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# The KIT and PDGFRA switch-control inhibitor DCC-2618 blocks growth and survival of multiple neoplastic cell types in advanced mastocytosis

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## ABSTRACT

Systemic mastocytosis is a complex disease defined by abnormal growth and accumulation of neoplastic mast cells in various organs. Most patients exhibit a D816V-mutated variant of *KIT*, which confers resistance against imatinib. Clinical problems in systemic mastocytosis arise from mediator-related symptoms and/or organ destruction caused by malignant expansion of neoplastic mast cells and/or other myeloid cells in various organ systems. DCC-2618 is a spectrum-selective pan KIT and PDGFRA inhibitor which blocks KIT D816V and multiple other kinase targets relevant to systemic mastocytosis. We found that DCC-2618 inhibits the proliferation and survival of various human mast cell lines (HMC-1, ROSA, MCPV-1) as well as primary neoplastic mast cells obtained from patients with advanced systemic mastocytosis ($IC_{50}$ <1 μM). Moreover, DCC-2618 decreased growth and survival of primary neoplastic eosinophils obtained from patients with systemic mastocytosis or eosinophilic leukemia, leukemic monocytes obtained from patients with chronic myelomonocytic leukemia with or without concomitant systemic mastocytosis, and blast cells obtained from patients with acute myeloid leukemia. Furthermore, DCC-2618 was found to suppress the proliferation of endothelial cells, suggesting additional drug effects on systemic mastocytosis-related angiogenesis. Finally, DCC-2618 was found to downregulate IgE-mediated histamine release from basophils and tryptase release from mast cells. Together, DCC-2618 inhibits growth, survival and activation of multiple cell types relevant to advanced systemic mastocytosis. Whether DCC-2618 is effective *in vivo* in patients with advanced systemic mastocytosis is currently under investigation in clinical trials.

## Introduction

Systemic mastocytosis (SM) is a hematopoietic neoplasm with complex biology and pathology, and a variable clinical course.[1-7] The disease is characterized by abnormal expansion and accumulation of neoplastic mast cells (MC) in one or more

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internal organs, including the bone marrow.[1-3] Various types of SM have been recognized by the World Health Organization (WHO).[8-11] The indolent variant of SM is associated with 'hematologic stability' and thus with an almost normal life expectancy.[12-14] By contrast, the prognosis in patients with advanced SM, including SM with an associated hematologic neoplasm (AHN), aggressive SM (ASM) and MC leukemia (MCL) is unfavorable, with short survival times and poor responses to conventional therapy.[1-5,12,13,15] Current research is, therefore, focusing on therapeutic targets and the effects of novel antineoplastic drugs on various cell types relevant to advanced SM.[16] Since most patients with SM also suffer from mediator-related symptoms that may sometimes be severe or even life-threatening, such drugs are often selected based on their dual effects on MC growth and MC activation.

Most patients with SM express the D816V-mutated variant of the stem cell factor receptor, KIT, which mediates ligand-independent activation and autonomous growth and differentiation of MC.[17-22] The D816V *KIT* point mutation also confers resistance against several tyrosine kinase inhibitors, including imatinib.[23-26] Novel kinase blockers acting on KIT D816V have, therefore, been developed. The highlighting example is midostaurin (PKC412).[27,28] However, despite superior clinical efficacy seen in a global phase II trial,[28] patients with advanced SM often exhibit or acquire resistance.[28,29] A number of different mechanisms may underlie resistance against midostaurin. One obvious problem is that the drug does not suppress all clinically relevant sub-clones and cell-types, especially cells lacking KIT D816V.[28,29] Such sub-clones are often seen in the context of advanced SM. Over 50% of these patients have or develop an AHN.[30-32] Of these patients with an AHN, approximately 80-90% have an associated myeloid neoplasm, the most frequent ones being chronic myelomonocytic leukemia (CMML) and acute myeloid leukemia (AML).[8-11,30-32] In these patients, leukemic expansion of monocytes and/or blast cells is typically found. In other patients, an expansion of eosinophils, sometimes resembling chronic eosinophilic leukemia (SM-CEL), is found. In most of these patients, eosinophils display *KIT* D816V.[33] By contrast, expression of rearranged *PDGFR* variants is rarely seen in SM, although in some patients with a *FIP1L1/PDGFRA* fusion gene, the MC expansion has a histopathological picture indistinguishable from that of SM.[34] Treatment of SM-AHN represents a clinical challenge because the AHN-component is often resistant.[16,32]

DCC-2618 is a switch-control type II inhibitor of KIT, which arrests KIT in an inactive state, regardless of activating mutations, such as KIT D816V.[35] Moreover, several additional oncogenic kinases, including FLT-3, PDGFRA, PDGFRB, KDR, TIE2 and FMS are recognized by DCC-2618.[35] Recently, the first clinical trials with DCC-2618 (NCT02571036) were started in patients with kinase-driven malignancies. In addition, first preclinical studies have shown that DCC-2618 may exert antineoplastic effects on neoplastic MC.[36]

In our current study, we show that DCC-2618 is a potent inhibitor of growth and survival of neoplastic human MC expressing various *KIT* mutations. Furthermore, we show that DCC-2618 produces growth inhibition and apoptosis in other cell types that play a role in advanced SM. Finally, we show that DCC-2618 inhibits IgE-dependent histamine secretion from basophils and tryptase secretion from MC. All in all, our data suggest that DCC-2618 is a promising, novel drug for the treatment of advanced SM.

## Methods

### Reagents

The reagents used in this study are described in the *Online Supplement*. DCC-2618 and its active metabolite, DP-5439, were kindly provided by Dr. B. Smith (Deciphera Pharmaceuticals LLC, Lawrence, KS, USA).

### Isolation of primary neoplastic cells

Primary neoplastic cells were isolated from bone marrow samples of 11 patients with SM. The bone marrow cells were obtained during routine diagnostic investigations after written

**Table 1.** Characteristics of patients with systemic mastocytosis and response of neoplastic cells to DCC-2618 and DP-5439.

| Patient n. | Age | m/f | Diagnosis, SM variant | *KIT* D816V | Serum tryptase ng/mL | BM MC infiltration % | % MC in MNC | DCC-2618 $IC_{50}$ | DP-5439 $IC_{50}$ | PKC412 $IC_{50}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| #1 | 68 | m | ISM | + | 83.3 | n.a. | 1 | 114 nM | 414 nM | 56 nM |
| #2 | 56 | m | ISM | + | 103 | 20 | 5 | 240 nM | 390 nM | 35 nM |
| #3 | 49 | f | ISM | - | 22.9 | 10 | n.a. | 198 nM | 554 nM | n.a. |
| #4 | 66 | m | ISM-MPN-eo | + | 170 | 5 | n.a. | 394 nM | 1481 nM | n.a. |
| #5 | 82 | f | SSM | + | 284 | 50 | n.a. | 347 nM | 1584 nM | n.a. |
| #6 | 57 | f | ASM | + | 87.9 | 50 | n.a. | 331 nM | 366 nM | n.a. |
| #7 | 90 | m | ASM | + | 125 | 20 | 25 | 386 nM | 679 nM | 360 nM |
| #8 | 63 | m | ASM-AML | + | 33.9 | 15 | 8 | 393 nM | 554 nM | 66 nM |
| #9 | 65 | m | ASM-CMML | + | 220 | 50 | 30 | 460 nM | 907 nM | n.a. |
| #10 | 78 | m | ASM-MPN-eo | + | 45.9 | 15 | 5 | 83 nM | 64 nM | 114 nM |
| #11 | 91 | m | MCL | + | 330 | 20 | 50 | 321 nM | 592 nM | 65 nM |

Diagnoses were established according to WHO criteria. Primary bone marrow cells were incubated with various concentrations of DCC-2618, DP-5439 or midostaurin (PKC412) at 37°C for 48 h. Proliferation was then determined by measuring uptake of $^3$H-thymidine and $IC_{50}$ values were calculated. WHO: World Health Organization; m: male; f: female; SM: systemic mastocytosis; PB, peripheral blood; BM, bone marrow; MC: mast cells; MNC, mononuclear cells, nM, nanomolar; n.a., not available; $IC_{50}$, half maximal inhibitory concentration; ISM: indolent SM; MPN: myeloproliferative neoplasms; SSM: smoldering SM; ASM: aggressive SM; CMML: chronic monomyelocytic leukemia; MCL: mast cell leukemia.

informed consent had been given. Patients were classified as having indolent SM (ISM; n=3), smoldering SM (SSM; n=1), ASM (n=2), SM-AHN (n=4) and MCL (n=1) according to WHO criteria.[8-11] In addition, neoplastic cells were obtained from ten patients suffering from CMML, ten with AML and three with hypereosinophilia. The patients' characteristics are shown in Table 1 (SM patients) and *Online Supplementary Table S1* (other hematologic disorders). Heparinized bone marrow cells were layered over Ficoll to isolate mononuclear cells. The study was approved by the ethics committee of the Medical University of Vienna.

### Culture of human cell lines

The following human MCL-like cell lines were employed in this study: HMC-1.1 and HMC-1.2,[37] three ROSA sub-clones (ROSA$^{KIT\ WT}$, ROSA$^{KIT\ D816V}$, ROSA$^{KIT\ K509I}$)[38] and four MCPV-1 sub-clones (MCPV-1.1, MCPV-1.2, MCPV-1.3, MCPV-1.4).[39] In addition, we examined several AML cell lines, the CEL-related cell line EOL-1, the microvascular endothelial cell line HMEC-1, and cultured human umbilical vein endothelial cells (HUVEC). A description of cell lines is provided in the *Online Supplement*.

### Evaluation of growth, survival of neoplastic cells

Drug-exposed cells (cell lines or primary cells) were analyzed for proliferation and survival. The bioassays employed are described in the *Online Supplementary Methods*.

### Western blotting

For evaluation of KIT and BTK signaling, HMC-1.1, HMC-1.2, ROSA$^{KIT\ WT}$ and ROSA$^{KIT\ D816V}$ cells were incubated in control medium or in DCC-2618 (0.5-5 μM) for 4 h at 37°C. Western blotting was performed essentially as described elsewhere.[26,40] For evaluation of downstream signaling pathways of KIT, HMC-1.1, HMC-1.2, ROSA$^{KIT\ WT}$ and ROSA$^{KIT\ D816V}$ cells were first pre-incubated overnight in Iscove modified Dulbecco medium devoid of fetal calf serum and of stem cell factor. Cells ($10^6$) from each line were then treated with DCC-2618 (0.001-10 μM) for 90 min at 37°C. At the end of the treatment, ROSA$^{KIT\ WT}$ cells were stimulated with stem cell factor-containing supernatants (10%) of Chinese hamster ovary cells transfected with the murine *scf* (*kl*) gene (CHO-KL) at room temperature for 10 min. Thereafter, Western blotting was performed essentially as described previously.[26,40] Antibodies against phosphorylated (p)Kit, STAT5, pSTAT5, ERK1/2, pERK1/2 were purchased from Cell Signaling (Danvers, MA, USA), antibodies against pBTK were bought from NovusBiologicals (Littleton, CO, USA) and antibodies against total KIT and total BTK were from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

### Measurement of mediator release

Drug-exposed cells (blood basophils obtained from healthy individuals and HMC-1 cells) were analyzed for histamine- and tryptase release as described in the *Online Supplementary Methods*.

### Evaluation of apoptosis in basophils

Drug-exposed blood basophils obtained from healthy donors by dextran sedimentation were analyzed for cell survival by flow cytometry. Technical details are described in the *Online Supplementary Methods*.

### Statistical analysis

To determine the level of significance the Student *t*-test was applied. Results were considered to be statistically significantly different when $P$ was <0.05.

## Results

### DCC-2618 and its metabolite DP-5439 inhibit proliferation of neoplastic mast cells

DCC-2618 and its active metabolite, DP-5439 were found to inhibit $^3$H-thymidine uptake and thus proliferation in a dose-dependent manner in all MC lines tested, with slightly lower $IC_{50}$ values obtained in HMC-1.1 cells lacking KIT D816V and ROSA$^{KIT\ WT}$ cells compared to the KIT D816V-positive cell lines HMC-1.2 and ROSA$^{KIT\ D816V}$ (Figure 1A and Table 2). $IC_{50}$ values obtained in HMC-1.1 cells with DCC-2618 were also lower than $IC_{50}$ values obtained with midostaurin.[25,26] In addition, DCC-2618 and DP-5439 were found to inhibit proliferation of ROSA$^{KIT\ K509I}$ cells with lower $IC_{50}$ values (DCC-2618, $IC_{50}$: 34±10 nM) compared to ROSA$^{KIT\ D816V}$ cells (Figure 1A). Unexpectedly, DCC-2618 and its metabolite also produced growth-inhibition in the multi-resistant MC lines MCPV-1.1, MCPV-1.2, MCPV-1.3 and MCPV-1.4 (Figure 1B and Table 2). Finally, we were able to show that DCC-2618 and DP-5439 induced dose-dependent inhibition of growth of primary neoplastic bone marrow cells obtained from patients suffering from various forms of SM, including ASM and MCL (Figure 1C, Table 1). Interestingly, the effects of DCC-2618 on primary neoplastic BM cells and the related $IC_{50}$ values obtained in different SM variants were comparable (Figure 1C, Table 1).

### DCC-2618 inhibits KIT, STAT5, AKT, and ERK activation in neoplastic mast cells

As expected, DCC-2618 was found to suppress phosphorylation of KIT in ROSA$^{KIT\ WT}$ and ROSA$^{KIT\ D816V}$ cells as well as in both HMC-1 sub-clones (Figure 2A). In addition, DCC-2618 was found to decrease the expression of phosphosphorylated (p)STAT5, pAKT and pERK1/2 in all cell lines tested (Figure 2B). As expression levels of pSTAT5 in

**Table 2. Effects of DCC-2618 and DP-5439 on growth of various human cell types.**

| Cell line / cell type | DCC-2618, $IC_{50}$ | DP-5439, $IC_{50}$ |
|---|---|---|
| HMC-1.1 | 12.3±3.7 nM | 13±4 nM |
| HMC-1.2 | 123±36 nM | 96±23 nM |
| ROSA KIT WT | 41±5 nM | 56±21 nM |
| ROSA KIT D816V | 168±65 nM | 188±60 nM |
| ROSA KIT K509I | 34±10 nM | 28±13 nM |
| MCPV-1.1 | 298±77 nM | 357±179 nM |
| MCPV-1.2 | 1000±932 nM | 913±378 nM |
| MCPV-1.3 | 724±511 nM | 756±435 nM |
| MCPV-1.4 | 670±418 nM | 697±410 nM |
| MOLM-13 | 132±95 nM | 73±31 nM |
| MV4-11 | 147±88 nM | 76±24 nM |
| KG-1 | 2.7±3.7 μM | 5.2±4.4 μM |
| U937 | 7.8±5.4 μM | 2.5±0.3 μM |
| EOL-1 | 1.8±1.3 nM | 1±0.8 nM |
| HMEC-1 | 3.7±2.2 μM | 2±1.3 μM |
| HUVEC | 707±224 nM | 605±222 nM |

Cell lines were incubated with various concentrations of DCC-2618 or DP-5439 at 37°C for 48 h. Then, proliferation was determined by measuring uptake of $^3$H-thymidine and $IC_{50}$ values were calculated. Values represent the mean±S.D. from three independent experiments. $IC_{50}$: half maximal inhibitory concentration.

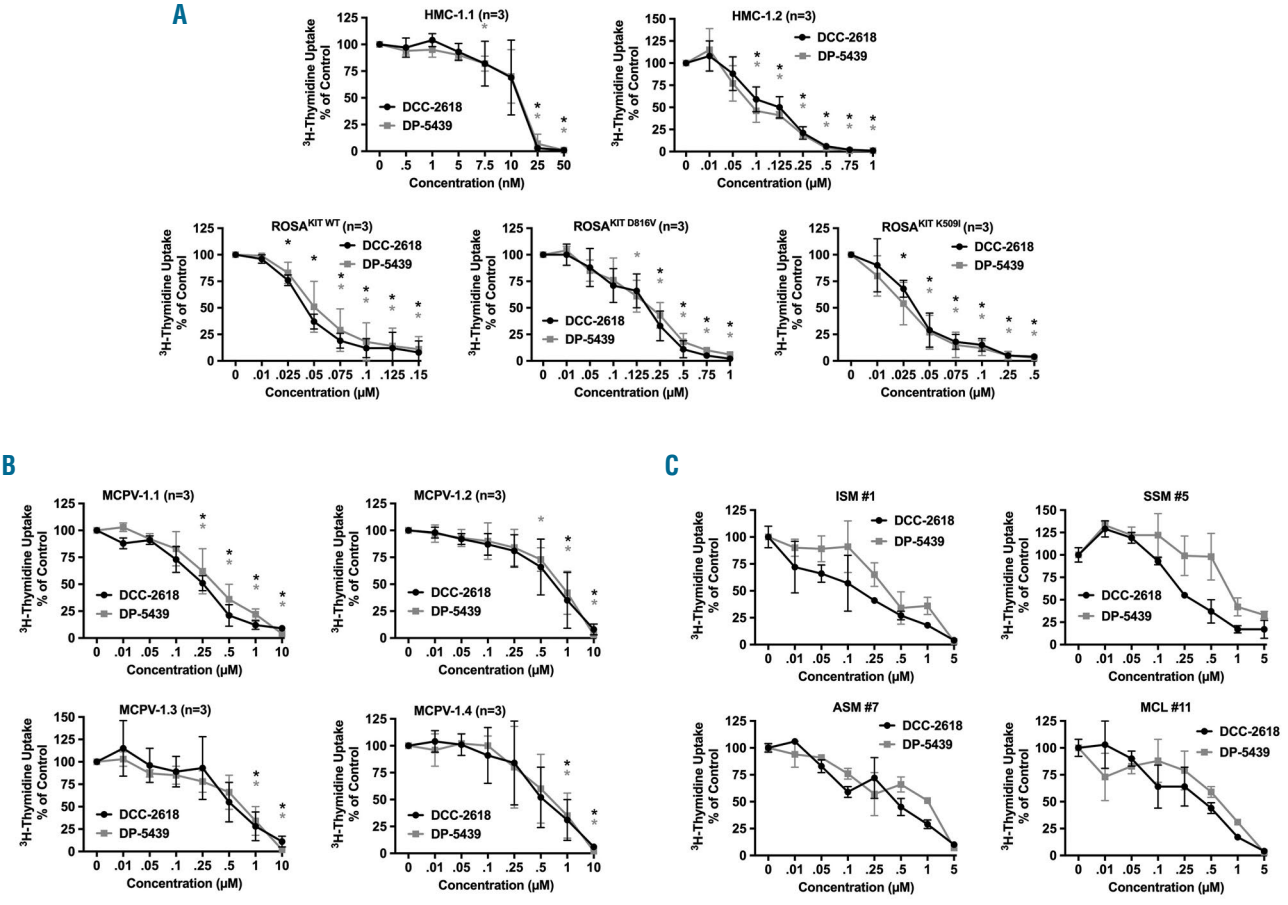
HMC-1.1 cells were rather low and difficult to quantify by Western blotting, we also performed intracellular flow cytometry-staining experiments using an antibody against pSTAT5. In these experiments, DCC-2618 was found to counteract pSTAT5 expression in HMC-1.1 and HMC-1.2 cells in a dose-dependent manner (*Online Supplementary Figure S1*). DCC-2618 did not inhibit phosphorylation of BTK, another important target of tyrosine kinase inhibitors expressed by neoplastic MC (Figure 2C).

## DCC-2618 induces apoptosis in neoplastic mast cells

To explore the mechanism of drug action, we analyzed the effects of DCC-2618 on the survival of neoplastic MC. As assessed by light microscopy, DCC-2618 induced apoptosis in HMC-1.1, HMC-1.2, ROSA$^{KIT\ WT}$, ROSA$^{KIT\ D816V}$ and ROSA $^{KIT\ K509I}$ cells in a dose-dependent manner (Figure 3A). The effects of DCC-2618 on survival were more pronounced in KIT D816V-negative MC lines than in KIT D816V-positive MC lines (Figure 3A). DCC-2618 was also found to produce apoptosis in the multi-resistant MCPV-1 cell lines (Figure 3B). The apoptosis-inducing effect of DCC-2618 on MC was confirmed by combined annexin V/propidium-iodide staining (Figure 3A,B and *Online Supplementary Figure S2A,B*). The metabolite DP-5439 was found to be equally effective in producing apoptosis in MC lines compared to DCC-2618 (Figure 3A,B and *Online Supplementary Figure S2A,B*). Together, these data show that DCC-2618 is a novel potent anti-neoplastic compound inducing apoptosis and growth arrest in neoplastic MC.

## DCC-2618 produces synergistic growth-inhibitory effects with midostaurin and cladribine (2CdA) in neoplastic mast cells

In advanced SM, drug combinations may be required to suppress malignant cell growth. We found that DCC-2618 and midostaurin produce clear cooperative (synergistic) growth-inhibitory effects in HMC-1.1 cells (*Online Supplementary Figure S3A,C*). In HMC-1.2 cells, the drug combination also produced cooperative antineoplastic effects, but these effects were additive rather than synergistic as defined by Calcusyn software (*Online Supplementary Figure S3A,C*). In addition, we found that DCC-2618 and 2CdA induce clear synergistic growth-inhibitory effects on HMC-1.1 and HMC-1.2 cells (*Online Supplementary Figure S3B,D*).



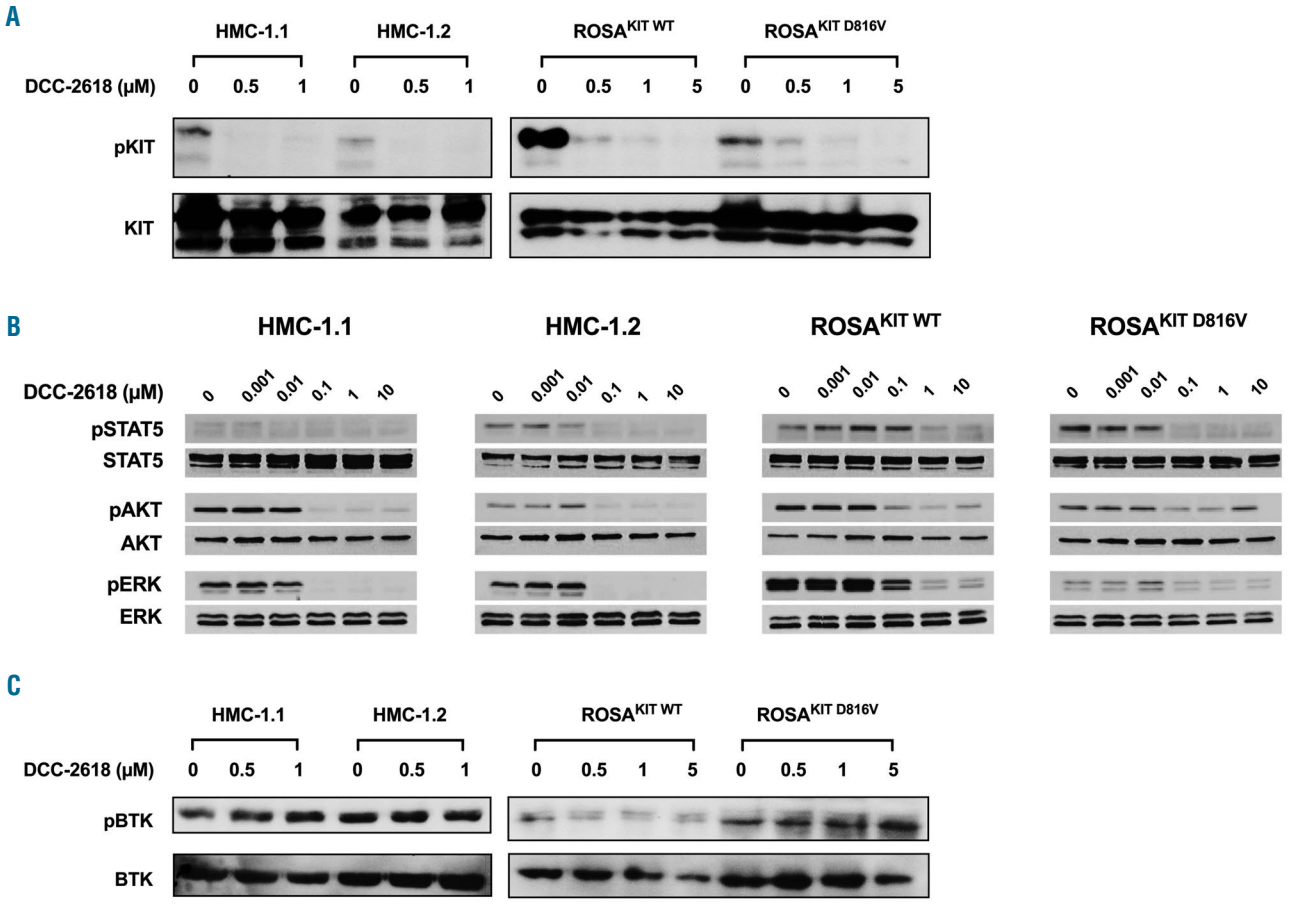

**Figures 1. DCC-2618 and its active metabolite DP-5439 inhibit proliferation of neoplastic mast cells.** HMC-1, ROSA (A), MCPV-1 (B) and primary neoplastic mast cells (C) obtained from patients with different variants of systemic mastocytosis (ISM, SSM, ASM, MCL) were incubated in control medium (0 nM) or medium containing various concentrations of DCC-2618 or DP-5439, as indicated, at 37°C for 48 h. Thereafter, $^{3}$H-thymidine uptake was measured. Results in (A) and (B) are expressed as percent of control and represent the mean±S.D. from three independent experiments. Results in (C) are expressed as percent of control and represent mean±S.D from triplicates. Asterisk (*): $P$<0.05 compared to control medium.

### DCC-2618 inhibits IgE-dependent histamine release from basophils and spontaneous tryptase release from neoplastic mast cells

Since patients with SM often suffer from symptoms caused by mediators released from neoplastic MC and/or basophils, we evaluated the effect of DCC-2618 on anti-IgE-induced histamine release. We found that DCC-2618 (0.1-1.0 μM) slightly inhibited anti-IgE mediated histamine release from normal human blood basophils (Figure 4A). This drug effect was found to be specific in that DCC-2618 did not inhibit C5a- or calcium ionophore-induced histamine release from basophils (*Online Supplementary Figure S4A*). As expected, DCC-2618 did not affect the viability of basophils between 0.1 and 1.0 μM and did not induce histamine secretion within 30 min of incubation (*Online Supplementary Figure S4B*). In consecutive experiments, we also found that DCC-2618 suppresses the spontaneous (baseline) secretion of tryptase from HMC-1.1 and HMC-1.2 cells during the entire incubation period (days 1 through 6) (Figure 4B).

### DCC-2618 counteracts growth and survival of leukemic monocytes and blast cells

We next explored the effects of DCC-2618 on AHN cell-types. In a first step, we examined AML cell responses. DCC-2618 was found to inhibit the proliferation of all AML cell lines tested, with considerably lower $IC_{50}$ values obtained with the *FLT3*-mutated cell lines MOLM-13 (132±95 nM) and MV4-11 (147±88 nM) compared to KG-1 and U937 cells (Table 2, Figure 5A). Similar effects were seen with DP-5439 (Figure 5A). DCC-2618 was also found to induce apoptosis in MOLM-13, MV4-11 and KG-1 cells (Figure 5B and *Online Supplementary Figure S5*). Finally, we found that DCC-2618 and DP-5439 produced dose-dependent inhibition of growth in primary leukemia cells obtained from patients with AML or CMML (*Online Supplementary Table S1* and Figure 5C). In one patient with ASM-CMML, we isolated mononuclear cells and found that DCC-2618 and DP-5439 induced growth inhibition of these cells in the same way as in mononuclear cells obtained from patients with CMML without SM (Table 1

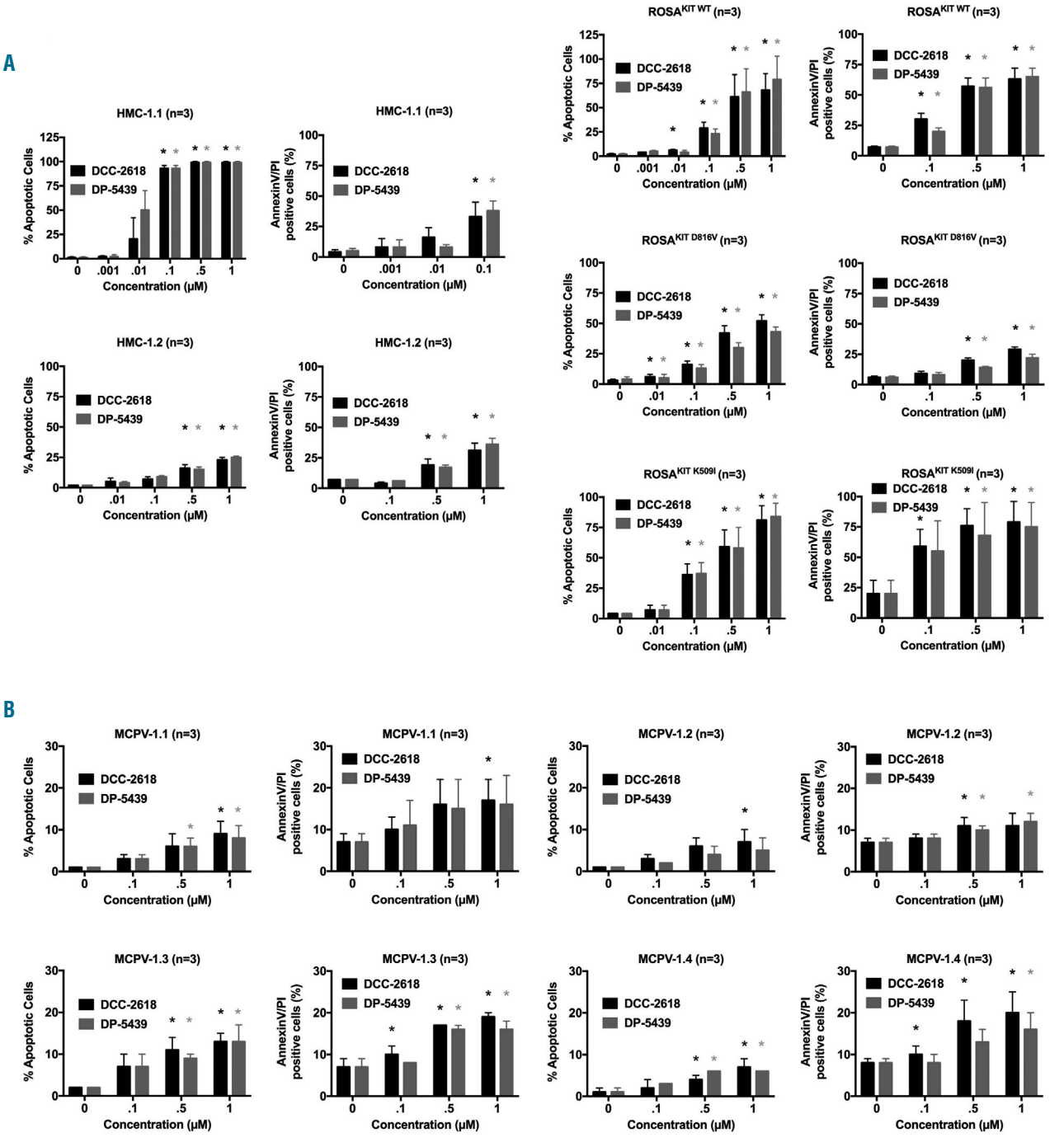



**Figures 2. DCC-2618 inhibits phosphorylation of KIT and other targets in neoplastic mast cells.** (A,C) HMC-1 and ROSA cells were incubated in control medium (ROSA$^{KIT\ WT}$: Iscove modified Dulbecco medium (IMDM) with stem cell factor, SCF; ROSA$^{KIT\ D816V}$: IMDM without SCF) or medium containing various concentrations of DCC-2618, as indicated, at 37 °C for 4 h. Thereafter, cells were harvested and Western blotting was performed as described in the text using antibodies against phosphorylated (p)KIT, total KIT, pBTK and total BTK. (B) HMC-1 and ROSA cells were first pre-incubated overnight in IMDM devoid of fetal calf serum and of SCF. Cells were then treated with DCC-2618 (0.001-10 μM) for 90 min at 37 °C. At the end of the treatment, ROSA$^{KIT\ WT}$ cells were stimulated with SCF (10% CHO-KL) at room temperature for 10 min. Thereafter, cells were harvested and Western blotting was performed as described in the text using antibodies against pSTAT5, total STAT5, pAKT, total AKT, pERK1/2, total ERK1/2. Western blot experiments were performed at least twice. Western blots in this figure show one representative experiment.

and Figure 5C). Together, these data suggest that DCC-2618 counteracts growth of AHN cells, including CMML monocytes and AML blasts.

### DCC-2618 inhibits the proliferation of neoplastic eosinophils

Advanced SM is often accompanied by eosinophilia. In addition PDGFRA is a known target of DCC-2618. We analyzed the effects of DCC-2618 on proliferation and survival of the FIP1L1-PDGFRA (F/P) positive EOL-1 cell line. DCC-2618 was found to inhibit proliferation in EOL-1 cells at low nanomolar range of concentrations ($IC_{50}$: 1.8±1.3 nM) (*Online Supplementary Figure S6A*). Similar effects were seen with DP-5439 (*Online Supplementary Figure S6A*). DCC-2618 also induced apoptosis in EOL-1 cells (*Online Supplementary Figure S6C*). Next, we examined the effects of DCC-2618 on growth of primary eosinophils. In these experiments, DCC-2618




**Figures 3. DCC-2618 and DP-5439 induce apoptosis in neoplastic mast cells.** HMC-1, ROSA (A) and MCPV-1 (B) were incubated in control medium (0 μM) or medium containing various concentrations of DCC-2618 and DP-5439, as indicated, at 37 °C for 48 h. Cells were then harvested and the percentage of apoptotic cells was quantified morphologically on Wright-Giemsa-stained cytospin preparations (left panels) or by flow cytometry (determination of annexinV/PI-positive cells, right panels). Results represent the mean±S.D. of three independent experiments. Asterisk (*): $P<0.05$ compared to control medium.

was found to inhibit the proliferation of neoplastic bone marrow cells obtained from a patient with ASM (Table 1 and *Online Supplementary Figure S6B*). In addition, DCC-2618 was found to block the growth of bone marrow cells obtained from patients with secondary hypereosinophilic syndromes (*Online Supplementary Table S1* and *Online Supplementary Figure S6B*).

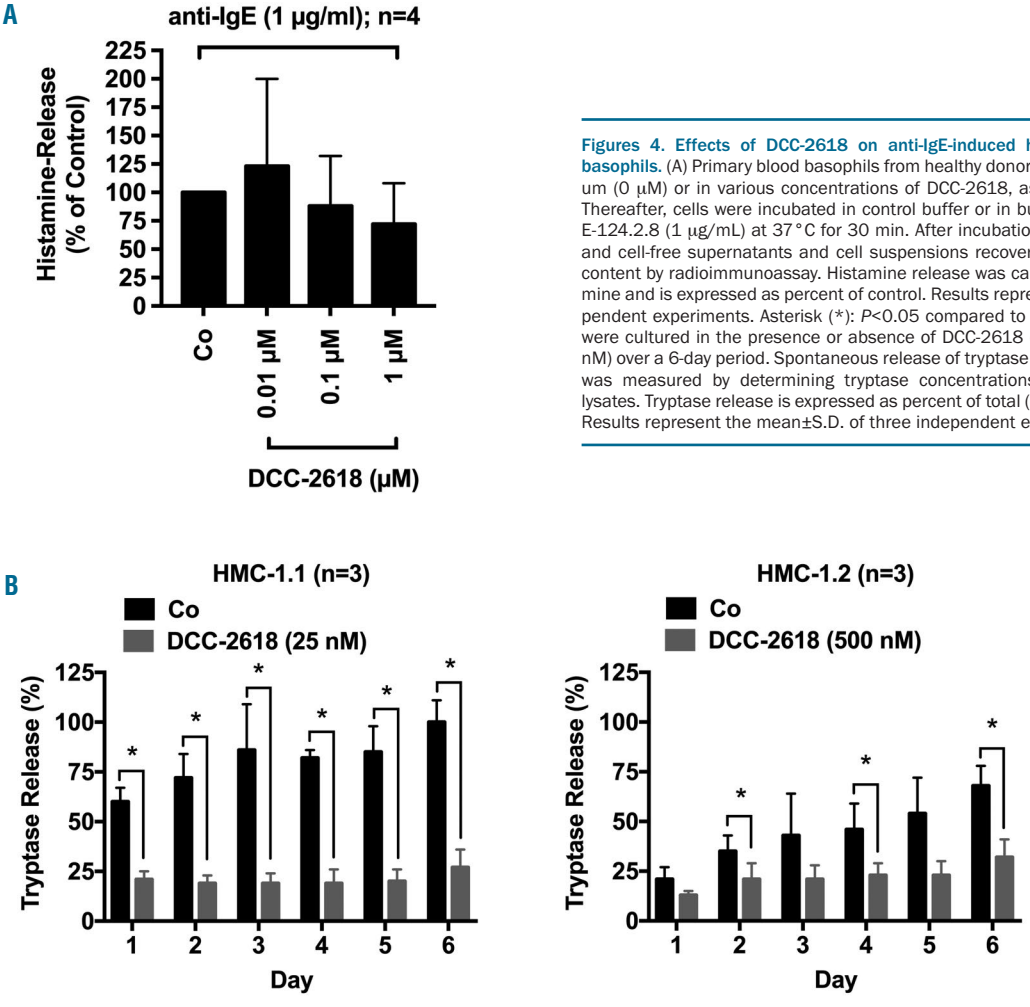
### DCC-2618 inhibits growth of human endothelial cells

Increased bone marrow angiogenesis has been implicated in the pathogenesis of SM.[41] To investigate potential effects of DCC-2618 on angiogenesis, we explored drug effects on growth of HUVEC and the microvascular endothelial cell line HMEC-1. As assessed by $^{3}$H-thymidine uptake, DCC-2618 and its metabolite were found to inhibit the proliferation of HUVEC and HMEC-1 cells in a dose-dependent manner (*Online Supplementary Figure S7*). DCC-2618 exerted stronger effects on HUVEC (707±224 nM) than on HMEC-1 cells (3.7±2.2 μM).

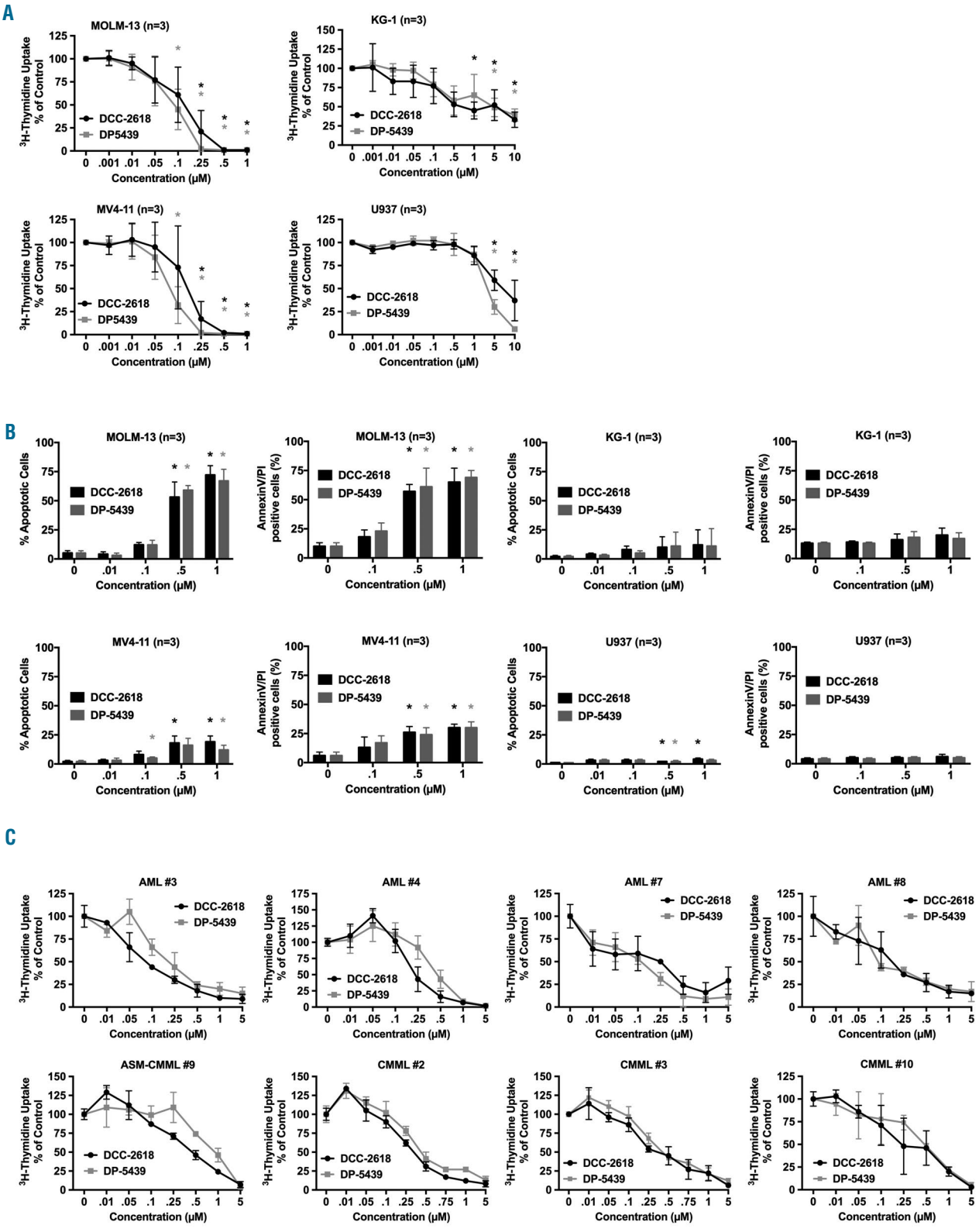
## Discussion

Due to the poor response to conventional drugs, treatment of patients with advanced SM is still a major challenge in clinical practice. Despite the availability of new drugs the prognosis of these patients remains poor with short survival times.[10,16,28,29] Research is, therefore, seeking new effective drugs and novel treatment concepts. DCC-2618 is a novel switch-control type II blocker that exerts inhibitory effects on KIT D816V, other KIT mutants, and several other critical target kinases, such as FLT3, PDGFRA and KDR.[35] We here describe that DCC-2618 inhibits the proliferation of nine different human MC lines, with lower $IC_{50}$ values obtained in HMC-1.1 cells and ROSA$^{KIT\ WT}$ cells than in KIT D816V-positive HMC-1.2 and ROSA$^{KIT\ D816V}$ cells. In addition, DCC-2618 was found to block the proliferation of primary neoplastic MC obtained from patients with ASM or MCL. Moreover, DCC-2618 exerted major antineoplastic effects on AHN cells and endothelial cells, all of which may be relevant in the pathogenesis of advanced SM. Based on these observations DCC-2618 is a novel emerging drug candidate for advanced SM. Indeed, clinical trials with DCC-2618 have been started recently.

The multi-kinase inhibitor midostaurin (PKC412) is effective against the D816V-mutated variant of *KIT* and has shown promising results in patients with advanced SM in a global phase II trial, with an overall response rate of 60%.[28] In addition, midostaurin was found to suppress mediator-related symptoms and IgE-dependent histamine release from basophils[28,42] However, despite clinical effi-




**Figures 4. Effects of DCC-2618 on anti-IgE-induced histamine release from normal basophils.** (A) Primary blood basophils from healthy donors were incubated in control medium (0 μM) or in various concentrations of DCC-2618, as indicated, at 37°C for 30 min. Thereafter, cells were incubated in control buffer or in buffer containing anti-IgE antibody E-124.2.8 (1 μg/mL) at 37°C for 30 min. After incubation, cells were centrifuged at 4°C, and cell-free supernatants and cell suspensions recovered and examined for histamine-content by radioimmunoassay. Histamine release was calculated as percent of total histamine and is expressed as percent of control. Results represent the mean±S.D. of four independent experiments. Asterisk (*): $P<0.05$ compared to control medium. (B) HMC-1 cells were cultured in the presence or absence of DCC-2618 (HMC-1.1: 25 nM; HMC-1.2: 500 nM) over a 6-day period. Spontaneous release of tryptase from HMC-1.1 and HMC-1.2 cells was measured by determining tryptase concentrations in cell-free supernatants and lysates. Tryptase release is expressed as percent of total (intra- and extra-cellular) tryptase. Results represent the mean±S.D. of three independent experiments. Asterisk (*): $P<0.05$.




**Figures 5. Effects of DCC-2618 and DP-5439 on proliferation and survival of acute myeloid leukemia (AML) and chronic myelomonocytic leukemia (CMML).** (A,C) MOLM-13, MV4-11, KG-1, U937 and primary leukemic cells were incubated in control medium (0 μM) or medium containing various concentrations of DCC-2618 or DP-5439, as indicated at 37°C for 48 h. Thereafter, $^3$H-thymidine uptake was determined. Results in (A) are expressed as percent of control and represent the mean±S.D. from three independent experiments. Asterisk (*): $P<0.05$ compared to control medium. Results of (C) are expressed as percent of control and represent the mean±S.D. from triplicates. (B) MOLM-13, MV4-11, KG-1 and U937 cells were incubated with control medium (0 nM) or various concentrations of DCC-2618 and DP-5439, as indicated, for 48 h. Thereafter cells were harvested and the percentage of apoptotic cells was quantified morphologically on Wright-Giemsa-stained cytospin preparations (left panels) or by flow cytometry (determination of annexinV/PI-positive cells, right panels). Results represent the mean±S.D. of three independent experiments. Asterisk (*): $P<0.05$ compared to control medium.

cacy, midostaurin is unable to produce long-lasting complete remission in all patients.[28] Therefore, new drugs and drug-combinations are currently being tested in the context of advanced SM. DCC-2618 might be a promising candidate for several reasons. First, DCC-2618 exhibits a broad target profile and is able to block growth of various neoplastic cells.[36] In the current study, DCC-2618 was found to block growth of neoplastic cells obtained from patients with ASM and MCL. In addition, the drug produced growth inhibition in all MCL-like cell lines tested, including KIT-mutated cells and cell lines in which other oncogenic pathways (such as the RAS pathway) trigger malignant cell growth. Moreover, unlike other KIT-targeting drugs, DCC-2618 is able to suppress the growth and survival of other cell types relevant to advanced SM and AHN, including monocytes, blast cells, neoplastic eosinophils and endothelial cells. The concentrations required to mediate these cellular inhibitory effects are readily achievable based on the recent report of clinical exposure of 5 μM or higher in patients with gastrointestinal stroma tumors.[43]

After intake, DCC-2618 is considered to be converted to one active metabolite, DP-5439. We therefore investigated whether DP-5439 is also able to counteract growth and survival of neoplastic cells. In these experiments, we were able to show that DP-5439 is able to suppress growth and survival of neoplastic MC and of other leukemic (non-MC-lineage) cells in the same way (and with comparable $IC_{50}$ values) as DCC-2618. These data suggest that DCC-2618 treatment should be effective even if the DP-5439 metabolite may accumulate over time.

It is well known that about one-third of all patients with advanced SM have an AHN at diagnosis. Of these patients, most have a myeloid neoplasm, often in the form of CMML or AML.[2-8,13,32] The treatment of these SM-AHN patients is a clinical challenge because the AHN is often drug-resistant. In fact patients with SM-AHN still have a poor prognosis with an overall survival time of about 24 months.[13,32] Because of its broad activity profile, we asked whether DCC-2618 might be a promising agent for patients with SM-AHN. In a first step, we found that DCC-2618 is a potent inhibitor of proliferation and survival of the FLT3-mutated AML cell lines MOLM-13 and MV4-11. DCC-2618 also inhibits the growth of other AML cell lines examined (KG-1 and U937), but at $IC_{50}$ values considerably higher than those for MOLM-13 or MV4-11 cells. We also found that DCC-2618 counteracts proliferation of primary leukemic cells obtained from patients with SM-AHN, AML or CMML (Table 1 and *Online Supplementary Table S1*). These findings suggest that DCC-2618 may be a promising agent for SM-AHN.

In SM patients, disease progression is often accompanied by expansion of neoplastic eosinophils, sometimes even resembling (chronic) eosinophilic leukemia. In most cases the eosinophils are of clonal origin as they express KIT D816V.[33] In rare cases, neoplastic eosinophils display the *FIP1L1/PDGFRA* fusion gene.[34] However, this fusion gene is usually detectable only in eosinophilic neoplasms, such as CEL. Since DCC-2618 is known to exert inhibitory effects against PDGFRA[35] we examined its effects on EOL-1 cells harboring FIP1L1-PDGFRA. DCC-2618 was found to exert strong anti-proliferative and apoptosis-inducing effects in EOL-1 cells, with $IC_{50}$ values in the low nanomolar range. In addition, DCC-2618 was found to inhibit growth of primary eosinophils obtained from patients with KIT D816V-positive SM or reactive hypereosinophilia. Together, these data suggest that DCC-2618 inhibits multiple AHN-related cell types, which may be relevant clinically as progression of SM is often accompanied by multilineage expansion of various sub-clones, including cells harboring or lacking KIT D816V.[15,28,29]

A number of different pro-oncogenic pathways and targets may be involved in KIT D816V-dependent expansion and accumulation of MC in advanced SM.[25,40,44-50] Several of these target pathways may be sensitive to therapy with tyrosine kinase inhibitors. We studied whether key target pathways in neoplastic MC can be disrupted by DCC-2618. As assessed by Western blotting, DCC-2618 was found to block the phosphorylation and thus activation of wild-type KIT and KIT D816V. In addition, we were able to show that DCC-2618 blocks the activation of AKT, ERK and STAT5, suggesting that multiple target pathways are accessible to this drug. By contrast, however, the drug did not disrupt activation of BTK, another important target displayed by neoplastic MC.[46]

Since the target spectrum of midostaurin (PKC412) and DCC-2618 is not identical, we were also interested to learn whether DCC-2618 and midostaurin can produce synergistic antineoplastic effects on neoplastic MC. Indeed, we found that both drugs induce cooperative or even synergistic growth-inhibitory effects on HMC-1.1 and HMC-1.2 cells.

Specific alterations in the microenvironment, including increased angiogenesis, are frequently detectable in advanced bone marrow neoplasms and are often considered to play an important role in disease progression. A typical finding in the affected bone marrow of patients with advanced SM is increased microvessel density.[41] We found that DCC-2618 inhibits the proliferation of human endothelial cells, including HUVEC and a microvascular endothelial cell line, HMEC-1. These data suggest that DCC-2618 also acts as an anti-angiogenic agent. Interestingly, the $IC_{50}$ values obtained for HMEC-1 cells were higher than those for HUVEC, which may be explained by the fact that HMEC-1 is a cell line, whereas HUVEC are primary cells. An alternative explanation would be the lack of key targets in HMEC-1 cells. Indeed, it is well known, that KDR, a key target of DCC-2618, is only expressed in HUVEC but not in HMEC-1 cells.

Patients with SM frequently suffer from symptoms produced by MC-derived mediators.[4-6,16] These mediators are released on IgE-dependent activation of MC and may cause severe problems or even lead to life-threatening anaphylaxis. Concomitant (IgE-dependent) allergies are, therefore, relevant comorbidities in the context of SM. We found that DCC-2618 counteracts IgE-dependent secretion of histamine from basophils obtained from healthy donors. In addition, we were able to show that DCC-2618 blocks IgE-independent, spontaneous release of tryptase from HMC-1.1 and HMC-1.2 cells *in vitro*. These results suggest that, apart from its antineoplastic effects, DCC-2618 might also have an impact on mediator release (and probably on the resulting symptoms) in patients with SM with concomitant allergies. Whether these data can be reproduced *in vivo* and whether the drug is able to suppress mediator symptoms in patients with advanced SM or SM with concomitant allergies remains to be determined. In fact, whereas the concentrations of

DCC-2618 required to block tryptase secretion in MC were rather low (<1.0 μM), the concentrations required to block IgE-dependent histamine release were rather high (1 μM).

Collectively, our data indicate that DCC-2618 is a novel promising agent that counteracts growth and survival of various cell types relevant to the pathogenesis of advanced SM. Whether DCC-2618 is able to block growth of neoplastic MC in patients with advanced SM is currently being explored in a clinical trial (NCT02571036).

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