Supplementary Methods

Patient samples

Progenitor cells were isolated with one of two methods. (A) Incubation with a de-bulking antibody cocktail (Stem Cell Technologies, Vancouver, Canada) against the lineage-specific markers CD2, CD3, CD14, CD15, CD16, CD19, CD24, CD56, CD66b, IgE and Glycophorin A and negative selection on an immunomagnetic column according to the manufacturer's protocol. Samples were 78% ± 11% $CD34^+$ following lineage depletion. (B) Alternatively, $CD34^+$ cells were isolated by positive selection using an automatic immunomagnetic cell sorter (Automacs, Miltenyi, Bergisch-Gladbach, Germany). Additional selection steps to isolate $CD38^+$, $CD38^-$ and $CD117^+$ cells were done on a Becton Dickinson FACSAria.

Construction of vectors

Lentiviral vectors for knockdown of human and murine KIT were constructed as follows. shKIT sequences in pLKO1 (Open Biosystems) were initially screened for maximal KIT knockdown. The optimal KIT targeting sequence (forward, GCGACGAGATTAGGCTGTTAT; reverse, ATAACAGCCTAATCTCGTCGC) was initially cloned into pCMVU6 and then transferred into the FUGW lentiviral vector (a kind gift of Dr. Richard Goodman, OHSU). The construct for simultaneous expression of p185BCR-ABL1 and shmKIT was derived through modification of the pmiRRaf1–BCR-ABL vector previously described and kindly provided by Justus Duyster, Freiburg/Germany[19]. Briefly, DNA oligos (CCCACTGTGATTCCGCCTTTA and TTCCGTGACATTCAACGTTTA) against murine kit were insert into LMP vector (kindly provided by Justus Duyster, Freiburg/Germany) between XhoI and EcoR I. Then the shRNA sequence together with flank sequence were amplified with primers (forward, TGAGGATCCTAGGGATAACAGGGTAATTG; reverse, ATGGGATCCAAAAAAGTGATTTAATTTATACC) and subcloned into pmiR-BCR-ABL vector.

Immunoblot analysis of cell lines and patient samples

To assess the sensitivity of BCR-ABL1 and KIT kinases to the various BCR-ABL1, KIT and dual inhibitors, 2 x 10^6 Mo7e cells or Mo7e cells stably expressing BCR-ABL1 (Mo7ep210$^{BCR-ABL1}$) were serum and cytokine starved for 16 hours then treated for four hours with the following inhibitors at concentrations expected to inhibit BCR-ABL1 kinase activity: 2 μM imatinib (a gift

of Novartis), 50 nM dasatinib (purchased from the OHSU pharmacy), and 1 μM PPY-A. KIT inhibition was accomplished using SCF-blocking antibody at a concentration of 200 ng/mL or BAW667 at 1 μM. Mo7e cells were additionally stimulated with 100 ng/mL SCF for the last 15 minutes of the culture period. For primary cells, 1-2 x 10^6 per condition Lin^- or $CD34^+$ cells from newly diagnosed CML patients were incubated overnight in serum free medium (IMDM, 20% BIT, 40 mg/mL LDL, 10^{-4} M β-mercaptoethanol) without inhibitors or with 2 μM imatinib, 1 μM PPY-A, 1 μM BAW667 or SCF-blocking antibody at a concentration of 200 ng/mL. Lysates were made by boiling cells in 3XGS loading dye.

Antibodies

Antibodies used in the various experiments were directed against $pKIT^{Y719}$, the murine equivalent of human $pKIT^{Y721}$ (Cell Signaling Technology, Beverly, MA), total KIT (Santa Cruz Biotech, Santa Cruz, CA), $pERK1/2^{Y202/204}$ (Cell Signaling Technology, Beverly, MA), $pAKT^{S473}$ (Cell Signaling Technology, Beverly, MA), $pSTAT5^{Y694}$ Cell Signaling Technology, Beverly, MA), $pABL^{Y402}$ (Cell Signaling Technology, Beverly, MA), total ABL (24-11, Santa Cruz), pCRKL (Cell Signaling Technology, Beverly, MA), CRKL (C-20, Santa Cruz), tubulin (B-5-1-2, Sigma) and total Foxo3A (Cell Signaling Technology, Beverly, MA).

Colony assays

Hematopoietic colonies assays were performed as described[17]. $5x10^4$ CML MNC or normal MNC were cultured in methylcellulose medium containing 50 ng/mL SCF, 10 ng/mL GM-CSF, 10 ng/mL IL-3 (Stem Cell Technologies) to assess granulocyte/macrophage colony formation (CFU-GM) or methylcellulose media additionally containing 3 U/mL erythropoietin (Stem Cell Technologies) to assess erythrocyte colony formation (BFU-E). In a number of experiments $CD34^+$ cells were used, typically at $5x10^3$ cells/dish. Imatinib (2 μM) or PPY-A (1 μM) were added at doses approximately equipotent with respect to BCR-ABL1 inhibition, with or without 200 ng/mL SCF-blocking antibody or 1 μM BAW667. Colonies were counted following 14 days of culture with > ~50 cells/colony as the criterion for positive colony scoring. To verify continuous inhibition of BCR-ABL1 by PPY-A, colonies from untreated and PPY-A-treated plates were resuspended, washed twice in IMDM and analyzed by immunoblot for CRKL and pCRKL as described above. To assess the contribution of individual cytokines to cell growth, cytokine-free methylcellulose (Stem Cell Technologies) was supplemented with the following

cytokine combinations: 1) “no SCF”: 10 ng/mL IL-3, 10 ng/mL GM-CSF, 2) “no IL-3”: 50 ng/mL SCF, 10 ng/mL GM-CSF and 3) “no GM-CSF”: 50 ng/mL SCF, 10 ng/mL IL-3.

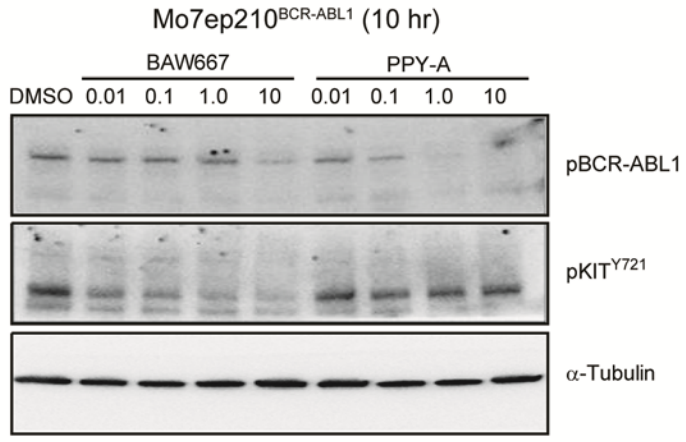
Cell proliferation assays

Viable cells were quantified using MTS, as described[20]. For details see Supplementary Methods. Mo7e cells or Mo7ep210$^{BCR-ABL1}$ cells were incubated or not with 1 μM PPY-A or 1 μM BAW667 or their combination, with or without 25 ng/ml SCF. In a separate experiment Mo7ep210$^{BCR-ABL1}$ cells were treated with 1 μM PPY-A, 20 μM MEK inhibitor PD98059 (or the PI3K inhibitor LY294002) or their combination in the presence or absence of 25 ng/ml SCF. In each experiment viable cells were quantified by MTS assay at 24, 48 and 72 hours.

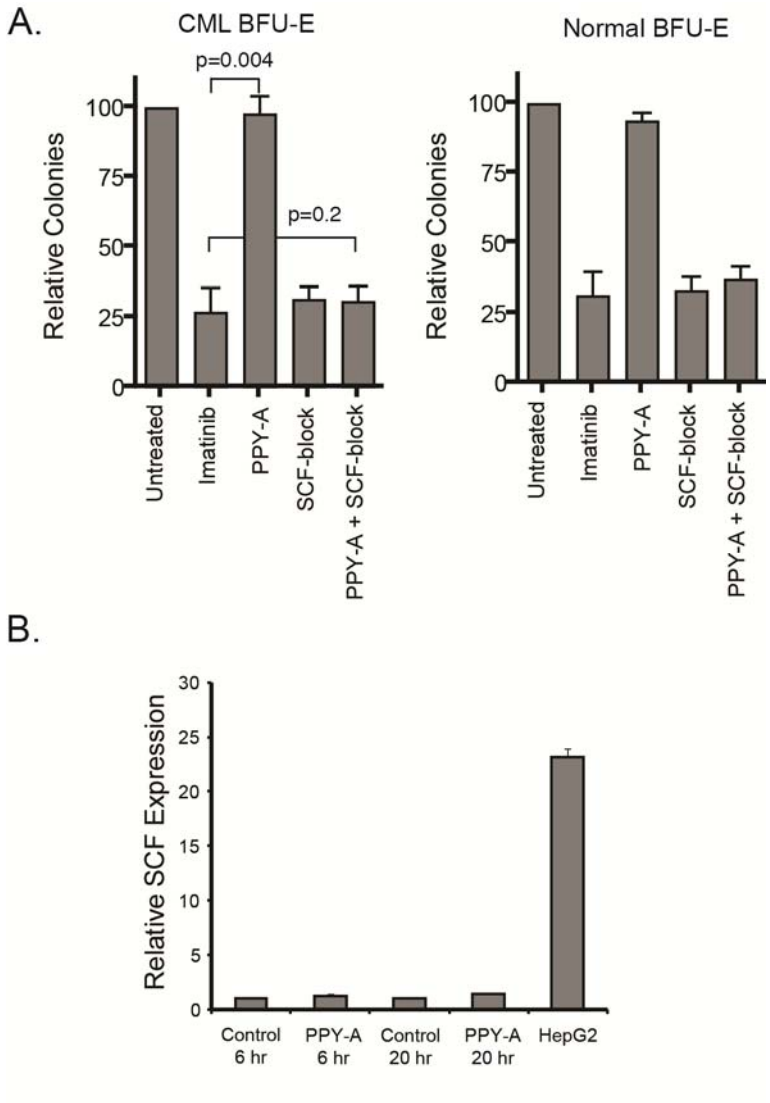
Quantitative RT-PCR for SCF

Up to 500000 CD34^{+} cells were lysed after 20h culture in the presence or absence of PP2Y. RNA was extracted as instructed by the manufacturer (RNeasy Micro Kit, QIAGEN). 100-200 ng RNA was converted to complementary DNA using iScript Reverse Transcription Supermix (Bio-Rad, Richmond, CA) as recommended by the manufacturer. SCF expression level was measured using SsoFast EvaGreen Supermix reaction (Bio-Rad, Richmond, CA) in a CFX96 Real Time System (Bio-Rad, Richmond, CA). SCF forward primer (5’-AGTGGATGACCTTGTGGAGTGCGTG-3’) and reverse primer (5’-GGAGTAAAGAGCCTGGGTTCTGGGC-3’) were used in this reaction. The expression level of SCF was normalized by calculating the ratio of SCF transcript to transcript level of the house keeping gene GUS (Forward:5’-GAAAATATGTGGTTGGAGAGCTCATT-3’, Reverse: 5’-CCGAGTGAAGATCCCCTTTTTA-3’) in this experiment.

Supplementary Figures

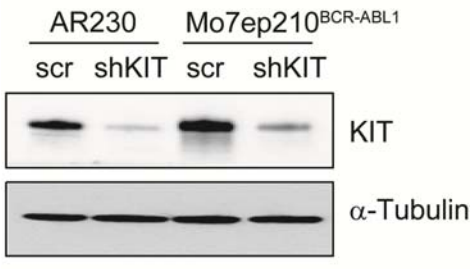


Supplementary Figure 1: Mo7ep210$^{BCR-ABL1}$ cells were treated with graded concentrations of BAW667 or PPY-A for 10 hours. Phospho-BCR-ABL1 and phospho-KIT were detected by immunoblot.

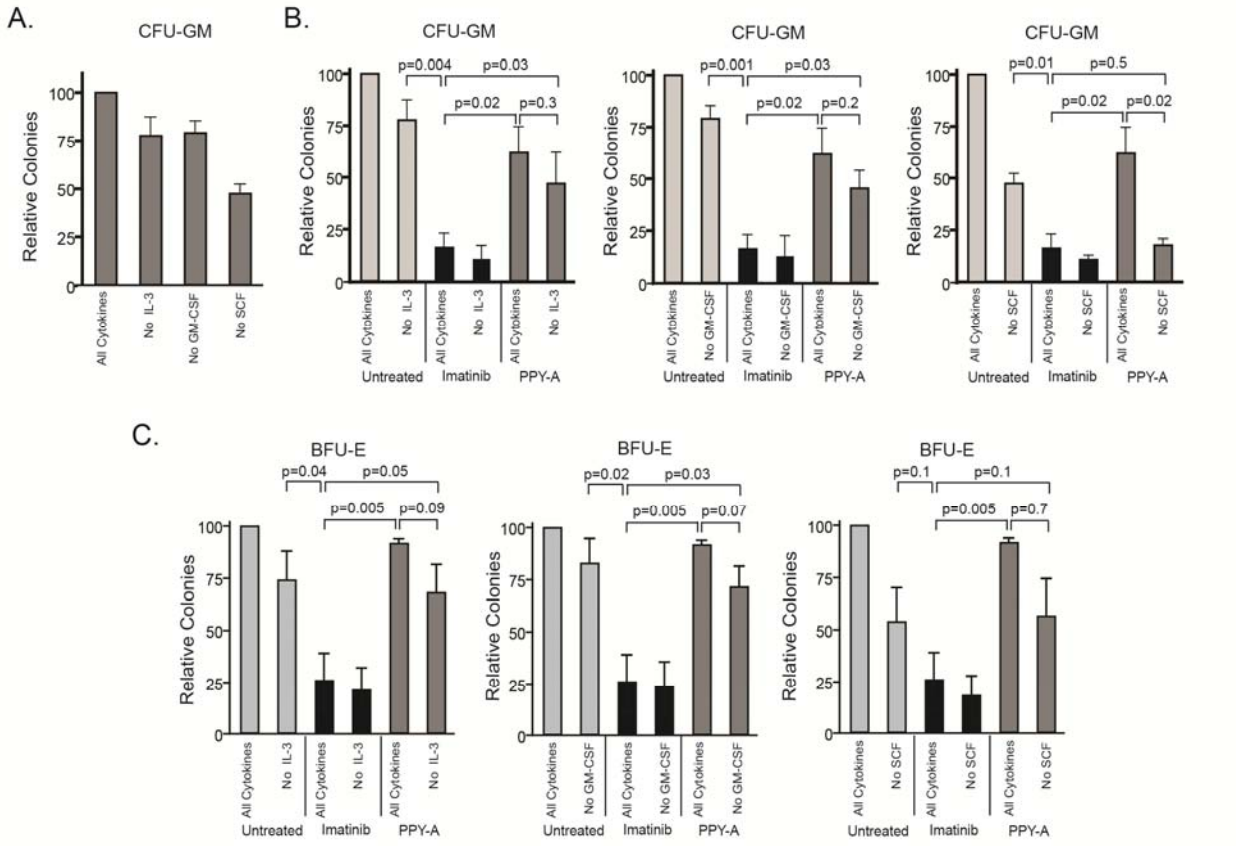


Supplementary Figure 2: (A) CFU-GM were assessed in samples (N=4) from newly diagnosed chronic phase CML patient cells (left) or normal mononuclear cells (right) cultured for 14 days in semisolid medium containing IL-3, GM-CSF, SCF and EPO. Imatinib, PPY-A, SCF-block or PPY-A + SCF-block were added as indicated. Mean colony number of triplicate plates is shown normalized relative to untreated samples. Error bars represent SEM. For individual CML samples, mean absolute colony numbers of the untreated controls were: CML1: 549, CML2: 27, CML3: 50, CML4: 143 BFU-E per $5x10^4$ MNC. **(B)** $CD34^+$ cells from a newly diagnosed CML-CP patient were cultured in the presence or absence of 1 μM PPY-A for up to 20 hours. Expression of SCF was measured by quantitative PCR. Results were identical in two additional

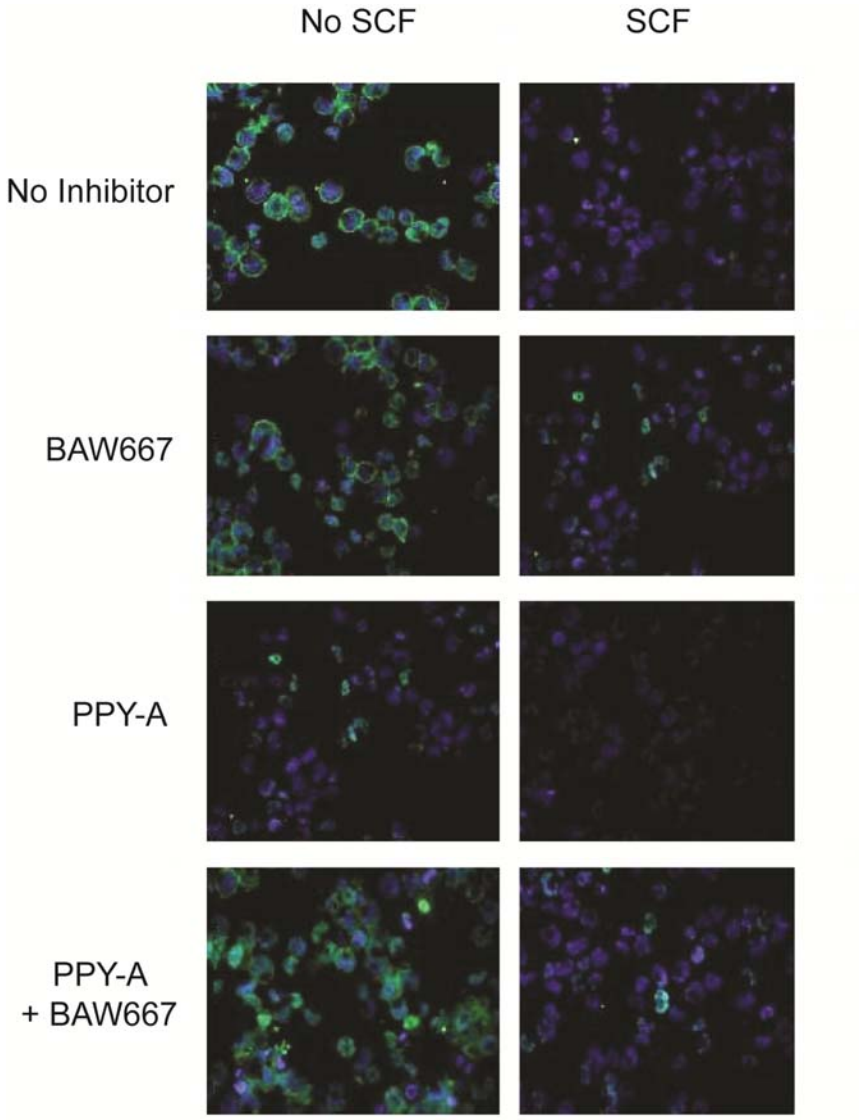
experiments. Moreover, analysis of a previously reported expression microarray dataset[28] revealed that SCF is not expressed by CML $CD34^+$ cells.



Supplementary Figure 3: AR230 and $Mo7ep210^{BCR\text{-}ABL1}$ cells were infected with lentivirus for co-expression of GFP with shKIT or shSCR. KIT expression was determined by immunoblot analysis of whole cell lysates from GFP-positive cells.



Supplementary Figure 4: (A) CFU-GM were assessed in MNC from newly diagnosed CML patients upon removal of IL-3, GM-CSF or SCF. Mean colony number of triplicate plates is shown relative to untreated for n=3 samples. Error bars represent SEM. Absolute colony numbers of controls were comparable to those reported in Figure 2. **(B)** CFU-GM were assessed following removal of individual cytokines in combination with imatinib or PPY-A treatment (n=3). Differences in colony growth were evaluated by Student's t-test. **(C)** BFU-E were assessed in Epo-containing colony assays on MNC from newly diagnosed CML-CP patients (n=3) upon removal of IL-3, GM-CSF or SCF. Cultures were carried out with 2 μM imatinib or 1 μM PPY-A. Mean colony number of triplicate plates is shown relative to untreated samples. Error bars represent SEM. Absolute colony numbers of controls were comparable to those reported in Figure 2.



Supplementary Figure 5: Mo7ep210$^{BCR\text{-}ABL1}$ were treated with inhibitors as indicated and then stimulated with 25 ng/mL SCF (see Figure 7B). Foxo3A was detected by immunofluorescence microscopy.

Supplementary Tables

Target	Cell ine	Nilotinib	BAW667
		Inhibition of cell proliferation (GI_{50} nM)	
K642E-KIT	GIST882	180	14.6
BCR-ABL	Ba/F3	47	> 10000
TEL-PDGFRα		< 3	5
TEL-PDGFRβ		< 3	< 3
ITD-FLT3		> 3000	< 3
TEL-CSF-1R		> 3000	45
NPM-ALK		> 3000	> 3000
V617F JAK2		> 8000	> 10000
Parental		> 10000	> 10000

Supplementary Table 1. Activity of BAW667 in comparison with nilotinib in cell lines expressing the respective kinases. Neither compound had any effect on the viability of Ba/F3 cell lines transfected to express the following TEL-fused kinases in the absence of IL-3 at concentrations < 2000 nM: FGFR-1/-2/-3/-4, IGF1R, InsR, LCK, LYN, MET, RET, RON, SRC, SYK, TIE-2, ZAP70.