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, ROSAKIT 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 ROSAKIT 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.
Mastocytosis designates a group of rare disorders characterized by a pathological accumulation of MC in one or more organs. 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). 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). 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. 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). 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). The pathophysiology of mastocytosis is complex and if acquiring 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-1 and its subclones (HMC-1.1 and HMC-1.2), LAD (subclones 1 through 5), LUVA, ROSA KIT WT and its subclone ROSA KIT WT and MCPV-1.1 through MCPV-1.4. 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, and MCPV-1 are RAS-mutated cells. 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. Indeed, various KIT activating mutations have been described, initially in patients with SM, then in children with cutaneous mastocytosis. In adult SM patients, KIT mutations affect primarily exon 17 encoding for the phosphotransferase domain, usually D816V (>80% of all patients) (Figure 1). 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. 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 80% of all cases. Indeed, a significant percentage of children present with KIT mutants located in the extracellular domain (codons 8 and 9) (Figure 1). 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. Indeed, AKT and STAT5 are constitutively acti-
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. Other intra-cellular pathways and molecules, such as the Feline sarcoma oncprotein, or the mechanistic target of rapamycin (mTOR) complex, are also potential triggers of oncogenesis. In addition, the KIT mutant activates ERK independently of SRC, in contrast to KIT WT. Finally, LYN and BTK are found activated in neoplastic MC in a KIT-independent manner.

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. 

The HMC-1 cell line and subclones (HMC-1.1 and HMC-1.2) have been extensively used to study KIT-mutated MC. The doubling time was relatively short (48-72 h) in the presence of SCF. Furthermore, KIT D816V and KIT D816V-mutant outside exon 17, may potentially respond to imatinib. 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 ROSAKIT WT and ROSA KIT D816V subclones

The SCF-dependent ROSAKIT WT cell line was established from a CD34+ fraction of normal umbilical cord blood cells. 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. ROSA KIT WT cells are round cells with a relatively high nuclear-to-cytoplasm ratio and metachromatic cells containing histamine and tryptase. The original cell line presented with a complex karyotype, with a derivative chromosome 1 consisting of two subclones: one minor subclone carrying a partial trisomy 5 [+del(5)(q14q34)]. 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)]. In addition, molecular studies revealed that both ROSAKIT 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 ROSAKIT WT cells and provide a premalignant (permissive) cellular background sufficient to trigger proliferation when a driver, such as KIT D816V, is introduced.

Rosa KIT WT cells were further transfected with a lentivirus encoding for GFP + KIT (CD117), CD38, CD4, CD9, CD203c, and CD300a, consistent with a MC phenotype, while they did not express CD2 or CD25 (Tables 2 and 3). 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. 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)]. In addition, molecular studies revealed that both ROSAKIT 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 ROSAKIT WT cells and provide a premalignant (permissive) cellular background sufficient to trigger proliferation when a driver, such as KIT D816V, is introduced.
Tables 1-4), although, in contrast to ROSA<sub>KIT WT</sub> cells, repeated attempts to activate ROSA<sub>KIT D816V</sub> cells by crosslinking FcɛRI failed in our hands. Interestingly, KIT (CD117) is expressed at higher levels in ROSA<sub>KIT D816V</sub> cells than in ROSA<sub>KIT WT</sub> cells. While KIT phosphorylation in ROSA<sub>KIT WT</sub> cells needs the presence of SCF, KIT is constitutively phosphorylated in ROSA<sub>KIT D816V</sub> cells. In addition, STAT5 and AKT are constitutively phosphorylated in ROSA<sub>KIT D816V</sub> cells, as in primary neoplastic MC. Interestingly, inhibition of AKT or STAT5 decreases ROSA<sub>KIT D816V</sub> cell proliferation. As expected, ROSA<sub>KIT WT</sub> cells responded to imatinib, while ROSA<sub>KIT D816V</sub> cells were resistant to imatinib, but sensitive to dasatinib or midostaurin (PKC412), 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<sub>KIT D816V</sub> cells were reported to engraft NOD/SCID IL-2Rγ<sup>−/−</sup> (NSG) mice efficiently, giving rise to an ASM/MCL-like disease in vivo, described later in this manuscript. Thus, the ROSA<sub>KIT D816V</sub> 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<sub>KIT WT</sub>, 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. In each case, the cells became SCF-independent, and KIT was found constitutively phosphorylated.

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

| Cell line      | Date of first description | Origin             | Doubling time  | KIT status          | SCF-dependence | FcɛRI expression | Authentication by DNA fingerprinting | Karyotype                                                                 | Presence of non-KIT somatic mutation(s) |
|----------------|---------------------------|--------------------|----------------|--------------------|----------------|-------------------|--------------------------------------|--------------------------------------------------------------------------------|----------------------------------------|
| HMC-1          | 1988                      | BM of a patient    | Long           | KIT WT             | Yes            | Yes               | No                                   | Complex (46 < 2n > XX, ins(10;16) (q25;q22q12), add(13)(q33))                | u.k.                                   |
|                |                           | with CB            | (2 weeks)      |                    |                |                   | No                                   |                                                                                  |                                        |
| Subclones      |                           |                    |                |                    |                |                   | No                                   |                                                                                  |                                        |
| HMC-1.1        | 2003                      | PB of a patient    | Short (48-72 h) | KIT V560G          | No             | No                | No                                   | Complex (46 < 2n > XX, ins(10;16) (q25;q22q12), add(13)(q33))                | u.k.                                   |
| HMC-1.2        |                           | with MCL           |                | KIT D816V          | No             | No                | No                                   |                                                                                  |                                        |
| LAD subclones  | 2003                      | BM cells of a      | Long           | KIT WT             | Yes            | Yes               | Yes                                  | Complex (41-72,XX, -7,-2[11], +2[11], +5[7], +16[3], +18[2], -21[14] cpi5)   | u.k.                                   |
| (1-5)*         |                           | patient with MCL   | (2 weeks)      |                    |                |                   | No                                   |                                                                                  |                                        |
| LUVJ           | 2011                      | PB of a patient    | Short (48-72 h) | KIT WT             | No             | Yes               | No                                   |                                                                                  | u.k.                                   |
|                |                           | with allergic      |                |                    |                |                   | No                                   |                                                                                  |                                        |
| ROSA<sup>KIT</sup> | 2014                    | Normal CB-derived | Short (48-72 h) | KIT WT             | Yes            | Yes               | Yes                                  | Complex (see description in the text)                                        | SF3B1 K700E                             |
| ROSA<sup>KIT</sup>  |                           | CD34<sup>+</sup>   |                | KIT D816V          | No             | Yes               | No                                   |                                                                                  |                                        |
|                |                           | cells               |                |                    |                |                   | No                                   |                                                                                  |                                        |
| MCPV-1 subclones | 2014                    | Normal CB-derived  | Short (48-72 h) | KIT WT             | No             | No                | No                                   |                                                                                  | u.k.                                   |
| (1-1.4)        |                           | MC progenitors     |                |                    |                |                   | No                                   |                                                                                  |                                        |

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.
nuclei characteristic of MC precursors.16 MCPV-1 cells are bi-, tri-, or multi-lobed (often cloverleaf-like-shaped) and cultured for more than 2 years to demonstrate immortality. 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 cytoplasma, 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 surface FcεRI.18 MCPV-1 cells grow independently of SCF and produce a MCL-like disease in NSG mice.

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.19 Single-cell clones were then isolated and cultured for more than 2 years to demonstrate immortality. 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.

| CD     | Name          | HMC-1 | ROSA** D816V | MCPV-1.1 |
|--------|---------------|-------|--------------|----------|
| CD16   | FcγRII        | -     | -            | -        |
| CD23   | FcεRI        | -     | -            | -        |
| CD26   | DPP4         | n.t.  | -            | -        |
| CD27   | TNFRSF7      | n.t.  | -            | -        |
| CD32   | FcγRII        | +     | +            | +        |
| CD50   | MIRL         | +     | n.t.         | +        |
| CD63   | LAMP-3        | +     | +            | +        |
| CD64   | FcγRI        | +/-   | -            | -        |
| CD66a  | BGP-1        | +     | +            | +        |
| CD69   | AIM           | +/-   | +            | +        |
| CD71   | TIR1         | +     | +            | +        |
| CD95   | FAS           | +     | n.t.         | +        |
| CD105  | ENDOGLIN      | -     | -            | -        |
| CD114  | G-CSFR        | -     | -            | +/-      |
| CD115  | M-CSFR        | -     | -            | -        |
| CD116  | GM-CSFRααα   | -     | -            | -        |
| CD127  | IL-7R        | -     | n.t.         | -        |
| CD129  | IL-9R        | -     | n.t.         | +        |
| CD135  | FLT3          | n.t.  | +/−          |         |
| CD138  | SYND1        | -     | -            | -        |
| CD164  | MGC-24       | +     | +/-          | +        |
| CD184  | CXCRI        | +/-   | n.t.         |          |
| CD203c | ENPP3        | -     | +            | +        |
| CD215a | IL-15Rxαα   | -     | -            | -        |
| CD218a | IL-18Rx      | -     | -            | -        |
| CD245  | 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.

| CD     | Name          | HMC-1 | ROSA** D816V | MCPV-1.1 |
|--------|---------------|-------|--------------|----------|
| CD2    | LFA2         | +     | n.t.         | -        |
| CD3    | TCR           | -     | -            | -        |
| CD4    | T4            | -     | +            | -        |
| CD5    | T1            | -     | n.t.         | -        |
| CD8    | T8            | -     | +            | -        |
| CD9    | MRPI          | +     | +            | -        |
| 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.

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

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.41 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
foming new standard of therapy in advanced SM is midostaurin (PKC412). 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. 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. Among conventional antineoplastic drugs, doxorubicin and cytosine arabinoside were the most active agents. Other effective agents were vincristine, etoposide and mitomycin. 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. HMC-1 cell numbers decreased in the presence of interferon-γ but were unaffected by interferon-α, contrasting with the activity of interferon-α in a subset of patients with advanced SM. 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, but it was not until 2006 that the in vitro effects of 2CdA on HMC-1 cells were described. While 2CdA alone produced growth-inhibitory effects on HMC-1 cells, the drug was also found to cooperate with midostaurin. 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.

**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 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. In 2003, these findings were confirmed using HMC-1.2 cells and patient-derived KIT D816V-MC. More recently, it was also confirmed that ROSA KIT D816V cells are insensitive to imatinib. Masitinib, another TKI active on KIT WT, although devoid of activity on KIT D816V in vitro, 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. Interestingly, masitinib improved mediator-related symptoms in a subset of patients as compared to placebo-treated patients, regardless of KIT mutational status. This clinical activity was linked to the in vitro inhibitory effects of masitinib on two molecules involved in MC activation, namely LYN and FYN.

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 PDGFRα. 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. 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. However, when evaluated in vivo in clinical trials or in individual SM patients, dasatinib unexpectedly demonstrated only marginal activity. 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. In addition, the drug abrogated KIT phosphorylation in MCL-like cell lines harboring KIT D816V and induced their apoptosis. Moreover, midostaurin suppressed the growth of primary human KIT D816V neoplastic MC. Finally, midostaurin was found to block IgE-dependent histamine release from MC and basophils. Based on these data,

### 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<sup>KIT WT</sup> | MCPV-1 |
|-----|-----------|-------|------------------------|--------|
| CD13| APN       | +     | +                      | +      |
| CD25| IL-3Rα    | -     | -                      | -      |
| CD30| Ki-1      | -     | -                      | -      |
| CD33| SIGLEC-3  | +     | +                      | +      |
| CD34| HPCA-1    | -     | -                      | -      |
| CD44| PGP-1     | +     | +                      | +      |
| CD52| CAMPATH-1 | +/-   | +                      | +      |
| CD67| UPAR      | +     | +                      | +      |
| CD117| KIT     | +     | +                      | +      |
| CD123| IL-3Rα   | -     | +/-                    | -      |

+: strong expression; +/-: weak expression; -: no expression.
clinical trials have been conducted to determine the efficacy of midostaurin in patients with advanced SM, with promising results. 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), EXEL-0862 (a TKI active against fibroblast growth factor receptors, vascular endothelial growth factor receptors, platelet-derived growth factor receptors, FLT3 and KIT), triptolide (a diterpenoid), ponatinib (a multi-kinase blocker), and bosutinib (a LYN/BTK-inhibiting TKI), which was administered to a patient with advanced SM, with no benefit. Nilotinib, which showed some effects in vitro on mutant KIT, was recently administered to 61 SM patients, with transient activity in some patients.

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. In a recent study, it was found that DCC-2618 inhibits proliferation and survival of HMC-1, HMC-1.2 and ROSA KIT D816V cells at IC50<1 μM. BLU-285 is a TKI developed by Blueprint Medicines. At low concentrations, BLU-285 selectively inhibited KIT D816V enzymatic activity (IC50 = 0.27 nM). 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. 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).

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, 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. 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.

Another molecule, STAT5, seems critical for KIT D816V-driven proliferation in MCL-like MC lines as well as in neoplastic MC in SM patients. Chaix et al. reported that the KIT D816V mutant can directly phosphorylate STAT5 in vitro. Interestingly, STAT5 is transcriptionally active in the HMC-1 cell line and in ROSA KIT D816V cells, and drugs targeting STAT5 are effective in decreasing the growth rate of these cells. 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 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, [H]thymidine uptake was measured. Results are expressed as percent of [H]thymidine uptake compared to the control and represent the mean ± standard deviation of three different experiments.
clones. However, the mTOR inhibitor rapamycin induced apoptosis only in HMC-1.2 cells. To support this unexpected selectivity, the authors demonstrated that the phosphorylation of 4E-BP1, a downstream substrate of the mTOR pathway, only in HMC-1.2 cells. 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. 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.

Finally, aberrant accumulation of neoplastic MC in SM might result from deregulation of apoptosis pathways. Indeed, the anti-apoptotic molecules BCL-2, BCL-xL and MCL-1 are overexpressed in KIT D816V neoplastic MC in SM patients, while the expression of the pro-apoptotic molecule BIM is suppressed in these cells. It has also been reported that MCL-1 is detectable in HMC-1.1 and HMC-1.2 cells. 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. 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.

**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. 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, 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 epigenetic 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, CD44, CD52, CD117 and CD123, 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. 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. 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). In addition, brentuximab-vedotin produced apoptosis in primary CD30+ neoplastic MC. 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. Recently, it was reported that the CD52-targeting antibody alemtuzumab counteracts growth of MCPV-1.1 cells. 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. Given that neoplastic (leukemic) stem cells identified in advanced SM may also express CD52, it can be concluded that antibodies or small molecules targeting CD52 might also be of potential interest.

**Figure 3. Dose-dependent inhibition of the proliferation of KIT D816V human mast cell lines by STAT5 inhibitors in vitro**. ROSA<sup>kitD816V</sup> 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 (Ki >25 μM on STAT5), and of more specific and potent STAT5 inhibitors BP-1-102 and BP-1-108 (Ki >10 μM on STAT5). 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<sup>kitD816V</sup> 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.
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. In the light of the revival of gemtuzumab-ozogamicin, its clinical efficacy in patients with acute myeloid leukemia and its effects on neoplastic MC, 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. In addition, highly selective BET bromodomain inhibitors, including JQ1, I-BET151, and I-BET762 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. Independently of the grade or variant of disease, neoplastic MC exhibit nuclear BRD4. However, in ASM and MCL, neoplastic MC also express substantial amounts of cytoplasmic BRD4. In line with this observation, HMC-1 and ROSA cells express cytoplasmic and nuclear BRD4 as well. The KIT-TKI midostaurin and dasatinib suppressed the expression of BRD4 in all MC lines. BRD4-specific short hairpin RNA and JQ1 decreased the proliferation of HMC-1 and ROSA cells. 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, 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. 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 ROSAKIT D816V-Gluc, which naturally secretes Gaussia princeps luciferase (Gluc), used as a reporter. In this study, intravenous injection of NSG mice with ROSAKIT 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). 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-relat-
ed 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-inde-
dependent 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 tri-
als 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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