD-2,

LUVA, ROSA and MCPV-1. While none of these cell lines simultaneously expresses the *KIT* D816V mutant and a functional FcεRI, making them useless for testing MC-stabilizing drugs or drugs interfering with FcεRI-induced signaling in the context of *KIT* D816V⁺ SM, some of these cell lines may qualify as MCL-like since they harbor SM-related *KIT* variants and/or other oncogenic molecules relevant to SM. Among all MC lines, HMC-1 cells have been most frequently used, but other more recently established MC lines, such as ROSA and MCPV-1, are now available and are being used in various preclinical studies. For example, these cell lines have been used to analyze *in vitro* the growth-inhibitory effects of antineoplastic drugs, including various KIT-TKI, on neoplastic MC. However, because neoplastic MC in advanced SM are triggered by KIT-independent signaling pathways and additional genetic lesions that confer resistance against KIT-TKI, it might be interesting to establish *in vitro* models of multi-mutated neoplastic MC, starting from established human *KIT* mutant-positive MC lines in which additional lesions, such as the S/A/R

combination of molecular lesions might be introduced. Such multi-mutated neoplastic MC lines should be useful to test combination therapies *in vitro*, and later in clinical trials in patients with advanced SM. With these approaches, new therapeutic concepts should be established in order to improve therapy in advanced SM.

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