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

Mathias Schneeweiss,1,2 Barbara Peter,1,2 Siham Bibi,3 Gregor Eisenwort,1,2 Dubravka Smiljkovic,1 Katharina Blatt,1,2 Mohamad Jawhar,4 Daniela Berger,2 Gabriele Stefanzl,2 Susanne Herndlhofer,1,2 Georg Greiner,5 Gregor Hoermann,5 Emir Hadzijusufovic,1,2,6 Karoline V. Gleixner,1,2 Peter Bettelheim,7 Klaus Geissler,6 Wolfgang R. Sperr,1,2 Andreas Reiter,4 Michel Arock3 and Peter Valent1,2

1Ludwig Boltzmann Cluster Oncology, Medical University of Vienna, Austria; 2Department of Internal Medicine I, Division of Hematology and Hemostaseology, Medical University of Vienna, Austria; 3Laboratoire de Biologie et Pharmacologie Appliquée, CNRS UMR 8113, Ecole Normale Superieure de Cachan, France; 4Department of Hematology and Oncology, University Medical Centre Mannheim and Medical Faculty Mannheim, Heidelberg University, Germany; 5Department of Laboratory Medicine, Medical University of Vienna, Austria; 6Department for Companion Animals and Horses, University Clinic for Small Animals, Internal Medicine Small Animals, University of Veterinary Medicine Vienna, Austria; 7Elisabethinen Hospital Linz, Austria and 8Fifth Medical Department, Hospital Hietzing, Vienna, Austria

©2018 Ferrata Storti Foundation. This is an open-access paper. doi:10.3324/haematol.2017.179895

Received: September 1, 2017.
Accepted: January 31, 2018.
Pre-published: February 8, 2018.
Correspondence: peter.valent@meduniwien.ac.at
Supplementary Information to Schneeweiss et al:

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

Supplementary Methods

Reagents

The multi-kinase inhibitor DCC-2618 and its metabolite, DP-5439, were kindly provided by Dr. B. Smith (Deciphera Pharmaceuticals LLC, Lawrence, KS, USA). Midostaurin (PKC412) was purchased from LC Laboratories (Woburn, MA, USA) and cladribine (2CdA) from Janssen Cilag (Titusville, NJ, USA). Stock solutions of drugs were prepared by dissolving in dimethyl-sulfoxide (DMSO) (Merck, Darmstadt, Germany). RPMI 1640 medium, Iscove’s modified Dulbecco’s medium (IMDM), penicillin+streptomycin, and endothelial basal medium-2 (EBM-2) were from Lonza (Verviers, Belgium), fetal calf serum (FCS) from GE Healthcare (Piscataway, NJ, USA), MCDB 131 medium from Life Technologies (Paisley, UK), trypsin (0.05% EDTA) from Gibco life technologies (Gaithersburg, MD, USA), alpha-thioglycerol from Sigma (St. Louis, MO, USA), amphotericin from PAN Biotech (Aidenbach, Germany) and \(^{3}\)H-thymidine from Perkin Elmer (Boston, MA, USA). Annexin V/FITC and Propidium Iodide (PI) were obtained from eBioscience (San Diego, CA, USA) and Annexin V/APC from Biolegend (San Diego, CA, USA). The monoclonal anti-IgE antibody E124.2.8 (De2) was purchased from Immunotech (Marseille, France), C5a from R&D Systems (Minneapolis, MN, US), 4',6-diamidino-2-phenyindole (DAPI) and Calcium-Ionophore from Sigma (St. Louis, MO, USA).
Culture of human cell lines

The human MCL-derived cell line HMC-1 was kindly provided by Dr. J.H. Butterfield (Mayo Clinic, Rochester, MN, USA).\(^1\) Two sub-clones were used, namely HMC-1.1 exhibiting KIT V560G, and HMC-1.2 harboring KIT V560G and KIT D816V.\(^2,3\) HMC-1 cells were grown in IMDM with 10% FCS, alpha-thioglycerol and antibiotics. The recently established human MC lines ROSA\(^{\text{KIT WT}}\), ROSA\(^{\text{KIT D816V}}\), ROSA\(^{\text{KIT K509I}}\), and 4 MCPV-1 subclones (MCPV-1.1, MCPV-1.2, MCPV-1.3, MCPV-1.4 (MCPV-1 is a cell line derived from mast cell-committed cord blood progenitors transformed by h-Tert, Large-T and oncogenic HRAS G12V) were cultured in IMDM containing 10% FCS.\(^4,5\) ROSA\(^{\text{KIT WT}}\) MCPV-1.1, MCPV-1.2, MCPV-1.3 and MCPV-1.4 cells were maintained in the presence of SCF-containing supernatants (10%) of chinese hamster ovary cells transfected with the murine scf (kl) gene (CHO-KL).\(^4,5\) ROSA\(^{\text{KIT K509I}}\) were established by lenti-viral transduction following a published protocol.\(^4\) The AML cell lines KG-1 and U937 as well as the FLT3-mutated AML cell lines MV4-11 and MOLM-13 were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). These cell lines were grown in RPMI 1640 medium plus 10% FCS. The eosinophilic cell line EOL-1 harbouring FIP1L1-PDGFR\(\alpha\) was also purchased from DMSZ. EOL-1 cells were maintained in RPMI 1640 medium and 20% FCS. Human cultured umbilical vein endothelial cells (HUVEC) were purchased from Technoclone (Vienna, Austria) or PromoCell (Heidelberg, Germany). HUVEC were cultured on 0.1% gelatine in EBM-2 medium (Lonza, Basel, Switzerland) supplemented with endothelial growth medium-2 (EGM-2; Lonza) at 37°C. The microvascular human endothelial cell line HMEC-1 was cultured in MCDB 131 medium (GIBCO, Life Technologies, Carlsbad, CA, USA) and
10% FCS. Endothelial cells were passaged using trypsin. HUVEC obtained from the 2nd, 3rd or 4th passage were used.

**Evaluation of drug effects on cell proliferation**

Primary neoplastic cells or cell lines were incubated in control medium, various concentrations of DCC-2618 (0.01 nM to 10 µM), various concentrations of its active metabolite DP-5439 (0.01 nM to 10 µM), or various concentrations of midostaurin (only primary neoplastic MC; 0.01 µM to 5 µM) in 96 well plates (TPP, Trasadingen, Switzerland) at 37°C for 48 hours. After incubation, 0.5 µCi ^3^H-thymidine were added for 16 hours. Cells were then harvested on filter membranes (Packard Bioscience, Meriden, CT, USA) and filter-bound radioactivity was counted in a Beta Counter (Top-Count NXT, Packard Bioscience). In a separate set of experiments, cell lines were incubated in the presence of various drug combinations. In these experiments HMC-1.1 and HMC-1.2 cells were exposed to various concentrations of DCC-2618 and midostaurin (PKC412) or DCC-2618 and cladribine (2CdA), either as single agents or in combination at a fixed ratio of drug concentrations. Drug combination effects (additive *versus* synergistic) were determined by calculating combination index (CI) values using CalcuSyn software (CalcuSyn; Biosoft, Ferguson, MO, USA).

**Evaluation of apoptosis by light microscopy and flow cytometry**

Cell lines were incubated in various concentrations of DCC-2618 (1 nM to 1 µM) at 37°C for 48 hours. After incubation, the percentage of apoptotic cells was quantified on Wright-Giemsa-stained cytospin-preparations. Apoptosis was defined according to conventional cytomorphologic criteria. For flow cytometric determination of
apoptosis, combined Annexin V/propidium iodide (PI) staining was performed. Cell lines were cultured in control medium or various concentrations of DCC-2618 or DP-5439 (1 nM to 1 µM) at 37°C for 48 hours. Then, cells were washed and incubated with Annexin V-APC (ROSA\textsuperscript{KITD816V}, ROSA\textsuperscript{KITK509I}) or Annexin V-FITC (other cell lines) in binding-buffer for 15 minutes. Cells were washed again and PI (1 mg/ml) was added. After washing, the percentage of apoptotic (Annexin V+/PI+) cells was determined on a FACSCalibur (Becton Dickinson, San Jose, CA, USA).\textsuperscript{3}

**Measurement of mediator release**

Dextran-enriched blood basophils obtained from healthy individuals (n=4) were incubated in control medium or DCC-2618 (0.1-10 µM) at 37°C for 30 minutes. Then, cells were incubated in histamine release buffer (HRB) or HRB containing anti-immunoglobulin E (anti-IgE) antibody E-124.2.8 (1 µg/mL), C5a (10 nM) or Calcium-Ionophore (10µg/ml) at 37°C for 30 minutes. Then, cells were centrifuged at 4°C. Cell-free supernatants and cell suspensions were recovered and analyzed for histamine content by radio-immunoassay. Histamine release was calculated as percent of released histamine compared to total (cellular+extracellular) histamine. Spontaneous release of tryptase from HMC-1 cells was studied by determining tryptase concentrations in cell-free supernatants and lysates every day over a time-period of 6 days. To evaluate the effect of DCC-2618 on tryptase release, HMC-1 cells were cultured in the presence or absence of DCC-2618 (HMC-1.1: 25 nM; HMC-1.2: 500 nM). Tryptase concentrations were measured by fluoroimmune enzyme assay. Tryptase release was expressed as percent of total (intra- and extracellular) tryptase.
**Evaluation of apoptosis in basophiles by flow cytometry**

Dextran-enriched blood basophils obtained from healthy individuals (n=3) were incubated in control medium or DCC-2618 (0.01-1 µM) at 37°C for 30 minutes. Then, cells were incubated with phycoerythrin (PE)-conjugated mAb 97A6 (CD203c) (Immunotech, Marseille, France) for 15 minutes. After incubation, cells were washed and DAPI (400 ng/ml) was added. The percentage of apoptotic CD203c+ cells (i.e. basophiles) was analyzed on a FACSCanto (Becton Dickinson, San José, CA).

**Evaluation of intracellular expression of pSTAT5 by flow cytometry**

Expression of pSTAT5 was examined in HMC-1.1 and HMC-1.2 cells using an intracellular flow cytometry staining protocol as reported. In brief, cells were fixed by incubation in 2% formaldehyde, followed by permeabilization in 100% methanol at -20°C. Subsequently, cells were stained with the Alexa Fluor 647-conjugated anti-pSTAT5 mAb 47 and the Alexa Fluor 647-conjugated isotype-matched control antibody (both Becton Dickinson, San Jose, CA, USA). Phosphorylated (p)STAT5 levels were then analyzed on a FACSCalibur (Becton Dickinson, San Jose, CA, USA). To evaluate the effects of DCC-2618, on expression of pSTAT5, HMC-1.1 and HMC-1.2 cells were incubated in control medium or in various concentrations of DCC-2618, at 37°C for 4 hours before being analyzed using flow cytometry.
Supplementary Table S1

**AML Patients**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age</th>
<th>Gender</th>
<th>Source</th>
<th>Diagnosis</th>
<th>Mutation</th>
<th>Cytogenetics</th>
<th>BM blast infiltration, %</th>
<th>DCC-2618, IC₅₀</th>
<th>DP-5439, IC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>54</td>
<td>m</td>
<td>BM</td>
<td>AML M4</td>
<td>NPM1</td>
<td>46, XY</td>
<td>25</td>
<td>1278 nM</td>
<td>n.t.</td>
</tr>
<tr>
<td>#2</td>
<td>74</td>
<td>f</td>
<td>BM</td>
<td>sec. AML M4</td>
<td>FLT3-ITD</td>
<td>46, XX</td>
<td>70</td>
<td>124 nM</td>
<td>200 nM</td>
</tr>
<tr>
<td>#3</td>
<td>57</td>
<td>m</td>
<td>BM</td>
<td>AML</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>95 nM</td>
<td>227 nM</td>
</tr>
<tr>
<td>#4</td>
<td>76</td>
<td>m</td>
<td>BM</td>
<td>AML M4</td>
<td>BCR-ABL1, TP53</td>
<td>46, XY</td>
<td>50</td>
<td>242 nM</td>
<td>469 nM</td>
</tr>
<tr>
<td>#5</td>
<td>21</td>
<td>m</td>
<td>BM</td>
<td>AML M3</td>
<td>PML/RARA</td>
<td>46, XY, t(15;17)</td>
<td>80</td>
<td>322 nM</td>
<td>1022 nM</td>
</tr>
<tr>
<td>#6</td>
<td>83</td>
<td>m</td>
<td>BM</td>
<td>sec. AML</td>
<td>none</td>
<td>46, XY</td>
<td>22</td>
<td>714 nM</td>
<td>1082 nM</td>
</tr>
<tr>
<td>#7</td>
<td>65</td>
<td>f</td>
<td>LP</td>
<td>AML M4</td>
<td>FLT3-ITD</td>
<td>46, XX</td>
<td>76</td>
<td>96 nM</td>
<td>82 nM</td>
</tr>
<tr>
<td>#8</td>
<td>65</td>
<td>m</td>
<td>BM</td>
<td>AML M4 relapse</td>
<td>CBFB-MYH11A;</td>
<td>46, XY, inv 16</td>
<td>11</td>
<td>151 nM</td>
<td>143 nM</td>
</tr>
<tr>
<td>#9</td>
<td>60</td>
<td>f</td>
<td>BM</td>
<td>AML M2</td>
<td>FLT3-ITD, NPM1</td>
<td>46, XX</td>
<td>90</td>
<td>62 nM</td>
<td>1 nM</td>
</tr>
<tr>
<td>#10</td>
<td>67</td>
<td>f</td>
<td>BM</td>
<td>AML with myelodysplasia-related changes</td>
<td>none</td>
<td>45,XX, -7</td>
<td>30</td>
<td>385 nM</td>
<td>n.t.</td>
</tr>
</tbody>
</table>

Abbreviations: m, male; f, female; PB, peripheral blood; BM, bone marrow; LP, cells obtained during leukapheresis; inv, inversion; t, translocation; nM, nanomolar; IC₅₀, half maximal inhibitory concentration; n.a., not available. Responses of cells to DCC-2618 or DP-5439 were assessed by ³H-thymidine uptake.
### CMML Patients

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age</th>
<th>Gender</th>
<th>Source</th>
<th>Diagnosis</th>
<th>Mutation</th>
<th>BM blast infiltration, %</th>
<th>% Blasts in PB</th>
<th>% Monocytes in PB</th>
<th>DCC-2618, IC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>DP-5439, IC&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>66</td>
<td>m</td>
<td>PB</td>
<td>CMML-1</td>
<td>none</td>
<td>3</td>
<td>1</td>
<td>29</td>
<td>392 nM</td>
<td>n.t.</td>
</tr>
<tr>
<td>#2</td>
<td>63</td>
<td>m</td>
<td>PB</td>
<td>CMML-2</td>
<td>none</td>
<td>n.a.</td>
<td>9</td>
<td>58</td>
<td>331 nM</td>
<td>472 nM</td>
</tr>
<tr>
<td>#3</td>
<td>66</td>
<td>m</td>
<td>PB</td>
<td>CMML-1</td>
<td>none</td>
<td>n.a.</td>
<td>2</td>
<td>10</td>
<td>363 nM</td>
<td>451 nM</td>
</tr>
<tr>
<td>#4</td>
<td>66</td>
<td>f</td>
<td>PB</td>
<td>CMML-1</td>
<td>JAK2 V617F</td>
<td>n.a.</td>
<td>&lt;1</td>
<td>7</td>
<td>194 nM</td>
<td>305 nM</td>
</tr>
<tr>
<td>#5</td>
<td>69</td>
<td>m</td>
<td>PB</td>
<td>CMML</td>
<td>n.a.</td>
<td>n.a.</td>
<td>7</td>
<td>3</td>
<td>221 nM</td>
<td>511 nM</td>
</tr>
<tr>
<td>#6</td>
<td>77</td>
<td>m</td>
<td>PB</td>
<td>CMML-1</td>
<td>none</td>
<td>n.a.</td>
<td>0</td>
<td>22</td>
<td>203 nM</td>
<td>108 nM</td>
</tr>
<tr>
<td>#7</td>
<td>75</td>
<td>f</td>
<td>PB</td>
<td>CMML-2</td>
<td>none</td>
<td>n.a.</td>
<td>0</td>
<td>14</td>
<td>476 nM</td>
<td>636 nM</td>
</tr>
<tr>
<td>#8</td>
<td>77</td>
<td>f</td>
<td>PB</td>
<td>CMML-1</td>
<td>none</td>
<td>n.a.</td>
<td>0</td>
<td>49</td>
<td>445 nM</td>
<td>367 nM</td>
</tr>
<tr>
<td>#9</td>
<td>81</td>
<td>m</td>
<td>BM</td>
<td>CMML</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>95 nM</td>
<td>615 nM</td>
</tr>
<tr>
<td>#10</td>
<td>77</td>
<td>f</td>
<td>BM</td>
<td>CMML</td>
<td>none</td>
<td>1</td>
<td>0</td>
<td>19</td>
<td>288 nM</td>
<td>425 nM</td>
</tr>
</tbody>
</table>

Abbreviations: m, male; f, female; PB, peripheral blood; BM, bone marrow; n.a., not available; n.t., not tested; IC<sub>50</sub>, half maximal inhibitory concentration. Responses of cells to DCC-2618 or DP-5439 were assessed by <sup>3</sup>H-thymidine uptake.

### Hypereosinophilia Patients

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age</th>
<th>Gender</th>
<th>Source</th>
<th>Diagnosis</th>
<th>BM blast infiltration, %</th>
<th>% Blasts in PB</th>
<th>% Eos in PB</th>
<th>DCC-2618, IC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>DP-5439, IC&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>67</td>
<td>f</td>
<td>BM</td>
<td>Churg-Strauss Syndrome</td>
<td>1</td>
<td>&lt;0.1</td>
<td>51</td>
<td>226 nM</td>
<td>491 nM</td>
</tr>
<tr>
<td>#2</td>
<td>23</td>
<td>f</td>
<td>BM</td>
<td>DRESS-Syndrome</td>
<td>1</td>
<td>0</td>
<td>14</td>
<td>92 nM</td>
<td>290 nM</td>
</tr>
<tr>
<td>#3</td>
<td>54</td>
<td>m</td>
<td>BM</td>
<td>Eosinophilic Esophagitis</td>
<td>1</td>
<td>0</td>
<td>19</td>
<td>320 nM</td>
<td>216 nM</td>
</tr>
</tbody>
</table>

Abbreviations: m, male; f, female; PB, peripheral blood; BM, bone marrow; Eos, Eosinophiles; n.a., not analyzed; IC<sub>50</sub>, half maximal inhibitory concentration. Responses of cells to DCC-2618 or DP-5439 were assessed by <sup>3</sup>H-thymidine uptake.
**Supplementary Figures**

**Schneeweiss et al., Figure S1**

**Figure S1:**
**Effects of DCC-2618 on intracellular pSTAT5 expression in HMC-1 cells**

HMC-1.1 cells and HMC-1.2 cells were incubated in control medium (0) or medium containing various concentrations of DCC-2618, as indicated, at 37°C for 4 hours. Then, expression of pSTAT5 in HMC-1 cells was analyzed by flow cytometry using a mAb against pSTAT5. Results show the mean fluorescence intensity (MFI) values and represent the mean±SD of 3 independent experiments. Asterisk (*): p<0.05 compared to control medium.
Schneeweiss et al., Figure S2A
Figure S2: Effects of DCC-2618 and DP-5439 on the survival of MC lines
HMC-1 cells, ROSA cells (A) and MCPV-1 cells (B) 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 hours. Thereafter, the percentage of AnnexinV/PI-positive cells was analyzed by flow cytometry. Dot plots show one exemplified experiment for each cell line. Bar graphs represent the mean±S.D. of 3 independent experiments of each corresponding cell line. Asterisk (*): p<0.05 compared to control medium.
Figure S3: DCC-2618 synergizes with midostaurin and 2CdA in inducing growth inhibition in HMC-1 cells
A: HMC-1.1 and HMC-1.2 cells were incubated with control medium (0 nM) or medium containing various concentrations of DCC-2618, midostaurin or the combination of both drugs (as indicated) at 37°C for 48 hours. B: HMC-1.1 and HMC-1.2 cells were incubated with control medium (0 nM) or medium containing various concentrations of DCC-2618, 2CdA or the combination of both drugs (as indicated) at 37°C for 48 hours. Thereafter, ³H-thymidine was added. After 16 hours, cells were harvested on filters and the bound radioactivity was measured in a β-counter. Results are expressed as percent of control and represent the mean±S.D from triplicates. C,D: To determine the nature of drug combination effects (additive versus synergistic) combination index (CI) values were calculated using CalcuSyn software as described (reference 6). A CI value of 1 indicates an additive effect, whereas CI values below 1 indicate synergistic drug effects. C: HMC-1.1 and HMC-1.2 cells were incubated with control medium (0 nM) or medium containing various concentrations of DCC-2618, midostaurin or the combination of both drugs as shown in Figure S3A. D: HMC-1.1 and HMC-1.2 cells were incubated with control medium (0 nM) or medium containing various concentrations of DCC-2618, 2CdA or the combination of both drugs as shown in Figure S3B.
Figure S4A:
Effects of DCC-2618 on C5a- and Ca-Ionophore-induced Histamine Release in normal basophils
Primary blood basophils from healthy donors were incubated in control medium or in various concentrations of DCC-2618, as indicated, at 37°C for 30 minutes. Then, cells were incubated in control buffer or in buffer containing C5a (10 nM) or Calcium-Ionophore A23187 (10 µg/ml) at 37°C for 30 minutes. After incubation, cells were centrifuged at 4°C, and cell-free supernatants and cell suspensions recovered and examined for histamine-content by RIA. Histamine release was calculated as percent of total histamine and is expressed as percent of control. Results represent the mean±S.D. of 3 independent experiments. The percentage of released histamine amounted to 49±22% of total histamine at 10 nM C5a and to 70±25% of total histamine at 10 µg/ml A23187.
Schneeweiss et al., Figure S4B

**Figure S4B:**
**Effects of DCC-2618 on the survival of basophiles**
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 minutes. After incubation cells were incubated with PE-conjugated monoclonal antibody against CD203c for 15 minutes. Thereafter, cells were washed and DAPI was added. The percentage of apoptotic CD203c+ cells (i.e. basophiles) was analyzed by flow cytometry. Bar graphs (upper panel) represent the mean±S.D. of 3 independent experiments. Dot plots show one representative experiment.
Figure S5: Effects of DCC-2618 and DP-5439 on survival of AML cells
MOLM-13, MV4-11, KG-1, and U937 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 hours. Thereafter, the percentage of Annexin V/PI-positive cells was analyzed by flow cytometry. Dot plots show one representative experiment for each cell line. Bar graphs (right panels) represent the mean±S.D. of 3 independent experiments in each cell line. Asterisk (*): p<0.05 compared to control medium.
Schneeweiss et al., Figure S6A

Schneeweiss et al., Figure S6B
Figure S6
Effects of DCC-2618 on proliferation and survival of neoplastic eosinophils
A and B: EOL-1 cells and primary neoplastic eosinophils 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 hours. Thereafter, ³H-thymidine was added. After 16 hours, cells were harvested on filters and the bound radioactivity was measured in a β-counter. Results of EOL-1 cells are expressed as percent of control and represent the mean±S.D. from 3 independent experiments. Results of primary cells are expressed as percent of control and represent the mean±S.D. from triplicates. C: EOL-1 cells were incubated in control medium (0 μM) or various concentrations of DCC-2618, as indicated, for 48 hours. Upper left panel: EOL-1 cells were harvested and the percentage of apoptotic cells was quantified on Wright-Giemsa-stained cytospin slides. Results show the percentage of apoptotic cells and represent the mean±S.D. of 3 independent experiments. Upper right panel: the percentage of AnnexinV/PI-positive cells was analyzed by flow cytometry. Results represent the mean±S.D. of 3 independent experiments. Lower panels: dot blots of Annexin V/PI stainings in one typical experiment. Asterisk (*): p<0.05 compared to control medium.
Schneeweiss et al., Figure S7

Figure S7

**DCC-2618 and DP-5439 inhibit proliferation of human endothelial cells**

HUVEC and HMEC-1 cells were incubated in control medium (0 µM) or in medium containing various concentrations of DCC-2618 or DP-5439, as indicated, at 37°C for 48 hours. Then, ³H-thymidine uptake was measured. Results are expressed as percent of control and represent the mean±S.D. from 3 independent experiments. Asterisk (*): p<0.05 compared to control medium.
References


