



# Autocrine Activation of the MET Receptor Tyrosine Kinase in Acute Myeloid Leukemia

## Citation

Kentsis, Alex, Casie Reed, Kim L. Rice, Takaomi Sanda, Scott J. Rodig, Eleni Tholouli, Amanda Christie, et al. 2012. Autocrine activation of the MET receptor tyrosine kinase in acute myeloid leukemia. *Nature Medicine* 18(7): 1118-1122.

## Published Version

doi:10.1038/nm.2819

## Permanent link

<http://nrs.harvard.edu/urn-3:HUL.InstRepos:10612870>

## Terms of Use

This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA>

## Share Your Story

The Harvard community has made this article openly available.  
Please share how this access benefits you. [Submit a story](#).

[Accessibility](#)



Published in final edited form as:

*Nat Med.* 2012 July ; 18(7): 1118–1122. doi:10.1038/nm.2819.

## Autocrine activation of the MET receptor tyrosine kinase in acute myeloid leukemia

Alex Kentsis<sup>1</sup>, Casie Reed<sup>1</sup>, Kim L. Rice<sup>2</sup>, Takaomi Sanda<sup>1</sup>, Scott J. Rodig<sup>3</sup>, Eleni Tholouli<sup>4</sup>, Amanda Christie<sup>1,5</sup>, Peter J.M. Valk<sup>6</sup>, Ruud Delwel<sup>6</sup>, Vu Ngo<sup>7</sup>, Jeffery L. Kutok<sup>3</sup>, Suzanne E. Dahlberg<sup>8</sup>, Lisa A. Moreau<sup>1</sup>, Richard J. Byers<sup>4,9</sup>, James G. Christensen<sup>10</sup>, George Vande Woude<sup>11</sup>, Jonathan D. Licht<sup>2</sup>, Andrew L. Kung<sup>1,5</sup>, Louis M. Staudt<sup>12</sup>, and A. Thomas Look<sup>1</sup>

<sup>1</sup>Department of Pediatric Oncology, Dana-Farber Cancer Institute, and Division of Hematology/Oncology, Children's Hospital Boston, Harvard Medical School, Boston, MA, USA <sup>2</sup>Division of Hematology/Oncology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA <sup>3</sup>Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA <sup>4</sup>Department of Haematology, Manchester Royal Infirmary, Central Manchester University Hospitals NHS Foundation Trust, and Manchester Academic Health Science Centre, Manchester, UK <sup>5</sup>Lurie Family Imaging Center, Dana-Farber Cancer Institute, Boston, MA, USA <sup>6</sup>Department of Hematology, Erasmus Medical Center, Rotterdam, The Netherlands <sup>7</sup>Division of Hematopoietic Stem Cell and Leukemia Research, City of Hope National Medical Center, Duarte, CA, USA <sup>8</sup>Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA, USA <sup>9</sup>School of Cancer and Enabling Sciences, Faculty of Medical and Human Sciences, University of Manchester, Manchester, UK <sup>10</sup>Department of Research Pharmacology, Pfizer Global Research and Development, La Jolla, CA, USA <sup>11</sup>Department of Molecular Oncology, Van Andel Research Institute, Grand Rapids, MI, USA <sup>12</sup>Center for Cancer Research, National Cancer Institute/NIH, Bethesda, MD, USA

### Abstract

Although the treatment of acute myeloid leukemia (AML) has improved significantly, more than half of all patients develop disease that is refractory to intensive chemotherapy<sup>1,2</sup>. Functional genomics approaches offer a means to discover specific molecules mediating aberrant growth and survival of cancer cells<sup>3–8</sup>. Thus, using a loss-of-function RNA interference genomic screen, we identified aberrant expression of the hepatocyte growth factor (HGF) as a critical factor in AML pathogenesis. We found HGF expression leading to autocrine activation of its receptor tyrosine kinase, MET, in nearly half of the AML cell lines and clinical samples studied. Genetic depletion of HGF or MET potently inhibited the growth and survival of HGF-expressing AML cells. However, leukemic cells treated with the specific MET kinase inhibitor crizotinib developed resistance due to compensatory upregulation of HGF expression, leading to restoration of MET signaling. In cases of AML where MET is coactivated with other tyrosine kinases, such as

---

Correspondence should be addressed to A. Thomas Look: 617-632-5826 (phone), 617-632-6989 (fax), thomas\_look@dfci.harvard.edu.

#### Author Contributions

A.K., C.R., K.L.R., T.S., A.C., V.N., L.M. performed experiments; A.K., S.R., P.V., R.D., J.K., S.D., R.B., J.C., G.W., J.D.L., A.K., L.S., A.T.L. analyzed data, A.K and A.T.L. wrote the manuscript.

#### Competing Financial Interests

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at <http://www.nature.com/naturemedicine/>.

#### Methods

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/naturemedicine/>.

fibroblast growth factor receptor 1 (FGFR1)<sup>9</sup>, concomitant inhibition of FGFR1 and MET blocked compensatory HGF upregulation, resulting in sustained logarithmic cell kill both *in vitro* and in xenograft models *in vivo*. Our results demonstrate widespread dependence of AML cells on autocrine activation of MET, as well as the importance of compensatory upregulation of HGF expression in maintaining leukemogenic signaling by this receptor. We anticipate that these findings will lead to the design of additional strategies to block adaptive cellular responses that drive compensatory ligand expression as an essential component of the targeted inhibition of oncogenic receptors in human cancers.

---

We used a doxycycline-inducible retroviral RNAi library of 5,087 bar-coded shRNAs targeting 1,740 human genes to screen for functional pathway dependence in OCI/AML-2 cells derived from a patient with complex-karyotype AML (Supplementary Fig. 1a)<sup>10</sup>. Among the top 30 genes specifically required for the growth of OCI/AML-2 cells but not cells from a variety of non-myeloid hematologic malignancies was HGF, the ligand of the receptor tyrosine kinase MET<sup>11</sup>. Targeting of *HGF* and downstream mediators of the MET signaling pathway, such as *STAT3* and *MAPK1*, with two independent shRNAs, significantly suppressed the growth of AML but not non-myeloid hematologic cancer cells (Supplementary Fig. 1b–d).

In tests of the functional consequences of HGF expression in AML cell lines, we found that four of seven cell lines, but not normal CD34<sup>+</sup> cells, expressed HGF that was associated with MET activation (Fig. 1b). These data indicate that HGF expression by AML cells is aberrant, while expression of MET is lineage appropriate. Knockdown of *HGF* using two independent specific shRNAs inhibited the growth of OCI/AML-2 cells (Fig. 1c and Supplementary Fig. 2a). This effect could be rescued with recombinant HGF protein or by the transduction of complementary DNA (cDNA) encoding *HGF* (Fig. 1c). OCI/AML-2 cell growth was also inhibited by the addition of a neutralizing antibody against HGF to the culture medium (Fig. 1c). We also demonstrated the requirement for HGF/MET signaling in three additional AML cell lines (HEL, SKNO-1, KG-1) by depleting *HGF* and *MET* using specific shRNAs and inhibiting MET kinase signaling using the kinase inhibitor SU11274 (Supplementary Fig. 3–5). Inhibition of HGF/MET signaling led to a significant increase in the apoptosis of HGF-expressing cells (Fig. 1d), without the induction of cell cycle arrest (Supplementary Fig. 3). In addition, treatment with the specific MET kinase inhibitor crizotinib led to decreased colony formation of HGF-expressing primary AML samples (Fig. 1e–f). Taken together, our findings indicate that cell-autonomous production of HGF causes autocrine activation of MET and is necessary for the proliferation or survival of HGF-expressing AML cells.

To estimate the prevalence of aberrant HGF/MET signaling in patients with AML, we used immunohistochemistry to detect the coexpression of HGF and MET in bone marrow biopsy specimens from 138 adults with a broad spectrum of AML subtypes (Fig. 2a–c, Supplementary Fig. 6a–e). These proteins were expressed together in 58 (42%) of the patients (Fig. 2c), often in association with specific genetic abnormalities, including the *PML-RARA* and *AML1-ETO* translocations (Supplementary Fig. 6f). Using capillary isoelectric focusing electrophoresis nanoimmunoassay (NIA), which allows precise quantitation of differences in protein expression and phosphorylation, we observed expression of HGF and activation of MET in 5 (38%) of 13 viably frozen bone marrow aspirate specimens (Supplementary Fig. 7), with confirmation by flow cytometry (Supplementary Fig. 8). In the cohort we examined, there was no statistically significant difference in survival between patients with and without aberrant HGF expression (Supplementary Fig. 6g). Additional analysis of the gene expression profiles of primary AML blasts from 285 patients with AML also revealed the expression of high levels of HGF

in a subset of patients with AML<sup>12</sup>, including cases with *PML-RARA* and *AML1-ETO* (Supplementary Fig. 9).

Because HGF expression is associated with specific biologic subtypes of AML, we hypothesized that it might be induced by chimeric transcription factors that act *trans* on the *HGF* locus to drive AML pathogenesis. Consistent with this concept, we did not detect any copy number changes (Supplementary Fig. 10a–b), mutations of the *HGF* promoter (Supplementary Fig. 10c), or allelic skewing of single nucleotide polymorphism expression (Supplementary Fig. 10d) in human AML cell lines with aberrant HGF expression. To test this predicted *trans*-acting mechanism directly, we transduced primary lineage-depleted mouse hematopoietic cells with fusion protein-encoding retroviruses, and monitored for expression of HGF and activation of MET using nanoimmunoassays. Cells transduced with *PML-RARA*, *PLZF-RARA*, and *AML1-ETO* expressed HGF (Fig. 2d, Supplementary Fig. 11), and exhibited phosphorylation of MET (Fig. 2e, Supplementary Fig. 11). Transformed cells were sensitive to MET kinase inhibition in serial replating colony-formation assays (Fig. 2f, Supplementary Fig. 11), while showing downregulation of MET phosphorylation (Fig. 2g, Supplementary Fig. 11). Thus, distinct chimeric transcription factors can induce expression of HGF, leading to aberrant MET activation and functional dependence on HGF/MET signaling.

To assess the potential of HGF/MET signaling as a therapeutic target, we investigated the sensitivity of HGF/MET-dependent AML cell lines to chemical inhibition of the MET kinase using the specific MET/ALK inhibitor crizotinib<sup>13</sup>, which permits targeting of MET in AML because ALK is not expressed by hematopoietic cells or any of the AML cell lines studied to date (Supplementary Fig. 3b). The growth of AML cell lines with aberrant HGF expression and MET activation was strongly inhibited by treatment with crizotinib, while that of cell lines lacking HGF expression and MET activation was unaffected (Fig. 1d, Supplementary Fig. 4e–f). However, cells treated for more than 6 days with crizotinib appeared to regain their normal growth rate (Fig. 3a, Supplementary Fig. 12a–b).

Further experiments to determine the origin of the acquired resistance to crizotinib using quantitative nanoimmunoassays showed profound inhibition of MET activation within 12 hours of crizotinib treatment (Supplementary Fig. 13a) that was associated with the induction of apoptosis (Supplementary Fig. 14). In addition, we observed a surprising 13-fold upregulation of HGF in crizotinib versus control-treated OCI/AML-2 cells (Supplementary Fig. 13b), which occurred in concert with the recovery of phospho-MET levels after 10 days of treatment (Supplementary Fig. 13a), accounting for the restoration of pre-treatment cell growth rates (Fig. 3a). This finding, confirmed in 3 different AML cell lines (Supplementary Fig. 12c), reflects increased biallelic expression of *HGF* mRNA (Supplementary Fig. 15). The recovery of MET phosphorylation corresponded with a recovery in the abundance of phosphorylated CRKL, STAT3, and ERK1/2 (Supplementary Fig. 13d–f), all associated with marked upregulation of HGF (Supplementary Fig. 13b). Depletion of HGF with a specific shRNA partially mitigated the compensatory upregulation of HGF in response to MET kinase inhibition, but this strategy was only partially successful in inhibiting leukemia growth *in vivo* due to the intrinsic variability in knockdown efficiency (Supplementary Fig. 16–17). While the rapid development of crizotinib resistance was somewhat surprising, the selective pressure to maintain MET phosphorylation by upregulation of HGF reinforces our original conclusion that specific types of AML require aberrant HGF-mediated activation of MET signaling for sustained growth and survival.

Since activation of MET can occur in AMLs that also harbor aberrant activation of other receptor tyrosine kinases, we reasoned that combined inhibition of the signaling pathways co-activated with MET might be required to block compensatory upregulation of HGF. In

this study, we focused on the co-activation of FGFR1 and HGF/MET in KG-1 cells, which bear a *FOP2-FGFR1* chromosomal translocation, derived from aggressive 8p11 myeloproliferative syndrome/stem cell leukemia<sup>9</sup>. After treating KG-1 cells with various concentrations of crizotinib and PD173074, a specific and potent inhibitor of the FGFR1 tyrosine kinase<sup>14</sup>, we analyzed the effects using isobologram analysis (Supplementary Fig. 18a). Nearly all dose combinations of PD173074 and crizotinib produced synergistic effects, as indicated by their low combination index values (Supplementary Fig. 18a). We demonstrated that the effect of PD173074 was mediated specifically by inhibition of FGFR1, by finding that depletion of FGFR1 sensitized KG-1 cells to treatment with crizotinib (Supplementary Fig. 19).

Combination treatment of KG-1 cells with 100 nM crizotinib and 20 nM PD173074 (corresponding to their individual  $IC_{50}$  values) prevented compensatory upregulation of HGF (Fig. 3b), leading to sustained inhibition of MET phosphorylation (Fig. 3c), and sustained blockade of downstream signaling pathways (Supplementary Fig. 18b–f). This strategy also led to potent induction of apoptosis and logarithmic cell kill that was sustained for 14 days of treatment (Fig. 3e–f). We confirmed the on-target effect of PD173074 by specifically depleting cells of FGFR1 by shRNA knockdown, and showing that cells depleted of FGFR1, but not those transduced with vector control, fail to upregulate HGF in response to chronic crizotinib treatment (Supplementary Fig. 19c). Thus, FGFR1 activity is required for the compensatory upregulation of HGF in response to MET inhibition.

Finally, we evaluated simultaneous inhibition of MET and blockade of compensatory HGF expression in KG-1 cells modified to express luciferase for bioluminescence imaging, and engrafted into immunocompromised mice via tail vein injection. Leukemic mice were treated with vehicle control, crizotinib ( $50 \text{ mg}^{-1} \text{ kg}^{-1}$ ) alone, PD173074 ( $25 \text{ mg}^{-1} \text{ kg}^{-1}$ ) alone, or combined treatment with these agents by daily oral gavage 10 days after transplantation of leukemia cells. Mice treated with single drugs or vehicle control continued to show exponentially growing leukemia, whereas animals treated with both crizotinib and PD173074 had significant disease regression after 10 days of therapy, as measured by bioluminescence (Fig. 4a–b). These results were confirmed by flow cytometry, demonstrating near-complete ablation of CD45<sup>+</sup> human cells in the peripheral blood and bone marrow of mice treated with the combination of crizotinib and PD173074, but not in those treated with either drug alone or vehicle control (Fig. 4c–d). Although PD173074 had more potent effects on cell growth than crizotinib, they were only modest at best, indicating that the impressive therapeutic synergy of this combination stems primarily from the PD173074-induced blockade of compensatory HGF upregulation in response to crizotinib treatment (Fig. 4e–f).

We have identified aberrant HGF/MET signaling as a requisite pathway in the growth and survival of AML cells, in nearly half of a large group of primary clinical samples. Besides *HGF*, our genome-wide shRNA screen identified a number of additional genes as critical factors in the biochemical processes that drive AML pathogenesis, which with proper validation could offer a functional taxonomy of AML cells, provide powerful insights into the pathophysiology of the disease, and ultimately targets for improved therapy.

How does aberrant production of HGF contribute to the pathobiology of AML? We show here that dysregulated expression of this secreted growth factor, due in part to the activity of distinct AML-associated transcription factors, leads to autocrine activation of the MET receptor and, in turn, to autocrine receptor tyrosine kinase signaling, as originally postulated on the basis of first-principle considerations<sup>15</sup>. Instead of direct mutational activation<sup>16</sup>, we find that MET is activated in AML as a result of aberrant autocrine signaling by HGF, which

appears to be dynamically controlled, as indicated by the upregulation of HGF in response to chronic MET kinase inhibition. Since constitutive activation of growth factor signaling pathways can have maladaptive cellular effects, such as cell cycle arrest and senescence<sup>17</sup>, these findings suggest that HGF deregulation and autocrine signaling provide a mechanism by which MET activity can be modulated to levels that optimize the fitness of AML cells, a property that may not be achieved by mutational activation of MET. In addition, *HGF* appears to be one of the most differentially expressed genes in the leukemia-initiating cells of AML, as compared to normal hematopoietic stem cells<sup>18</sup>, suggesting that aberrant HGF/MET signaling may contribute to the growth and survival of AML stem cells, thus strengthening the rationale for targeting this pathway.

Our study also raises the intriguing possibility that ligand-induced receptor activation may provide a general mechanism through which cancer cells can develop resistance to the therapeutic inhibition of receptor tyrosine kinase signaling. Autocrine signaling is widely prevalent in human cancers, affecting the EGFR, IGFR, PDGFR, FGFR, TRK, EPH, and TIE receptor families, many of which are currently being explored as therapeutic targets<sup>19</sup>. Indeed, ligand-dependent activation of receptor tyrosine kinases has been observed with other leukemogenic receptor tyrosine kinases, most notably KIT and FLT3<sup>20</sup>. Treatment with FLT3 kinase inhibitors leads to upregulation of FLT3 ligand<sup>21</sup>, which may be responsible, at least in part, for the diminished clinical efficacy of FLT3 inhibitors in patients with AML<sup>22</sup>. Autocrine or paracrine ligand-induced receptor activation will likely mitigate the effects of targeted kinase inhibitors of these receptors, in a manner analogous to the mechanisms by which HGF antagonizes inhibition of the MET kinase by crizotinib. Adaptive increases in ligand expression levels provide a means for cancer cell populations to re-establish the signal transduction pathways that existed prior to the onset of inhibitor treatment.

Clinical strategies will need to be developed to effectively overcome ligand-mediated resistance to targeted therapies. In the case of 8p11 stem cell leukemia involving *FGFR1* translocations, *FGFR1* activity is required for compensatory upregulation of HGF in response to MET inhibition. Combined inhibition of these co-activated pathways is highly synergistic due to the blockade of compensatory HGF upregulation, which leads to the sustained logarithmic cell kill that is required for clinically effective therapy.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

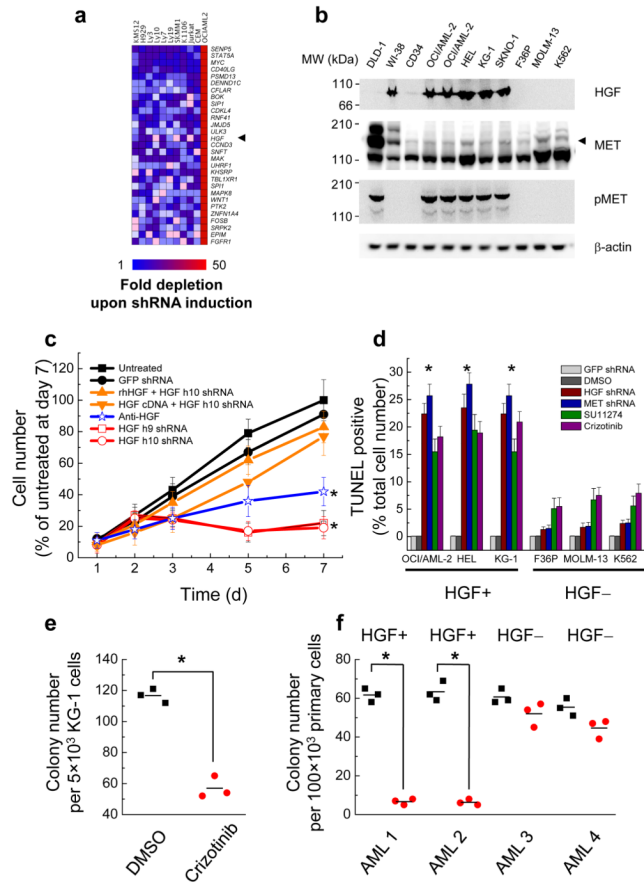
## Acknowledgments

We thank A. Gutierrez, M. Mansour and E. Gjini for critical discussions, and J. Gilbert for editorial advice. This research was supported by the National Institutes of Health K08CA160660 (A.K.), William Lawrence and Blanche Hughes Foundation (T.S.), Samuel Waxman Cancer Research Foundation (J.D.L.), the V Foundation (A.T.L.), and the Intramural Research Program of the National Cancer Institute, Center for Cancer Research (L.S.).

## References

1. Grimwade D, et al. The predictive value of hierarchical cytogenetic classification in older adults with acute myeloid leukemia (AML): analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 trial. *Blood*. 2001; 98:1312–1320. [PubMed: 11520776]
2. Burnett A, Wetzler M, Lowenberg B. Therapeutic advances in acute myeloid leukemia. *J Clin Oncol*. 2011; 29:487–494. [PubMed: 21220605]
3. Westbrook TF, Stegmeier F, Elledge SJ. Dissecting cancer pathways and vulnerabilities with RNAi. *Cold Spring Harb Symp Quant Biol*. 2005; 70:435–444. [PubMed: 16869781]

4. Bernards R, Brummelkamp TR, Beijersbergen RL. shRNA libraries and their use in cancer genetics. *Nat Methods*. 2006; 3:701–706. [PubMed: 16929315]
5. Ngo VN, et al. A loss-of-function RNA interference screen for molecular targets in cancer. *Nature*. 2006; 441:106–110. [PubMed: 16572121]
6. Whitehurst AW, et al. Synthetic lethal screen identification of chemosensitizer loci in cancer cells. *Nature*. 2007; 446:815–819. [PubMed: 17429401]
7. Turner NC, et al. A synthetic lethal siRNA screen identifying genes mediating sensitivity to a PARP inhibitor. *EMBO J*. 2008; 27:1368–1377. [PubMed: 18388863]
8. Scholl C, et al. Synthetic lethal interaction between oncogenic KRAS dependency and STK33 suppression in human cancer cells. *Cell*. 2009; 137:821–834. [PubMed: 19490892]
9. Gu TL, et al. Phosphotyrosine profiling identifies the KG-1 cell line as a model for the study of FGFR1 fusions in acute myeloid leukemia. *Blood*. 2006; 108:4202–4204. [PubMed: 16946300]
10. Wang C, Curtis JE, Minden MD, McCulloch EA. Expression of a retinoic acid receptor gene in myeloid leukemia cells. *Leukemia*. 1989; 3:264–269. [PubMed: 2538684]
11. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. MET, metastasis, motility, and more. *Nature Reviews Molecular Cell Biology*. 2003; 4:915–925.
12. Valk PJ, et al. Prognostically useful gene-expression profiles in acute myeloid leukemia. *N Engl J Med*. 2004; 350:1617–1628. [PubMed: 15084694]
13. Zou HY, et al. An orally available small-molecule inhibitor of c-Met, PF-2341066, exhibits cytoreductive antitumor efficacy through antiproliferative and antiangiogenic mechanisms. *Cancer Res*. 2007; 67:4408–4417. [PubMed: 17483355]
14. Mohammadi M, et al. Crystal structure of an angiogenesis inhibitor bound to the FGF receptor tyrosine kinase domain. *EMBO J*. 1998; 17:5896–5904. [PubMed: 9774334]
15. Sporn MB, Todaro GJ. Autocrine secretion and malignant transformation of cells. *N Engl J Med*. 1980; 303:878–880. [PubMed: 7412807]
16. Loriaux MM, et al. High-throughput sequence analysis of the tyrosine kinome in acute myeloid leukemia. *Blood*. 2008; 111:4788–4796. [PubMed: 18252861]
17. Haq R, et al. Constitutive p38HOG mitogen-activated protein kinase activation induces permanent cell cycle arrest and senescence. *Cancer Res*. 2002; 62:5076–5082. [PubMed: 12208764]
18. Majeti R, et al. Dysregulated gene expression networks in human acute myelogenous leukemia stem cells. *Proc Natl Acad Sci U S A*. 2009; 106:3396–3401. [PubMed: 19218430]
19. Robinson DR, Wu YM, Lin SF. The protein tyrosine kinase family of the human genome. *Oncogene*. 2000; 19:5548–5557. [PubMed: 11114734]
20. Zheng R, Klang K, Gorin NC, Small D. Lack of KIT or FMS internal tandem duplications but co-expression with ligands in AML. *Leuk Res*. 2004; 28:121–126. [PubMed: 14654075]
21. Zhou J, et al. Enhanced activation of STAT pathways and overexpression of survivin confer resistance to FLT3 inhibitors and could be therapeutic targets in AML. *Blood*. 2009; 113:4052–4062. [PubMed: 19144991]
22. Sato T, et al. FLT3 ligand impedes the efficacy of FLT3 inhibitors in vitro and in vivo. *Blood*. 2011; 117:3286–3293. [PubMed: 21263155]

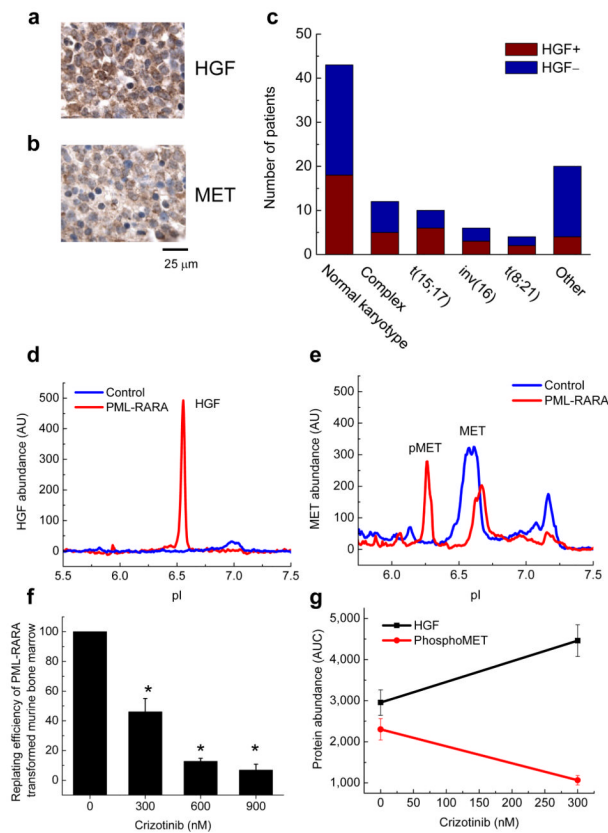


**Fig 1. Aberrant HGF expression by AML cells is associated with MET activation and is necessary for cell growth and survival**

(a) Heat map of the 30 top-ranking genes in the RNAi screen, whose depletion reduced the growth of OCI/AML-2 cells but not diffuse large B-cell lymphoma (Ly3, Ly10, Ly7, Ly19, K1106), myeloma (KMS12, H929, SKMM1), or T-cell acute lymphoblastic leukemia (Jurkat, CEM) cell lines. Relative cell depletion is represented by a blue/red color gradient. The master myeloid transcription factor *SPI1* served as the internal positive control; *HGF* is denoted with an arrowhead. (b) Western blot analysis of lysates of colon carcinoma DLD-1 cells with *MET* amplification, WI-38 fibroblasts expressing HGF, normal human CD34<sup>+</sup> cells, and seven AML cell lines; OCI/AML-2 is duplicated. HGF is detected with an apparent mobility of 90 kDa, corresponding to its intracellular pro-form, while MET is detected as both pro- and mature forms in DLD-1 cells (180 and 140 kDa, respectively), and predominantly as the mature form (140 kDa) in AML cells (arrowhead). (c) Growth of OCI/AML-2 cells is inhibited by transduction of specific shRNAs targeting HGF (h9 and h10) or by treatment with a neutralizing anti-HGF antibody (100 nM), but not by transduction of control shRNA (GFP) or by concomitant rescue with *HGF* cDNA or recombinant human HGF (0.1 nM). Measurements are normalized to the value for untreated cells at day 7, and shown as means and standard deviations of three biologic replicates. \*  $p < 0.05$  versus untreated control. (d) TUNEL analysis of AML cells that express HGF and activate MET (OCI/AML-2, HEL, KG-1) versus those that lack HGF expression (F36P, MOLM-13, K562) as a function of depletion of HGF or MET using RNAi, treatment with the MET kinase inhibitor SU11274 (1  $\mu$ M) or crizotinib (0.1  $\mu$ M) for 48 hours. Transduction with *GFP* shRNA and treatment with DMSO served as controls. Values are means and standard deviations of three biologic replicates. \*  $p < 0.05$  versus DMSO or *GFP* shRNA control.

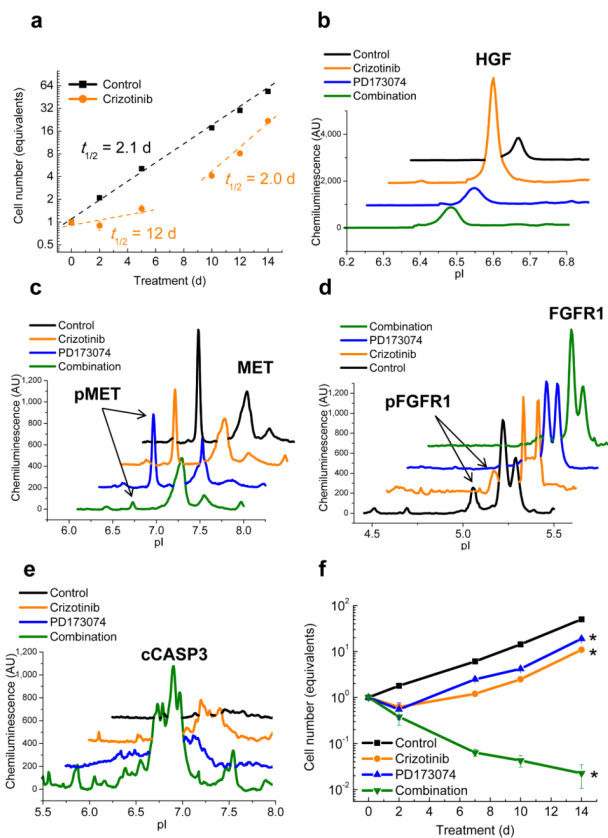


**(e,f)** Methylcellulose colony-forming assays of KG-1 cells (e) in the presence of DMSO control (black box) or crizotinib (0.1 $\mu$ M, red circle), and primary AML specimens (f) with aberrant HGF expression (AML 1, AML 2) versus those lacking HGF (AML 3, AML 4). Individual data points and means (bars) of three biologic replicates are shown. \*  $p < 0.05$  versus DMSO control.



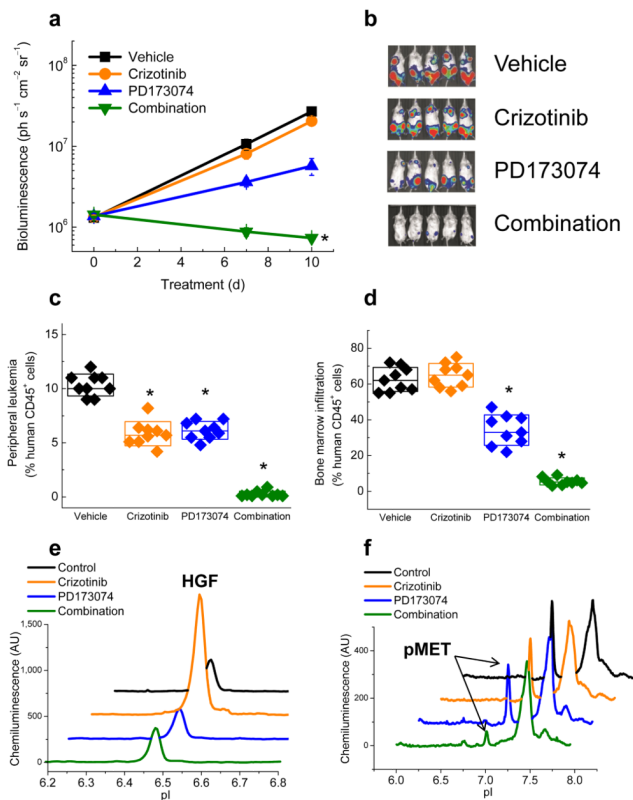
**Fig 2. HGF and MET are co-expressed in the leukemic blasts of patients with AML and are induced by leukemogenic transcription factors in primary mouse hematopoietic progenitor cells, conferring susceptibility to MET kinase inhibition**

(a,b) Immunohistochemical analysis of diagnostic bone marrow AML biopsy, demonstrating intracellular staining of HGF and pericytoplasmic membrane staining of MET in leukemic blasts, consistent with autocrine activation of MET. Scale bar = 25  $\mu$ m. (c) Distribution of primary AML specimens that co-express HGF and MET (HGF<sup>+</sup>) versus those that lack HGF expression (HGF<sup>-</sup>) by immunohistochemistry among patients with a normal karyotype, complex karyotype (complex), and other cytogenetic abnormalities, demonstrating aberrant HGF expression in 58 (42%) of the cases. (d) Abundance of mouse HGF at 7 days after retroviral transduction of mouse hematopoietic progenitors with *PML-RARA* (red) or vector control (blue), showing induction of HGF expression as measured with nanoimmunoassays. (e) Abundance of MET and phospho-MET (pMET) 7 days after retroviral transduction of mouse hematopoietic progenitors with *PML-RARA* (red) or vector control (blue), showing activation of MET upon induction of HGF expression. Equal protein loading was confirmed by the use of  $\beta_2$ -microglobulin as the loading control. (f) Colony replating efficiency of *PML-RARA* transformed mouse hematopoietic progenitor cells, as a function of increasing concentration of crizotinib. Values are normalized to the number of colonies in mock-treated cells and plotted as means and standard deviations of three biologic replicates. \*  $p < 0.05$  for comparison to DMSO treated cells. (g) Abundance of HGF (black) and phospho-MET (red) in *PML-RARA*-transformed mouse hematopoietic progenitor cells treated with 0 and 300 nM crizotinib, demonstrating inhibition of MET phosphorylation and upregulation of HGF.



**Fig 3. Restoration of leukemic cell growth upon chronic MET kinase inhibitor treatment is due to compensatory upregulation of HGF and MET re-activation, which can be overcome by inhibiting compensatory upregulation of HGF**

(a) Kinetics of growth of OCI/AML-2 cells treated with crizotinib (0.1 μM in DMSO) or vehicle (DMSO), demonstrating that acute crizotinib treatment leads to significant reduction in AML cell growth (doubling time of 2.1 versus 12 days,  $p < 0.05$ ), while with chronic treatment (10 days), the doubling time is 2.0 days. Cells were split and culture medium changed to maintain a constant cell density of 1 million cells  $\text{ml}^{-1}$ . (b) Abundance of HGF in KG-1 cells treated for 10 days with DMSO (black), 100 nM crizotinib (orange), 20 nM PD173074 (blue), or a combination of crizotinib and PD173074 (green), as measured by quantitative nanoimmunoassay, with  $\beta_2$ -microglobulin as the loading control, also see (Supplementary Fig. 16e). (c) MET activation as assessed by the abundance of phospho-MET (pMET) in KG-1 cells treated for 10 days with the indicated drugs, demonstrating maintenance of MET signaling in cells treated with 100 nM crizotinib or 20 nM PD173074, but not in cells exposed to combination treatment. (d) FGFR1 activation as assessed by abundance of phospho-FGFR1 (pFGFR1) in KG-1 cells treated for 10 days with the indicated drugs, demonstrating lack of effect of crizotinib on FGFR1 activity. (e) Induction of apoptosis as assessed by the abundance of cleaved caspase 3 (cCASP3) in KG-1 cells treated for 10 days demonstrating substantially greater induction of apoptosis in cells treated with combination of 100 nM crizotinib and 20 nM PD173074 as compared to either drug alone. (f) Combined treatment of KG-1 cells with crizotinib and PD173074 leads to sustained logarithmic cell kill as compared to either drug alone. Values are means and standard deviations of three biologic replicates. \*  $p < 0.05$  versus either drug alone.



**Fig 4. Combined inhibition of MET and FGFR1 blocks compensatory upregulation of HGF, leading to sustained inhibition of MET in KG-1 cells and to near-complete regression of AML *in vivo***

(a) Bioluminescence measurements of leukemic mice engrafted with luciferase-modified KG-1 cells and treated with vehicle control (black),  $50 \text{ mg}^{-1} \text{ kg}^{-1}$  crizotinib alone (orange),  $25 \text{ mg}^{-1} \text{ kg}^{-1}$  PD173074 (blue), or a combination of crizotinib and PD173074 by daily oral gavage (green). Values are means and standard deviations of each treatment group ( $n = 9$ ). \*  $p < 0.05$  versus each remaining group. (b) Bioluminescent photographs of representative mice from each treatment group (blue-to-red color gradient represents increasing bioluminescence intensities). (c,d) Scatter plots of the fraction of human CD45<sup>+</sup> KG-1 cells in the peripheral blood (c) and bone marrow (d) of mice after 10 days of treatment, demonstrating near-complete elimination of human AML cells in mice treated with the combination of crizotinib and PD173074. Boxes denote means and standard deviation for each group ( $n = 9$ ). \*  $p < 0.05$  versus vehicle control group. (e,f) Abundance of HGF (e) and phospho-MET (f) in human CD45-selected KG-1 cells isolated from the bone marrow of mice after 10 days of treatment as indicated, demonstrating blockade of compensatory HGF upregulation in response to crizotinib treatment by the combined inhibition of MET and FGFR1, and sustained inhibition of MET activation.