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Genetic and Epigenetic Heterogeneity in Acute Myeloid Leukemia

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Abstract

Genetic and epigenetic heterogeneity is emerging as a fundamental property of human cancers. Reflecting the genesis of tumors as an evolutionary process driven by clonal selection. The complexity of clonal architecture has been known for many years in the setting of acute myeloid leukemia (AML), based on karyotyping studies. However the true complexity of AMLs is only now being understood thanks to in depth genome sequencing studies in humans, which reveal that heterogeneity is a multilayered and involves not only the genome but also the epigenome. Here, we review recent advances in genetic and epigenetic heterogeneity and clonal dynamics in AML and their relevance to biology, clinical outcomes and therapeutic implications. Special attention is focused on somatic mutations affecting regulators of cytosine methylation, since these tend to occur early in disease evolution, reprogram the epigenome of hematopoietic stem cells, and are linked to unfavorable outcome.

Introduction

Acute myeloid leukemias (AML) are tumors that arise from hematopoietic stem and progenitor cells. During the course of malignant transformation, these cells undergo continuous genetic and epigenetic evolution and clonal diversification. As a consequence, AMLs are composed of heterogeneous populations of malignant cells. Subclones of leukemia cells typically contain distinct sets of cytogenetic abnormalities and somatic mutations, resulting in considerable genetic complexity. More recently it has been appreciated that diversification in the epigenome can also endow subpopulations of cells

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with unique characteristics. Genetic and epigenetic heterogeneity in AML is multifaceted and includes (but is not limited to): (1) Inter-patient mutational heterogeneity, (2) intra-patient sequential acquisition of mutations, including different mutations in the same genes, (3) clonal diversification and complexity, (4) functional hierarchical complexity (i.e. subpopulations of cells with greater stem cell potential, etc), (5) epigenetic signatures, some of which may be linked to specific somatic mutations, (6) global epigenetic heterogeneity, and (7) focal epigenetic allelic diversity at specific genomic loci. Understanding how genetic and epigenetic heterogeneity develop in leukemia, whether and how they are interrelated, and what they contribute to the disease from a clinical standpoint is an important new area of research. Herein, we will consider the genetic and epigenetic heterogeneity of AML, with a special focus on intersections between these two layers. In particular we will highlight the impact of mutations in epigenetic modifier genes, which occur early in disease and can profoundly disrupt the epigenetic landscape and phenotype of hematopoietic cells, thus creating a functional link between genetic and epigenetic heterogeneity.

Recurrent Somatic Mutations in Acute Myeloid Leukemia

Characterization of AML genomes by next generation sequencing has revealed that these tumors exhibit a relatively low recurrent somatic mutation rate as compared to other tumor types [1]. From the functional perspective, somatic mutations in AML can be classified in several ways. One approach splits genetic lesions into two classes. The first class corresponded mostly to mutation in genes from the receptor tyrosine kinase family, including Fms-Related Tyrosine Kinase 3 (*FLT3*)[2], V-Kit Hardy-Zuckerman 4 Feline Sarcoma Viral Oncogene Homolog (*KIT*), and Neuroblastoma RAS Viral (V-Ras) Oncogene Homolog (*NRAS*). These mutations mainly enhance proliferation and survival of the hematopoietic progenitors through activation of signaling pathways. The second class of mutations mainly occurs in transcription factors such as *RUNX1*, *CEBPA* and *RARA*. Mutations or translocations affecting these factors hinder cell differentiation and create an accumulation of immature progenitors. Gilliland & Griffin proposed a “double-hit” model of leukemogenesis based on the frequent observation of mutations in each of these two classes in AML. They proposed that either class of mutation is insufficient to cause malignant transformation of hematopoietic stem cells. Only the co-occurrence of a class I and class II mutation would cooperate to form leukemias. However, there are limitations to the double-hit model, in that not every AML harbors mutations that strictly correspond to these two classes of mutations[1]. Indeed, a recent epigenomics study showed that combination of a class I and class II mutations, rather than cause additive effects, instead cooperated in a synergistic manner to induce distinct epigenetic and transcriptional programming effects than what was caused by either mutation alone[3]. Current sequencing efforts have thoroughly defined the recurrent mutations in AML and classified them according to the functions of the proteins they disrupt[1]. Here we focus mainly on somatic mutations in epigenetic modifiers.

Sequential acquisition of mutations in AML

Each case of AML is a complex mosaic of cells containing different combinations of genetic lesions and epigenetic variants [4-6]. Clonal evolution within each AML patient appears to

be a dynamic process whereby there is continuous acquisition and loss of specific mutations, sometimes occurring in different timepoints. The end result is simultaneous evolutionary convergence and divergence among particular clones and subclones during the course of disease [4,5]. Exposure to chemotherapy regimens places an enormous stress on AML cell populations, and may be more toxic to certain clones than others. It has been observed that clonal composition of AMLs can change quite markedly after therapy in relapsed disease, with selection occurring at both the genetic and epigenetic levels [4,6]. Along these lines, the genetic composition of clones has been proven to be functionally significant. Within the same AML patient certain clones may exhibit distinct morphology, differentiation markers, and engraftment potential in immunocompromised mice [7]. Among AML cells it is the subpopulation of more immature leukemia stem cells that are believed to perpetuate and repopulate the disease, and which are more chemotherapy resistant. Hence leukemia stem cell functionality may be a reflection of subclonal combinations of specific mutations, with the emergence of chemoresistance and/or tumor changes potentially driven by these clonality shifts [6].

Other research points towards heterogeneity in the order of acquisition of mutations during AML pathogenesis. First of all, as individuals age there is accumulation of somatic mutations in their hematopoietic cells [8,9]. In older patients leukemia initiator mutations may occur in the context of hematopoietic cells that already harbor other somatic variants, which then become “passenger” lesions [9]. Somatic mutations in the epigenetic modifiers *DNMT3A*, *IDH1/2* and *TET2* are initiating mutations in AML, especially those with normal karyotype [9-11]. Whereas mutations of the *NPM1* gene that encode the aberrant *NPM1c* protein have also been described as initial hits [9], clonal evolution analysis of AML patients at relapse, results suggests that at least in cases with *DNMT3A* + *NPM1* that the *DNMT3A* mutations may precede *NPM1* [12], since *DNMT3A* mutation persisted at relapse in cases where *NPM1* mutation was lost. Shlush, *et al.* established a sequential order of mutation acquisition whereby *DNMT3A* and *IDH2* mutations exist in pre-leukemic HSCs and precede *NPM1c* and *FLT3-ITD* [10]. Concurrently, Corces-Zimmerman, *et al.* also found that somatic mutations in epigenetic modifiers that regulate cytosine methylation occur early in pre-leukemia cells and persist once AML goes in remission, whereas somatic mutations in signaling pathways that drive proliferation are later events in AML transformation [13]. These data suggest that disruption of epigenetic patterning is likely an early and prominent event during leukemogenesis (Figure 1).

Heterogeneous cytosine methylation patterning in AML

Although presenting relatively few somatic mutations, patients with AML exhibit profound and heterogeneous disruption of their cytosine methylation landscapes [14]. Indeed DNA methylation signatures can be used to classify AMLs into sixteen disease subtypes with distinct biological and clinical features [14]. Several of these epigenetic signatures were linked to known translocations or mutations in transcription factors. Others had no obvious link to genetic background. However examination of these signatures together with mutational profiling helped to show how newly discovered genetic lesions can directly perturb epigenetic programming of hematopoietic cells [15]. The nature of the DNA methylation defect in AML cases is quite variable with some patients exhibiting dominant

hypomethylation, others showing dominant hypermethylation, and yet others showing signatures with intermediate hypo and hypermethylation[14]. Recent genetic and mechanistic studies can explain at least in part the genesis of some of these diverse and opposing signatures, which can be linked back to the presence of epigenetic driver mutations that initiate malignant transformation of hematopoietic cells.

Mutations that perturb cytosine methylation patterning in AML

The presence of hypermethylated signatures could be linked to loss of mechanisms that remove methyl groups from cytosine. One particular hypermethylated signature was associated with somatic mutation of the metabolic enzymes isocitrate dehydrogenase 1 and 2 (IDH1/2)[15]. IDH enzymes normally catalyze the conversion of isocitrate into alpha ketoglutarate (aKG). aKG serves as a cofactor for dioxygenase enzymes including the 2-oxoglutarate (2OG)- and Fe(II)-dependent TET family of enzymes. TET enzymes catalyze the conversion of 5-methylcytosine to 5 hydroxymethylcytosine (5hmC), which is an intermediate step in the process of DNA demethylation [16]. *IDH1/2* mutations result in a gain of function that causes the enzyme to aberrantly convert aKG into 2-hydroxyglutarate (2HG), which can compete with aKG for binding to dioxygenase enzymes such as the TETs thus suppressing their function[17]. Notably, AMLs also feature frequent loss of function mutations of TET2, and these patients exhibit a similar DNA hypermethylation signature to *IDH1/2* patients[15,18]. *IDH1/2* and *TET2* mutations are virtually mutually exclusive, genetically confirming that they affect a common pathway to leukemic transformation[15]. Mouse models of IDH1 mutation or TET2 loss of function recapitulate the human epigenetic profiles, and center on gene pathways such as WNT and TGFb[3,19].

In addition to a common hypermethylation signature, *IDH1/2* and *TET2* mutant AMLs share corresponding reduction in 5hmC levels[18]. Genome wide profiling of 5hmC patterning showed that the pattern of 5hmC loss is also quite similar between IDH1/2 and TET2 mutant patients[18]. Somatic mutations in the transcription factor WT1 could also induce loss of 5hmC[18]. Strikingly, WT1 mutations were mostly exclusive of TET2 and IDH1/2 mutations. WT1 could interact with and recruit TET2 to its target genes, and its loss of function could complement TET2[18,20]. Collectively these findings suggest that mutations perturbing the IDH-TET2-WT1 axis all result in specific and focal loss of 5hmC as well as specific and focal increases in 5-methylcytosine. Both of these epigenetic marks may be important influences on gene expression since 5-methylcytosine was inversely correlated with gene expression and 5hmC is positively correlated with gene expression in these AML cases[18], with 5hmC predicting gene expression even better than 5mC. In this way, these apparently heterogeneous set of mutations and epigenetic signatures can be grouped together based on sharing a common mechanism of transcriptional regulation and cellular action.

Another major influence on DNA methylation patterning is the frequent somatic mutation of the DNA methylation writer DNMT3A, occurring in ~30% of AML patients [1,21]. Many of these lesions are focused on a particular hot spot resulting in a R882H mutation, whereas others may truncate the protein. Researchers further discovered that R882H DNMT3A mutation dominantly inhibits wild-type DNMT3A by blocking its ability to form active tetramers, and AML cases with this mutation exhibit focal hypomethylation [22-24].

DNMT3A has been reported as a critical mediator of epigenetic regulation of hematopoietic stem cell regulatory genes, thereby contributing to efficient differentiation[25]. Its regulatory effect has been linked to an activity in regulating the spreading of edges of hypomethylated regions that have been called methylation “canyons”[26]. Loss of DNMT3A results in increased stem cell self renewal and defective hematopoietic differentiation[25], perhaps linked in part to hypomethylation of the oncogenic transcription factor Meis1[27]. Moreover mice lacking DNMT3A can develop fatal myeloproliferative disease and may eventually also develop a variety of hematopoietic neoplasms [28-30].

TET2 and DNMT3A mutations are linked to inferior outcome in AML, suggesting that mutations that induce either hyper or hypo methylation can both result in more chemotherapy resistant disease. It is important to underline again that AMLs represent a heterogeneous population of cells from a functional standpoint, among which leukemia stem cells carry the greatest potential to maintain and repopulate disease after treatment. Along these lines it is interesting that TET2, IDH and DNMT3A mutations are proposed to be initial or early lesions in pre-leukemic hematopoietic stem cells, and may lead to a clonally expanded pool of pre-leukemic hematopoietic stem cells [9,10]. Elderly individuals feature an increased incidence of TET2 and DNMT3A mutation in their hematopoietic cells[11] and the presence of these mutations may predict the later onset of hematopoietic neoplasm [8]. These findings underline the power of somatic mutations in epigenetic modifiers for reprogramming normal cells towards a path that can lead to enhanced stem cell functionality and malignant transformation.

Yet DNMT3A, TET2 and IDH mutations are not enough to transform cells to create leukemia. This would seem to indicate that their downstream epigenetic effects are insufficient to reprogram the phenotype of HSCs. And indeed the presence of these mutations in individuals with normal hematopoiesis is consistent with this notion. The recent observation that TET2 alone exerts surprisingly little perturbation of cytosine methylation, but causes massive epigenetic reprogramming in the presence of FLT3 mutation [3] suggests that perhaps the real epigenetic “hit” in leukemia comes from combinatorial effects of mutations as opposed to the actions of individual genetic lesions. This notion was confirmed in the case of TET2 and FLT3, which in combination, but not individually, mediate hypermethylation and aberrant silencing of GATA2 [3]. Restoration of GATA2 expressed rescued and reversed the leukemic effect of TET2 and FLT3 combination [3]. Collectively these data suggest that many epigenetic signatures in AML - rather than being the product of individual translocations or mutations - are the product of their combinatorial, synergistic effects. Considering that AMLs are composed of multiple combinations of genetic lesions within different subclones, it then becomes likely that cytosine methylation patterns captured thus far by profiling unselected patient specimens must actually represent a composite of many different epigenetic signatures that occur in a subclone-specific manner. This may explain why and how genetically defined subclones can manifest distinct phenotypes as described by Klco et. al [7].

Global and focal epigenetic heterogeneity and allelic diversity in AML

Epigenetic marks characteristically display great plasticity. In addition to the effects of somatic mutations, cytosine methylation changes can occur naturally as cells replicate, as individuals age, or due to changes in nutrition, metabolism, environmental stimuli or inflammatory conditions[31]. Collectively these influences can cause cytosine methylation distribution to vary among cells within a given tissue. Much of this variability is likely of little functional consequence. However, there is some evidence that DNA methylation heterogeneity is not completely random and may occur preferentially at epigenetic hypervariable hotspots [32]. These sites tended to vary to an even greater degree in tumor cells and were accompanied by changes in the expression of nearby genes suggesting that these focal variability hotspots have functional relevance [32]. It is possible that sites of focal epigenetic variance could allow cells within the same population to sample different transcriptional states, resulting in greater evolutionary fitness. In this manner the presence of focal “epigenetic alleles,” or epialleles, could carry a similar significance to genetic alleles and perhaps follow similar subclonal distributions. Other studies used metrics to quantify global DNA methylation heterogeneity in lymphoid neoplasms and prostate cancer (e.g. [33-35]). The clinical significance of global DNA methylation heterogeneity was first shown in the context of DLBCL, where it was an independent factor associated with unfavorable outcome[36]. Subsequent studies measuring focal DNA methylation heterogeneity confirmed that these focal variability sites were also linked to inferior outcome both in DLBCL and CLL [34,35]. Very little is known of this form of epigenetic heterogeneity in the context of AML. However one recent study developed an entropy-based algorithm to measure shifts in epigenetic allelic diversity to study AML progression [6]. These studies revealed marked changes in epigenetic allele composition and diversity during disease progression. Not surprisingly epigenetic allelic diversity was increased in AML when compared to normal control bone marrow cells [6]. Further studies are needed to explore the biological and clinical significance of epigenetic allelic diversity in AML. It is not known whether genetic and epigenetic heterogeneity and allelic diversity are linked or independent of each other in any tumor type. However data available to date as explained above suggest that epigenetic and genetic lesions are not always associated. If this is indeed the case then there could be additive effects between the two layers of heterogeneity such that patients with the lowest overall heterogeneity could manifest relatively more favorable clinical outcomes than the patients with highest combined heterogeneity in the genetic and epigenetic compartments (Figure 2).

Conclusion

The many levels of genetic and epigenetic heterogeneity represent an extraordinary challenge to our ability to understand the composition, biology, phenotypic potential and therapeutic vulnerabilities of acute leukemias. Improved methods to track and characterize subclonal populations of cells in AML are required to gain a full understanding of these phenomena[37]. From the precision medicine perspective it is important to keep in mind that targeting mutant proteins that are not represented throughout the entire population of leukemic cells is unlikely to do more than partially control the disease. There is some hope that targeting initiator mutations such as IDH1, IDH2 could have broad effects. Recently

developed mutant IDH1 and IDH2 specific inhibitors seem to reverse the phenotypic and epigenetic effects of these mutations and are currently in clinical trials[38-40]. Nonetheless addressing through targeted therapy a genetically complex population of cells remains a daunting proposition. On the other hand, epigenetic heterogeneity could potentially be easier to tackle given that cytosine methylation and other marks are erasable. Epigenetic targeted therapy drugs could perhaps reduce epigenetic complexity of AMLs. Although drugs as DNA methyltransferase inhibitors have not been studied in this way, it seems warranted to explore their activity and significance in reducing epigenetic heterogeneity.

References

- 1•. Cancer Genome Atlas Research N. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med.* 2013; 368:2059–2074. The TCGA cancer consortium comprehensively profiled 200 adult AML patients with genetic, epigenetic, transcriptomic information. They summarized nine functional classes of mutations. The dataset provides invaluable asset for the computational biologist to explore and identify potential biomarkers for diagnosis and treatment. [PubMed: 23634996]
2. Gilliland DG, Griffin JD. The roles of FLT3 in hematopoiesis and leukemia. *Blood.* 2002; 100:1532–1542. [PubMed: 12176867]
- 3•. Shih AH, Jiang Y, Meydan C, Shank K, Pandey S, Barreyro L, Antony-Debre I, Viale A, Socci N, Sun Y, et al. Mutational cooperativity linked to combinatorial epigenetic gain of function in acute myeloid leukemia. *Cancer Cell.* 2015; 27:502–515. The Authors demonstrate for the first time that somatic mutations in AML can cooperate to synergistically reprogram the epigenome. The cooperativity of the two class of mutations which are sequentially acquired at disease initiation (TET2) and progression (FLT3-ITD) indicate that AML evolution is not necessarily the consequence of additive effects. They show that epigenetic synergy of these mutations results in aberrant silencing of key tumor suppressors, which are demonstrated to drive AML phenotype. [PubMed: 25873173]
4. Ding L, Ley TJ, Larson DE, Miller CA, Koboldt DC, Welch JS, Ritchey JK, Young MA, Lamprecht T, McLellan MD, et al. Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. *Nature.* 2012; 481:506–510. The authors using whole genome profiling determined the clonal architecture of 8 AML patients from diagnosis stage to relapse stage. They proposed two AML clonal evolution models based on the composition of clones at these clinical timepoints. [PubMed: 22237025]
5. Paguirigan AL, Smith J, Meshinchi S, Carroll M, Maley C, Radich JP. Single-cell genotyping demonstrates complex clonal diversity in acute myeloid leukemia. *Sci Transl Med.* 2015; 7:281re282.
- 6•. Li S, Garrett-Bakelman F, Perl AE, Luger SM, Zhang C, To BL, Lewis ID, Brown AL, D'Andrea RJ, Ross ME, et al. Dynamic evolution of clonal epialleles revealed by methclone. *Genome Biol.* 2014; 15:472. The authors developed an entropy-based approach to determine shifts in epigenetic allelic diversity. They show for the first time that epigenetic allelic diversity undergoes significant shifts during AML progression. [PubMed: 25260792]
- 7•. Klco JM, Spencer DH, Miller CA, Griffith M, Lamprecht TL, O'Laughlin M, Fronick C, Magrini V, Demeter RT, Fulton RS, et al. Functional heterogeneity of genetically defined subclones in acute myeloid leukemia. *Cancer Cell.* 2014; 25:379–392. The authors used flow cytometry and deep sequencing and functional assays to demonstrate for the first time that genetically distinct AML subclones may exhibit distinct phenotypes including leukemia repopulating potential. They also showed that the engraftment of AML cells in mice did not always fully represent AML evolution trajectory. [PubMed: 24613412]
- 8•. Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, Lindsley RC, Mermel CH, Burt N, Chavez A, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med.* 2014; 371:2488–2498. The authors demonstrate that somatic mutations in epigenetic modifiers can occur in hematopoietic stem cells during the aging process and may augur the development of hematologic malignancies. [PubMed: 25426837]

9. Welch JS, Ley TJ, Link DC, Miller CA, Larson DE, Koboldt DC, Wartman LD, Lamprecht TL, Liu F, Xia J, et al. The origin and evolution of mutations in acute myeloid leukemia. *Cell*. 2012; 150:264–278. [PubMed: 22817890]
- 10•. Shlush LI, Zandi S, Mitchell A, Chen WC, Brandwein JM, Gupta V, Kennedy JA, Schimmer AD, Schuh AC, Yee KW, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature*. 2014; 506:328–333. This article showed that somatic mutations present in the AML founding clone may be acquired by haematopoietic stem cells before the initiation of leukemogenesis. These early mutations include lesions of DNMT3A and IDH2, both of which are epigenetic modifiers. [PubMed: 24522528]
- 11•. Xie M, Lu C, Wang J, McLellan MD, Johnson KJ, Wendl MC, McMichael JF, Schmidt HK, Yellapantula V, Miller CA, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat Med*. 2014; 20:1472–1478. The authors find that somatic mutations in epigenetic modifiers occur in hematopoietic cells as individuals age and may be associated with development of hematologic malignancies. [PubMed: 25326804]
12. Kronke J, Bullinger L, Teleanu V, Tschurtz F, Gaidzik VI, Kuhn MW, Rucker FG, Holzmann K, Paschka P, Kapp-Schworer S, et al. Clonal evolution in relapsed NPM1-mutated acute myeloid leukemia. *Blood*. 2013; 122:100–108. [PubMed: 23704090]
- 13•. Corces-Zimmerman MR, Hong WJ, Weissman IL, Medeiros BC, Majeti R. Preleukemic mutations in human acute myeloid leukemia affect epigenetic regulators and persist in remission. *Proc Natl Acad Sci U S A*. 2014; 111:2548–2553. This article showed that somatic mutations in epigenetic modifiers are an early genetic event and somatic mutations that activate proliferative signaling are a later genetic event. [PubMed: 24550281]
14. Figueroa ME, Lugthart S, Li Y, Erpelinck-Verschueren C, Deng X, Christos PJ, Schifano E, Booth J, van Putten W, Skrabanek L, et al. DNA methylation signatures identify biologically distinct subtypes in acute myeloid leukemia. *Cancer Cell*. 2010; 17:13–27. [PubMed: 20060365]
15. Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, Li Y, Bhagwat N, Vasanthakumar A, Fernandez HF, et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell*. 2010; 18:553–567. [PubMed: 21130701]
16. Scourzac L, Mouly E, Bernard OA. TET proteins and the control of cytosine demethylation in cancer. *Genome Med*. 2015; 7:9. [PubMed: 25632305]
17. McKenney AS, Levine RL. Isocitrate dehydrogenase mutations in leukemia. *J Clin Invest*. 2013; 123:3672–3677. [PubMed: 23999441]
18. Rampal R, Alkalin A, Madzo J, Vasanthakumar A, Pronier E, Patel J, Li Y, Ahn J, Abdel-Wahab O, Shih A, et al. DNA hydroxymethylation profiling reveals that WT1 mutations result in loss of TET2 function in acute myeloid leukemia. *Cell Rep*. 2014; 9:1841–1855. [PubMed: 25482556]
19. Sasaki M, Knobbe CB, Munger JC, Lind EF, Brenner D, Brustle A, Harris IS, Holmes R, Wakeham A, Haight J, et al. IDH1(R132H) mutation increases murine haematopoietic progenitors and alters epigenetics. *Nature*. 2012; 488:656–659. [PubMed: 22763442]
20. Wang Y, Xiao M, Chen X, Chen L, Xu Y, Lv L, Wang P, Yang H, Ma S, Lin H, et al. WT1 recruits TET2 to regulate its target gene expression and suppress leukemia cell proliferation. *Mol Cell*. 2015; 57:662–673. [PubMed: 25601757]
21. Patel JP, Gonen M, Figueroa ME, Fernandez H, Sun Z, Racevskis J, Van Vlierberghe P, Dolgalev I, Thomas S, Aminova O, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N Engl J Med*. 2012; 366:1079–1089. [PubMed: 22417203]
22. Ribeiro AF, Pratcorona M, Erpelinck-Verschueren C, Rockova V, Sanders M, Abbas S, Figueroa ME, Zeilemaker A, Melnick A, Lowenberg B, et al. Mutant DNMT3A: a marker of poor prognosis in acute myeloid leukemia. *Blood*. 2012; 119:5824–5831. [PubMed: 22490330]
23. Russler-Germain DA, Spencer DH, Young MA, Lamprecht TL, Miller CA, Fulton R, Meyer MR, Erdmann-Gilmore P, Townsend RR, Wilson RK, et al. The R882H DNMT3A mutation associated with AML dominantly inhibits wild-type DNMT3A by blocking its ability to form active tetramers. *Cancer Cell*. 2014; 25:442–454. [PubMed: 24656771]
24. Holz-Schietinger C, Matje DM, Reich NO. Mutations in DNA methyltransferase (DNMT3A) observed in acute myeloid leukemia patients disrupt processive methylation. *J Biol Chem*. 2012; 287:30941–30951. [PubMed: 22722925]

25. Challen GA, Sun D, Jeong M, Luo M, Jelinek J, Berg JS, Bock C, Vasanthakumar A, Gu H, Xi Y, et al. Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat Genet.* 2012; 44:23–31. [PubMed: 22138693]
26. Jeong M, Sun D, Luo M, Huang Y, Challen GA, Rodriguez B, Zhang X, Chavez L, Wang H, Hannah R, et al. Large conserved domains of low DNA methylation maintained by Dnmt3a. *Nat Genet.* 2014; 46:17–23. [PubMed: 24270360]
27. Ferreira HJ, Heyn H, Vizoso M, Moutinho C, Vidal E, Gomez A, Martinez-Cardus A, Simo-Riudalbas L, Moran S, Jost E, et al. DNMT3A mutations mediate the epigenetic reactivation of the leukemogenic factor MEIS1 in acute myeloid leukemia. *Oncogene.* 2015
28. Celik H, Mallaney C, Kothari A, Ostrander EL, Eultgen E, Martens A, Miller CA, Hundal J, Klc JM, Challen GA. Enforced differentiation of Dnmt3a-null bone marrow leads to failure with c-Kit mutations driving leukemic transformation. *Blood.* 2015; 125:619–628. [PubMed: 25416276]
29. Mayle A, Yang L, Rodriguez B, Zhou T, Chang E, Curry CV, Challen GA, Li W, Wheeler D, Rebel VI, et al. Dnmt3a loss predisposes murine hematopoietic stem cells to malignant transformation. *Blood.* 2015; 125:629–638. [PubMed: 25416277]
30. Guryanova OA, Lieu YK, Garrett-Bakelman FE, Spitzer B, Glass JL, Shank K, Martinez AB, Rivera SA, Durham BH, Rapaport F, et al. Dnmt3a regulates myeloproliferation and liver-specific expansion of hematopoietic stem and progenitor cells. *Leukemia.* 2015
31. Shaknovich R, De S, Michor F. Epigenetic diversity in hematopoietic neoplasms. *Biochim Biophys Acta.* 2014; 1846:477–484. [PubMed: 25240947]
32. Hansen KD, Timp W, Bravo HC, Sabunciyar S, Langmead B, McDonald OG, Wen B, Wu H, Liu Y, Diep D, et al. Increased methylation variation in epigenetic domains across cancer types. *Nat Genet.* 2011; 43:768–775. [PubMed: 21706001]
33. Brocks D, Assenov Y, Minner S, Bogatyrova O, Simon R, Koop C, Oakes C, Zucknick M, Lipka DB, Weischenfeldt J, et al. Intratumor DNA methylation heterogeneity reflects clonal evolution in aggressive prostate cancer. *Cell Rep.* 2014; 8:798–806. [PubMed: 25066126]
34. Landau DA, Clement K, Ziller MJ, Boyle P, Fan J, Gu H, Stevenson K, Sougnez C, Wang L, Li S, et al. Locally disordered methylation forms the basis of intratumor methylome variation in chronic lymphocytic leukemia. *Cancer Cell.* 2014; 26:813–825. [PubMed: 25490447]
35. Pan H, Jiang Y, Boi M, Tabbo F, Redmond D, Nie K, Ladetto M, Chiappella A, Cerchiatti L, Shaknovich R, et al. Epigenomic evolution in diffuse large B-cell lymphomas. *Nat Commun.* 2015; 6:6921. [PubMed: 25891015]
36. De S, Shaknovich R, Riester M, Elemento O, Geng H, Kormaksson M, Jiang Y, Woolcock B, Johnson N, Polo JM, et al. Aberration in DNA methylation in B-cell lymphomas has a complex origin and increases with disease severity. *PLoS Genet.* 2013; 9:e1003137. [PubMed: 23326238]
37. Alizadeh AA, Aranda V, Bardelli A, Blanpain C, Bock C, Borowski C, Caldas C, Califano A, Doherty M, Elsner M, et al. Toward understanding and exploiting tumor heterogeneity. *Nat Med.* 2015; 21:846–853. [PubMed: 26248267]
38. Okoye-Okafor UC, Bartholdy B, Cartier J, Gao EN, Pietrak B, Rendina AR, Rominger C, Quinn C, Smallwood A, Wiggall KJ, et al. New IDH1 mutant inhibitors for treatment of acute myeloid leukemia. *Nat Chem Biol.* 2015; 11:878–886. The authors identified a novel IDH1 mutant specific inhibitor to target IDH1 mutant cells with activity against IDH1 mutant AML, resulting in epigenetic reprogramming of these cells. [PubMed: 26436839]
39. Wang F, Travins J, DeLaBarre B, Penard-Lacronique V, Schalm S, Hansen E, Straley K, Kernysky A, Liu W, Gliser C, et al. Targeted inhibition of mutant IDH2 in leukemia cells induces cellular differentiation. *Science.* 2013; 340:622–626. This article reports the first IDH2 mutant specific small molecule inhibitor. When administered to leukemia cells the compound reduced proliferation and induced differentiation. Given that IDH2 mutations might be initiating events in AML this drug may be able to suppress founding clones and pre-leukemic HSCs and reprogram the epigenome of these cells. [PubMed: 23558173]
40. Kernysky A, Wang F, Hansen E, Schalm S, Straley K, Gliser C, Yang H, Travins J, Murray S, Dorsch M, et al. IDH2 mutation-induced histone and DNA hypermethylation is progressively reversed by small-molecule inhibition. *Blood.* 2015; 125:296–303. [PubMed: 25398940]

Figure 1. Genetic and epigenetic evolution of AML

The illustration depicts the sequential acquisition of somatic mutations, with founding mutations enriched in epigenetic regulators, and late mutations enriched in proliferative activated signaling. Epigenetic states are altered accordingly impacted by the sequential acquisitions of somatic mutations. The epigenetic heterogeneity and epiallele shift evolves over time. Epigenetic heterogeneity refers to global variability in the methylation state of cytosine residues. Epiallele shift represents focal epigenetic allelic diversity.

Figure 2. Proposed epigenetic and genetic models with different leukemia cell fitness and clinical outcome

Model 1 is AML with low genetic and epigenetic heterogeneity. Perhaps these cases would display the most favorable clinical outcomes. Model 2 is AML with high genetic and but low epigenetic heterogeneity, with intermediate clinical outcome. Model 3 is AML with high genetic and but low epigenetic heterogeneity, also with putative intermediate clinical outcome. Model 4 is AML with high genetic and epigenetic heterogeneity, with possible unfavorable clinical outcome.