Diverse disease phenotypes are observed in tumors expressing activated oncogenes, but clinical resistance is clearly associated with reactivation of the target by a mutation that prevents drug binding. This phenomenon was first described in chronic myeloid leukemia (CML) (1), but this paradigm has been extended to multiple human cancers responsive to TKI therapy including epidermal growth factor receptor (EGFR)-mutant lung cancer (2), gastrointestinal stromal tumor (GIST) driven by c-KIT (3), and, recently, acute myeloid leukemia (AML) associated with mutations in Fms-like tyrosine kinase-3 (FLT3) (4). Many oncogenes effectively targeted by current clinical therapeutics encode kinases constitutively activated by mutation through a variety of mechanisms identified in clinical samples, including point mutations and in-frame deletions or duplications as observed in c-KIT-mutant GIST (5), EGFR-mutant lung cancer (6), and FLT3-mutant AML (7, 8). However, how individual mechanisms activating the same kinase (i.e., point mutation vs. in-frame duplication) may affect disease phenotype and response to therapy remains largely unexplored. In PNAS, Bailey et al. (9) describe a mouse knock-in model of FLT3 activated by an in-frame internal tandem duplication (ITD) in the juxtamembrane (JM) domain. This work provides clear evidence that different mutations, although they may result in constitutive activation of the same kinase, may not be equivalent and can result in diverse disease phenotypes.

FLT3 is a class III receptor tyrosine kinase that plays an important role in normal hematopoiesis (10) and is mutated in ~30% of AML. Recent large-scale genomic sequencing efforts have confirmed that FLT3 is the most commonly mutated gene in human AML (11), with ~20% of mutations consisting of ITD mutations in the JM domain (12) and with an additional subset (~7–10%) consisting of point mutations in the FLT3 tyrosine kinase domain (TKD), commonly at the activation loop residue D835 (8, 12). FLT3-ITD mutations have been clearly associated with poor prognosis (13), whereas the prognostic significance of FLT3 TKD mutations has been less clear (8, 12). Although both FLT3-ITD and FLT3 TKD mutations cause ligand-independent kinase activation, in vitro studies have identified differential autophosphorylation (14) and downstream signaling patterns for FLT3-ITD (15) compared with FLT3 TKD and native FLT3, in particular preferential activation of STAT5 (16) by FLT3-ITD, as well as increased proliferation and clonogenic growth potential in cellular models (16). It has been suggested that this differential signaling is the result, in part, of aberrant trafficking of FLT3-ITD mutant receptors resulting in prolonged retention in the endoplasmic reticulum (ER) and increased exposure to intracellular substrates such as STAT5 (17). In a murine bone marrow (BM) transduction and transplantation model, FLT3 D835Y yields an oligoclonal lymphoid disorder with longer disease latency distinct from the myeloproliferative neoplasm (MPN) observed with FLT3-ITD (18). It is also notable that, although FLT3-ITD and D835 mutations have rarely been known to co-occur de novo in individual patients, recent translational studies have established that secondary FLT3 D835 mutations co-occurring on the same allele as FLT3-ITD (FLT3-ITD/D835V/Y/F) are a frequent cause of acquired resistance to FLT3 inhibitors (4). At this point, it is unknown whether these compound FLT3-ITD/D835 mutations function more like FLT3-ITD or FLT3 D835 mutations in regard to signal transduction, proliferative and transforming potential, and effect on disease phenotype.

In the last decade, much attention in oncology drug development has focused on exploiting “oncogene addiction,” the premise that, despite multiple genetic lesions, some tumors remain reliant on a single oncogene for maintenance of a malignant phenotype associated with cellular proliferation and survival. Compelling evidence for the concept of oncogene addiction arises from genetically engineered mouse models in which an inducible oncogene expressed in a specific target tissue can give rise to tumorigenesis, but tumor regression is observed when the oncogene is effectively turned off again by genetic or pharmacologic means. Convincing support that prevents drug binding. This phenomenon was first described in chronic myeloid leukemia (CML) (1), but this paradigm has been extended to multiple human cancers responsive to TKI therapy including epidermal growth factor receptor (EGFR)-mutant lung cancer (2), gastrointestinal stromal tumor (GIST) driven by c-KIT (3), and, recently, acute myeloid leukemia (AML) associated with mutations in Fms-like tyrosine kinase-3 (FLT3) (4). Many oncogenes effectively targeted by current clinical therapeutics encode kinases constitutively activated by mutation through a variety of mechanisms identified in clinical samples, including point mutations and in-frame deletions or duplications as observed in c-KIT-mutant GIST (5), EGFR-mutant lung cancer (6), and FLT3-mutant AML (7, 8). However, how individual mechanisms activating the same kinase (i.e., point mutation vs. in-frame duplication) may affect disease phenotype and response to therapy remains largely unexplored. In PNAS, Bailey et al. (9) describe a mouse knock-in model of FLT3 activated by a point mutation in the FLT3 kinase activation loop, D835Y, that contrasts phenotypically with their previously described and otherwise genetically identical, knock-in model of FLT3 activated by an in-frame internal tandem duplication (ITD) in the juxtamembrane (JM) domain. This work provides clear evidence that different mutations, although they may result in constitutive activation of the same kinase, may not be equivalent and can result in diverse disease phenotypes.

FLT3 is a class III receptor tyrosine kinase that plays an important role in normal hematopoiesis (10) and is mutated in ~30% of AML. Recent large-scale genomic sequencing efforts have confirmed that FLT3 is the most commonly mutated gene in human AML (11), with ~20% of mutations consisting of ITD mutations in the JM domain (12) and with an additional subset (~7–10%) consisting of point mutations in the FLT3 tyrosine kinase domain (TKD), commonly at the activation loop residue D835 (8, 12). FLT3-ITD mutations have been clearly associated with poor prognosis (13), whereas the prognostic significance of FLT3 TKD mutations has been less clear (8, 12). Although both FLT3-ITD and FLT3 TKD mutations cause ligand-independent kinase activation, in vitro studies have identified differential autophosphorylation (14) and downstream signaling patterns for FLT3-ITD (15) compared with FLT3 TKD and native FLT3, in particular preferential activation of STAT5 (16) by FLT3-ITD, as well as increased proliferation and clonogenic growth potential in cellular models (16). It has been suggested that this differential signaling is the result, in part, of aberrant trafficking of FLT3-ITD mutant receptors resulting in prolonged retention in the endoplasmic reticulum (ER) and increased exposure to intracellular substrates such as STAT5 (17). In a murine bone marrow (BM) transduction and transplantation model, FLT3 D835Y yields an oligoclonal lymphoid disorder with longer disease latency distinct from the myeloproliferative neoplasm (MPN) observed with FLT3-ITD (18). It is also notable that, although FLT3-ITD and D835 mutations have rarely been known to co-occur de novo in individual patients, recent translational studies have established that secondary FLT3 D835 mutations co-occurring on the same allele as FLT3-ITD (FLT3-ITD/D835V/Y/F) are a frequent cause of acquired resistance to FLT3 inhibitors (4). At this point, it is unknown whether these compound FLT3-ITD/D835 mutations function more like FLT3-ITD or FLT3 D835 mutations in regard to signal transduction, proliferative and transforming potential, and effect on disease phenotype.

Fig. 1. Diverse disease phenotypes are observed in genetically identical knock-in murine models with (A) FLT3-ITD mutation and (B) FLT3 D835Y mutation.

Author contributions: C.C.S. wrote the paper. The author declares no conflict of interest. See companion article 10.1073/pnas.1310559110.

1 E-mail: csrsmith@medicine.ucsf.edu.
In light of previous in vitro and in vivo data suggesting signaling and phenotypic differences induced by FLT3-ITD and FLT3 TKD mutant isoforms, it is perhaps not surprising that Bailey et al. (9) confirmed in a knock-in mouse model where Flt3 is expressed under control of the endogenous promoter that disease induced by FLT3 D835Y is phenotypically distinct from disease induced by FLT3-ITD. In contrast to FLT3-ITD mice, which exclusively develop MPN (19), the FLT3 D835Y mice develop a MPN with longer latency and broader range of disease phenotypes, including some lymphoid disease such as associated extranodal B-cell masses and T cell–rich B-cell lymphoma. FLT3 D835Y mice also exhibited expansion of pre-Pro-B, early and late Pro-B-cell populations with a normal fraction of more mature B-cell populations, whereas FLT3-ITD mice exhibit a block between early and late Pro-B transition with associated decrease in later B-cell populations. Further examination of the expanded Pre-Pro-B and early Pro-B populations in both models showed increased Pax5 expression in the D835Y mice, whereas ITD mice demonstrated increased expression of myeloid transcription factors PU.1 and CCAAT/enhancer binding protein α (CEBPα) consistent with the marked myeloid expansion observed in the ITD model. Altogether, these data suggest an increased permissiveness of FLT3 D835Y for lymphoid neoplasms, whereas FLT3-ITD appears to be skewed more exclusively toward myeloid disease. It is notable that such a bias has also been observed in human leukemia where in acute lymphoblastic leukemia, FLT3 mutations, although rare, have largely manifested as activation loop mutations or insertions/deletions in the JM domain (20, 21) rather than the ITD mutations more commonly associated with AML (10).

Further confirming the ability of this model to recapitulate known features of FLT3 mutant leukemias, Lin− BM and sorted KSL (Lin− c-KIT+ Sca-1+) from FLT3 D835Y mice demonstrated decreased levels of STAT5 phosphorylation, and Lin− BM of D835Y mice showed decreased expression of STAT5 target genes compared with FLT3-ITD mice. Whole BM cells isolated from FLT3 D835Y mice demonstrated decreased proliferation and STAT5 phosphorylation in response to leukaemia, a FLT3 TKI with activity against FLT3 D835 mutations, but not to sorafenib, which has no activity against D835 mutations, suggesting that this model may effectively predict response to FLT3 TKIs in vivo.

It is hoped that knock-in mouse models of oncoprotein addiction such as the FLT3 D835Y model described by Bailey et al. can effectively model and recapitulate human disease and ultimately provide insight into disease pathogenesis that would be difficult to obtain by other means. Insight into the differential effect of FLT3 ITD and TKD mutations on hematopoietic stem cell (HSC) function and its potential role in disease pathogenesis is one advantage provided by such murine models. In their analysis of the stem cell compartment, Bailey et al. observe that, in contrast to their recent finding that FLT3-ITD causes expansion of the myeloid progenitor population by mobilization of the normally quiescent KSL SLAM (Lin− c-KIT+ Sca-1+ CD150+ CD48−) cells into cell cycle, effectively depleting their stem cell compartment, the HSC compartment of FLT3 D835Y mice appears relatively preserved with a normal frequency of KSL SLAM cells and preserved functional engrafment potential. The role of these differences in the HSC compartment in disease pathogenesis and how they might potentially be therapeutically exploited remain to be elucidated.

As noted by the authors, neither FLT3-ITD nor FLT3 D835Y mutations are sufficient to cause leukemia on their own, suggesting a clear role for cooperating mutations in leukemogenesis. Recent genomic sequencing efforts have identified clear links between subsets of FLT3 mutations and other genetic lesions in AML, notably the frequent co-occurrence of FLT3-ITD, NPM1, and DNMT3A mutations (11), as well as a recently described association between a novel activating kinase domain mutation, N676K, and the core-binding factor rearrangements (t(8,22) and inv(16)(t(16,16)) (22). Future efforts will need to focus on investigating the role of these and other cooperating lesions in modulating disease phenotype, prognosis, and response to therapy. Establishing genetically engineered murine models of AML through endogenous coexpression of FLT3 D835Y and known cooperating AML lesions will generate important tools to explore mechanisms of leukemogenesis, test novel therapeutic strategies, and model drug resistance in vivo.