Table W1. Characterization of Antibodies.

Antigen	CD	Clone* Name	Fluorochrome	Animal Source	Isotype	Provider Source
HPCA1	CD34	581	PE	Mouse	IgG1	BD Biosciences
ADP-RC	CD38	HIT2	APC	Mouse	IgG1	BD Biosciences
LCA	CD45	2D1	PerCP	Mouse	IgG1	BD Biosciences
ENNP	CD203c	97A6G	PE	Mouse	IgG1	Immunotech
c-Met	n.c.	95106	PE	Mouse	IgG1	R&D Systems
Active caspase 3	n.c.	C92605	PE	Rabbit	IgG1	BD Biosciences
rh Annexin	—	—	FITC	—	—	BD Biosciences
Isotype-Co	—	MOPC-21	PE	Mouse	IgG1	BD Biosciences
HGF	n.c.	H-145 (polyclonal)	—	Rabbit	IgG	Santa Cruz Biotechnology
Phospho-Met	n.c.	D26	—	Rabbit	IgG	Cell Signaling
Basogranulin	n.c.	BB1	—	Mouse	IgG	University of Southampton

ADP-RC indicates adenosine diphosphate ribosyl cyclase; APC, allophycocyanin; BD, Becton Dickinson; c-Met, c-mesenchymal epithelial transition factor; ENNP, ectonucleotide pyrophosphatase/phosphodiesterase 3; FITC, fluoresceine isothiocyanate; HPCA1, human precursor cell antigen 1; IgG, immunoglobulin G; LCA, leukocyte common antigen; PE, phycoerythrin; PerCP, peridinin chlorophyll protein.
*All antibodies except H-145 were monoclonal antibodies (mAb).

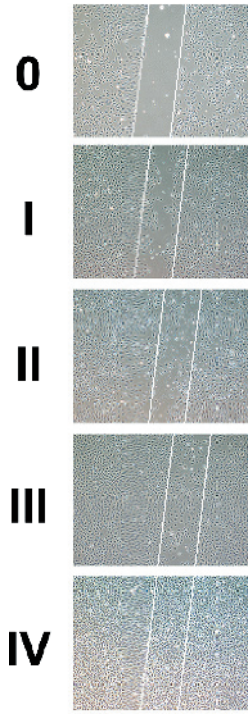
Table W2. Primer Sequences Used to Generate Northern Blot Probes.

Gene	Sequence
mu HGF-fwd	5′-GCCAGAAAG ATATCCCGACA-3′
mu HGF-rev	5′-AACTCGGATGTTTGGGTCAG-3′
mu-hu Actin-fwd	5′-GACGGCCAG GTCATCACTAT-3′
mu-hu Actin-rev	5′-AGGGAGACCAAAGCCTTCAT-3′
hu c-Met-fwd	5′-CAGGCAGTGCAGCATGTAGTG-3′
hu c-Met-rev	5′-TAAGGTGGGGCTCCT CTTGTCA-3′

fwd indicates forward; hu, human; mu, murine; rev, reverse.

Table W3. Oligonucleotide Primer Sequences for qPCR.

Gene	Sequence
mu HDC-fwd	5′-ACT CCA GTG CAG CCT GGA TAC C-3′
mu HDC-rev	5′-GGC TAG ATG CCC ACG TGA ATC CTA A-3′
mu HGF-fwd	5′-GGC ATC AAA TGC CAG CCT TG-3′
mu HGF-rev	5′-CGC GAT AGC TCG AAG GCA AA-3′
mu-hu Actin-fwd	5′-TCG ACA ACG GCT CCG GCA TG-3′
mu-hu Actin rev	5′-CCT CTC TTG CTC TGG GCC TCG TC-3′
hu c-met-fwd	5′-CGGACCCAATCATGAGCACTG-3′
hu c-met-rev	5′-ATCACGGCGCGCTTCACAG-3′
hu HDC-fwd	5′-GGA GAC ATG CTG GCT GAT GC-3′
hu HDC-rev	5′-TCT GTA CAC GCA GGG CTG GA-3′
hu HGF-fwd	5′-CAT CGC CAT CCC CTA TGC AG-3′
hu HGF-rev	5′-TGA TTA GGG TAG TCT TTG CTG ATT TT-3′
huVEGF-fwd	5′-GTC GGG CCT CCG AAA CCA TG-3′
hu VEGF-rev	5′-CTG GAT GAT TCT GCC CTC CTC CTT C-3′
hu Abl-fwd	5′-TGT ATG ATT TTG TGG CCA GTG GAG-3′
hu Abl-rev	5′-GCC TAA GAC CCG GAG CTT TTC A-3′



Score:

0	Scratch area completely empty (no cells migrated)
I	Few single endothelial cells in scratch wound
II	Multiple isolated endothelial cells in scratch lesion
III	Endothelial cells form aggregates and bridges in scratch wound
IV	Scratch wound completely "healed" (confluent layer)

Figure W1. Scores for evaluating endothelial migration in the scratch wound migration assay: 0, scratch area empty (no cells migrated); I, few single endothelial cells in scratch wound; II, multiple isolated endothelial cells in scratch lesion; III, endothelial cells form aggregates and bridges in scratch wound; IV, scratch wound completely "healed" (confluent layer).

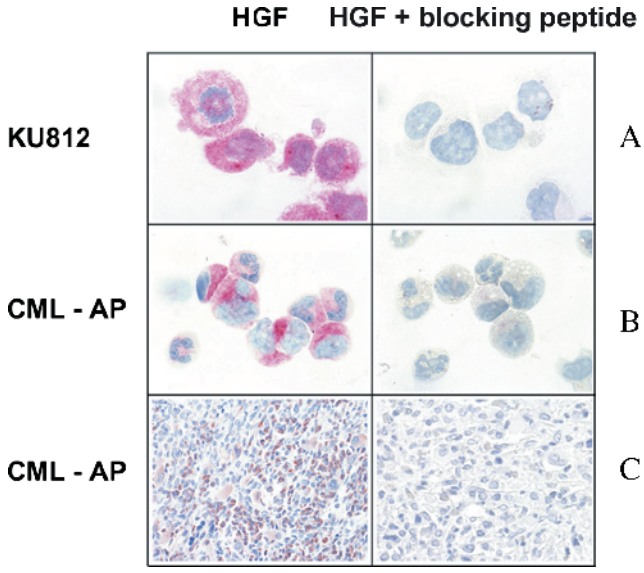


Figure W2. Reactivity of CML basophils with an anti-HGF antibody and effects of a HGF-specific blocking peptide. ICC detection of HGF in KU812 cells (A, upper panels) and isolated BM cells (B, middle panels) obtained from a patient with CML in AP. Before staining, the anti-HGF antibody was preincubated in control buffer (left panels) or with a HGF-specific blocking peptide (right panels). (C) Adjacent BM sections obtained from a patient with CML AP were stained with an anti-HGF antibody. Before staining, the anti-HGF antibody was preincubated in control buffer (left panel) or with a HGF-specific blocking peptide (right panel). Images were produced using an Olympus DP21 camera connected to an Olympus BX50F4 microscope equipped with 40×/0.85 UPlan-Apo objective lens (Olympus). Figures were prepared using Adobe Photoshop CS2 software version 9.0 (Adobe Systems, San Jose, CA) and PowerPoint software (Microsoft, Redmond, WA).

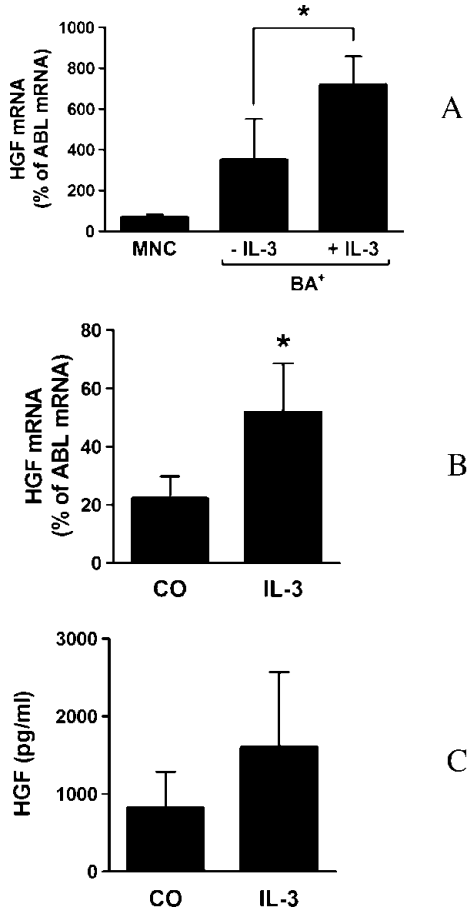


Figure W3. IL-3 regulates the expression and release of HGF in CML cells. (A) Expression of HGF mRNA in PB MNCs and highly purified (sorted CD203c$^+$) basophils (BA$^+$) from three patients with CML. Basophils were incubated in the presence and absence of IL-3 (100 ng/ml) for 30 minutes and then subjected to RNA isolation, cDNA synthesis, and qPCR using primers specific for HGF and ABL. Results show HGF mRNA expression levels as percent of ABL mRNA levels and represent the mean ± SD of three donors. *$P < .05$. (B) Expression of HGF mRNA in PB MNC of three patients with CML with marked basophilia (>10%). Cells were incubated in the presence or absence of IL-3 (100 ng/ml) for 8 hours. Then, RNA was isolated, and qPCR was performed using primers specific for HGF and ABL. Results show HGF mRNA expression levels as percent of ABL mRNA levels and represent the mean ± SD of three donors. *$P < .05$. (C) Measurement of HGF in supernatants of PB MNC of patients with CML with basophilia (>10%). Cells were incubated in the presence or absence of IL-3 (100 ng/ml). Supernatants were collected after 5 days, and HGF concentrations were determined by ELISA. Results represent the mean ± SD of three independent experiments.

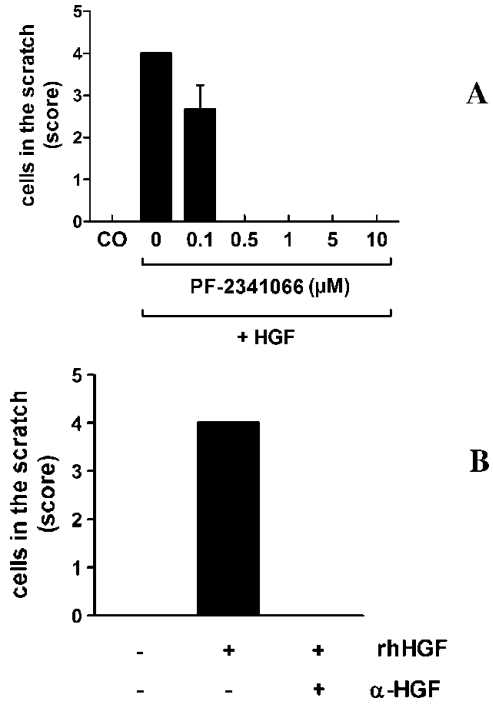


Figure W4. Effects of the c-Met inhibitor PF-2341066, rhHGF and an anti-HGF antibody on endothelial cell (HUVECs) migration in a scratch wound assay. (A) After producing a scratch wound into confluent layers of HUVECs by a pipette tip, cells were incubated with rhHGF (100 ng/ml) in the absence (CO) or presence of various concentrations of the c-Met inhibitor PF-2341066 at 37°C for 24 hours. Endothelial cell migration was examined under an inverted microscope (Eclipse TE 300; Nikon). Cell density in the scratch wound was scored from 0 to 4 (see also Figure W1): 0, scratch area empty (no cells migrated); I, few single endothelial cells in scratch wound; II, multiple isolated endothelial cells in scratch lesion; III, endothelial cells form aggregates and bridges in scratch wound; IV, scratch wound completely "healed" (confluent layer). Results represent the mean ± SD of three independent experiments. (B) After producing a scratch wound into confluent layers of HUVECs by a pipette tip, cells were incubated in the absence or presence of rhHGF (100 ng/ml) and a neutralizing anti-HGF antibody (αHGF; 60 ng/ml) at 37°C for 24 hours. Endothelial cell migration was examined under an inverted microscope (Eclipse TE 300; Nikon). Cell density in the scratch wound was scored from 0 to 4 (see also Figure W1). Results represent the mean ± SD of three independent experiments.

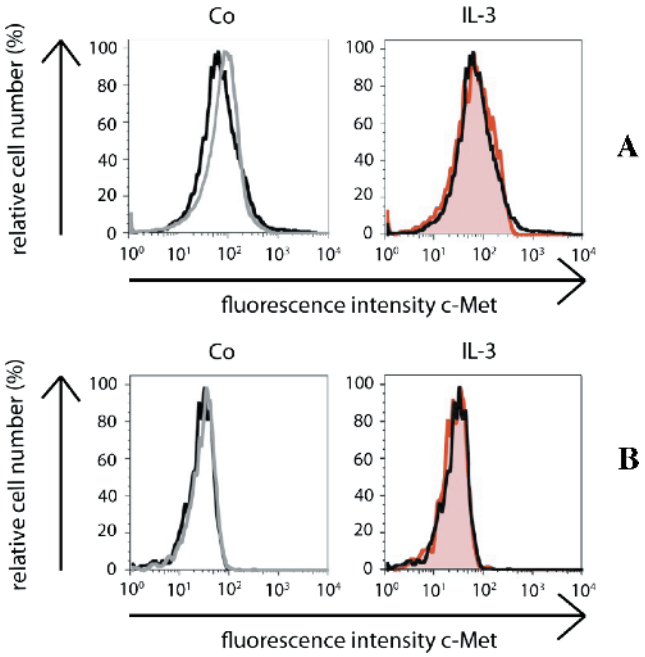


Figure W5. Surface expression of c-Met on CML basophils and CML $CD34^+/CD38^-$ cells after incubation with IL-3. (A) Surface expression of c-Met on CML basophils after incubation in the presence and absence (CO; black histograms) of IL-3 (100 ng/ml; red histograms) for 8 hours at 37°C. Cells were analyzed for expression of c-Met by multicolor flow cytometry. Expression of c-Met was controlled by an isotype-matched antibody (gray open histograms). (B) Surface expression of c-Met on CML $CD34^+/CD38^-$ cells after incubation in the presence and absence (CO) (black histograms) of IL-3 (100 ng/ml, red histograms) for 8 hours at 37°C. Cells were analyzed for expression of c-Met by multicolor flow cytometry. The expression of c-Met was controlled by an isotype-matched antibody (gray open histograms).

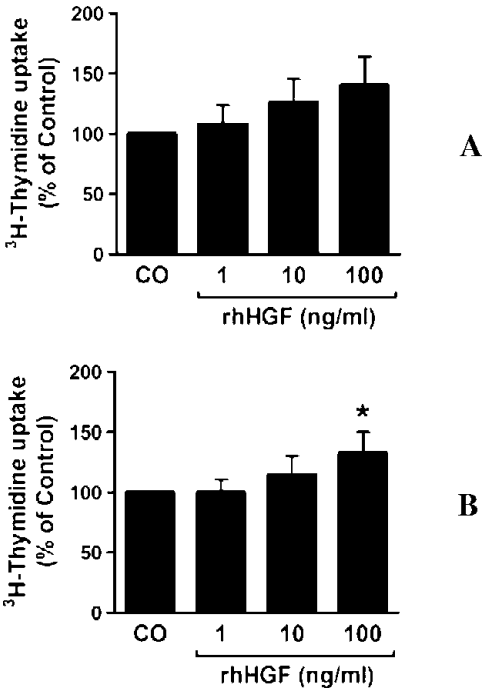


Figure W6. Effects of rhHGF on primary CML cells. Ficoll isolated mononuclear (MNC) BM cells (A) and PB cells (B) were incubated in control medium (CO) or in medium containing various concentrations of rhHGF at 37°C and 5% CO_2 for 48 hours. After incubation, 0.5 μCi of ^{3}H-thymidine was added. Twelve hours later, cells were harvested, and bound radioactivity was measured in a β-counter. Results are expressed as the percentage of control (CO) and represent the mean ± SD of three independent experiments. *$P < .05$.