Published in final edited form as:

Semin Hematol. 2019 April ; 56(2): 84–89. doi:10.1053/j.seminhematol.2018.08.001.

Genetic and epigenetic determinants of AML pathogenesis

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Abstract

Acute myeloid leukemia (AML) was one of the first cancers to be sequenced at the level of the whole genome. Molecular profiling of AML through targeted sequencing panels and cytogenetics has become a mainstay in risk-stratifying AML patients and guiding clinicians toward optimal therapies for their patients. The extensive high-resolution genomic data generated to characterize AML have been instrumental in revealing the tremendous biological complexity of the disease, dictated in part by mutational, clonal, and epigenetic heterogeneity. This is further complicated by the antecedent nonleukemic state of clonal hematopoiesis that nevertheless is associated with an increased risk of developing a hematologic malignancy and with a greater risk of mortality from ischemic cardiovascular disease. Here in this review, we discuss developments in the field of AML biology and therapeutics, with a focus on advances in our understanding of how genetic and epigenetic determinants of AML have influenced prognostication and recent shifts in treatment paradigms, particularly within the context of precision oncology, for this highly complex group of hematologic malignancies.

Keywords

AML; Acute myeloid leukemia; Clonal hematopoiesis; Epigenetics; Genetics; Targeted therapy

Introduction

It has been nearly a decade since the first whole genome sequence of a cancer–derived from a patient with cytogenetically normal acute myeloid leukemia (AML)–was reported by Ley et al. [1]. Since then, the application of next-generation sequencing technologies to molecularly profile and risk-stratify patients based on the presence of somatic mutations detected within the leukemia has become a routine and integral component of AML diagnostics [2]. These mutational data aid clinicians in delineating optimal therapies for

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Disclosure of Conflicts of Interest

R.L.L. is on the supervisory board of Qiagen and is a scientific advisor to Loxo, Imago, C4 Therapeutics and Isoplexis. He receives research support from and consulted for Celgene and Roche, research support from Prelude Therapeutics, and has consulted for Novartis and Gilead. He has received honoraria from Lilly and Amgen for invited lectures.

S.F.C. has consulted for Imago Biosciences.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1053/j.seminhematol.2018.08.001.

AML patients who are either newly diagnosed or found to be in relapse, particularly now that novel targeted agents have been approved for patients harboring certain mutations such as *FLT3* [3] and *IDH1/2* [4,5]. Sequencing efforts carried out in the AML field have unveiled remarkable insights about the mutational and clonal complexity of this disease as well as uncovered antecedent preleukemic states now defined as clonal hematopoiesis in healthy individuals. Furthermore, these efforts have also demonstrated the striking frequency of epigenetic derangements that contribute to AML pathogenesis. In this review, we highlight recent advances in our understanding of how both genetic and epigenetic determinants of AML influence the biology and current treatment paradigms for this heterogeneous group of blood cancers.

Clonal hematopoiesis

The identification of the preleukemic state characterized by an expanded lineage of cells or clones within the hematopoietic compartment, known as clonal hematopoiesis (CH), was built upon multiple lines of genomic evidence [6]. This was first characterized by the detection of age-dependent nonrandom X chromosome inactivation in women [7], followed by large-scale whole-exome [8-10] or targeted sequencing [11,12] of patients who otherwise did not have any evidence of hematologic disorders. All of these studies together confirmed several key features about CH. First, there is a strong age association with CH, such that the prevalence of CH within older patients is much higher relative to younger patients. Second, recurrent somatic mutations typically associated with myeloid malignancies and frequently including epigenetic modifiers, including alterations in *DNMT3A*, *TET2*, *JAK2*, *ASXL1*, *SF3B1*, *PPM1D*, *CBL*, *BCOR*, and *TP53* were identified in this population [10]. Furthermore, these patients with CH exhibited a greater likelihood of acquiring a hematologic malignancy [8]. In a separate study focused on assessing the frequency of CH in patients with nonhematologic malignancies, Coombs et al. [13] detected CH in an astonishingly high proportion (25%) of cancer patients and found additional associations with prior radiation therapy and smoking as well as a specific enrichment of *PPM1D* and *TP53* mutations in patients previously treated with chemotherapy, consistent with the observation that distinct therapeutic and environmental perturbations play an important role in altering stem cell fitness based on the mutational history of individual clones. The penetrance of CH progression to overt hematologic malignancy is incomplete and, given data from genetically engineered mouse models of myelodysplastic syndrome and AML showing the requirement of cooperative mutations to generate full-blown disease, suggests that stochastic acquisition of “second hits,” albeit under distinct selection pressures conferred by aging, therapy, or immunoediting, may be a critical determinant of progression to overt myelodysplastic syndrome or AML. This process of clonal evolution under selection pressure within the context of CH has been excellently reviewed by Bowman and colleagues [14].

In addition to a higher risk of hematologic malignancy, CH patients also had an increased risk of atherosclerotic cardiovascular disease as well as all-cause mortality [15]. A causal link between CH and coronary heart disease was established by performing transplantation experiments in which atherosclerosis-prone mice were engrafted with bone marrow cells from either wild-type or *Tet2*-knockout mice [15,16]. Recipient mice engrafted with

Tet2-deficient bone marrow cells had significantly larger atherosclerotic plaques, and *Tet2*-deficient macrophages exhibited more robust inflammatory cytokine production. Other correlative analyzes linked *TET2* mutations with other inflammatory conditions such as chronic obstructive pulmonary disease [17]. These studies together implicate CH as a significant contributor to inflammatory phenotypes that potentially form the nidus for a variety of chronic non-hematopoietic diseases.

In the relatively short wake of observing and defining the entity of CH in patients, a number of biological and clinical questions arise. Within this pool of individuals exhibiting CH resides a vulnerable patient population with increased risk of all-cause mortality, ischemic heart disease, and transformation to overt hematologic malignancy. What primary prevention measures are indicated in this population, and at what point in time should clinicians intervene to mitigate the adverse sequelae associated with clonal hematopoiesis? For instance, 2 recent studies have demonstrated the reversibility of both stem cell dysfunction and leukemogenicity conferred by *Tet2* deficiency either through reconstitution of Tet2 expression or through treatment of mice or cells with vitamin C, which serves as a co-factor for Fe^{2+} and α-ketoglutarate-dependent dioxygenases and restores Tet2 function [18,19]. In light of these findings, would CH patients with *TET2* mutations benefit from prophylactic administration of dietary vitamin C? Questions such as these can and will ultimately be addressed in prospective clinical trials, but that requires an infrastructure to be put in place to follow this at-risk patient population. Regardless, our current understanding of clonal hematopoiesis, enlightened through tractable, high-throughput genomic assessments of blood DNA from large patient populations, establishes a precedent that has potential applicability to other precancerous states and highlights the importance of continued mining of these rich datasets and validating the biology rigorously in preclinical models.

Precision oncology in AML

The past several decades have seen little change to the backbone of AML-directed therapy, comprised of an intensive anthracycline-based induction regimen for those who are fit enough to receive it, followed by risk-stratified postremission therapy with either consolidation cytarabine or allogeneic stem cell transplantation. In 2017, 4 agents newly approved by the Food and Drug Administration (FDA) entered the AML armamentarium and dramatically ushered the treatment landscape for myeloid malignancies into the sphere of precision oncology. A brief overview of these new drugs, their therapeutic targets, as well as the patient populations designated for each therapy is provided in Table. In this section, we highlight each of the individual new therapies and their targets and discuss the particular relevance of these agents within the context of AML genomics.

Midostaurin

Among these 4 new agents, the FDA approval of midostaurin for use in combination with standard upfront induction chemotherapy for AML patients harboring *FLT3* internal tandem duplications ($FLT3^{ITD}$) or *FLT3* tyrosine kinase domain ($FLT3^{TKD}$) mutations created a priority for ascertaining *FLT3* mutational status in newly diagnosed patients prior to initiating induction therapy. First described as a protein kinase C inhibitor, midostaurin was

also shown to have inhibitory activity against multiple tyrosine kinases [20]. A chemical screen subsequently revealed the ability of midostaurin to inhibit FLT3 kinase activity [21]. The pivotal RATIFY study led by Stone et al. [3] randomized newly diagnosed *FLT3*-mutated patients under the age of 60 years to standard induction and consolidation chemotherapy combined with either placebo or midostaurin, followed by maintenance therapy with placebo or midostaurin for 12 months. The trial showed that 4-year overall survival was significantly improved by 7% in the arm receiving chemotherapy plus midostaurin (51%) when compared to placebo (44%), data that ultimately led to the FDA approval of this kinase inhibitor. Concomitant with this approval, the FDA also approved the LeukoStrat CDx *FLT3* Mutation Assay, a commercial assay that can be used for rapid screening for $FLT3^{ITD}$ or $FLT3^{TKD}$ mutations at time of diagnosis. The requirement for confirming *FLT3* mutation status prior to initiating induction chemotherapy either with or without the addition of midostaurin has changed the workflow when evaluating a newly diagnosed AML patient and created a need for rapid and accurate turnaround of these molecular assays. The number of mutations needing to be profiled upfront will likely continue to expand as additional targeted agents are being added to induction regimens in clinical trials.

Gemtuzumab ozogamicin

The story of gemtuzumab ozogamicin (GO) is a case study on the critical importance of postmarket analysis. A humanized CD33 monoclonal antibody-drug conjugate, GO is conjugated to the potent DNA toxin calicheamicin. Upon binding to cell surface CD33, GO is internalized via lysosomes and, there in the acidic microenvironment, releases calicheamicin to induce DNA damage and cell death in GO-internalized cells. Accelerated approval of GO was granted by the FDA initially in 2000 for $CD33^+$ AML patients older than 60 years and in first relapse [22]. Subsequently, a SWOG-led phase 3 study (S0106) comparing 7 + 3 induction with GO at a dose of 6 mg/m^2 on day 4 vs 7 + 3 alone revealed higher mortality and lack of survival benefit [23]. These data led to the voluntary withdrawal of GO in 2010. Subsequently, 4 randomized trials were completed–MRC AML15 [24], ALFA-0701 [25], and NCRI AML1642 [26], and GOELAMS AML2006IR. Together, these European studies demonstrated an overall survival benefit with the addition of GO to induction therapy, and meta-analyzes and subgroup analyzes demonstrated particular benefit conferred to favorable to intermediate-risk disease [25,27]. Additionally, the NCRI AML1642 trial compared 3 mg/m^2 and 6 mg/m^2 GO and showed that the lower dose was associated with less veno-occlusive disease yet no decrement in survival benefit or relapse rate [28]. These findings culminated in the re-approval of GO by the FDA for newly diagnosed $CD33^+$ AML patients to be used in combination with standard induction and for relapsed or refractory $CD33^+$ AML patients to be used in monotherapy at the lower dose of 3 mg/m^2. More recently, correlative studies from the pediatric phase III trial AAML0531 defined genetic determinants of GO response, with patients having the rs12459419 CC splice variant of *CD33* exhibiting improved event-free survival, disease-free survival, and lower relapse rates relative to other patients having CT or TT genotypes [29]. These and other genomic features that may modulate responsiveness to GO therapy are reviewed by Godwin et al. [30].

Enasidenib and/or ivosidenib

Recurrent mutations in isocitrate dehydrogenase (IDH) 1 and IDH2 were first reported in AML patients by Mardis et al. in 2009 [31]. Similar hotspot mutations targeting the enzymatic domains were also described in other malignancies, including gliomas [32] and soft tissue tumors [33]. These mutations were found to confer neomorphic catalytic activity leading to aberrant conversion of α-ketoglutarate into the oncometabolite 2-hydroxyglutarate (2-HG) [34-36], which, in turn, poisons the enzymatic function of α-ketoglutarate-dependent enzymes including TET2 to impart a block in myeloid differentiation [37]. Consistent with the role of TET2 in DNA hydroxymethylation, a critical intermediary step leading to DNA demethylation, IDH-mutant AML phenocopied TET2 loss-of-function mutations and exhibited global DNA hyper-methylation [37]. The neomorphic activity of mutant IDH was an attractive therapeutic target and resulted in the development of several small molecule inhibitors including ivosidenib (AG-120) and enasidenib (AG-221) to target IDH-1 and IDH-2, respectively. Enasidenib was evaluated in a first-in-human phase 1 out of 2 study for relapsed or refractory IDH2-mutant AML patients [4]. Remarkably, in this heavily treated population, Stein et al. reported an overall response rate of 38.5% with a CR or CRi rate 26.6%. Consistent with the central mechanism of action of these agents being induction of myeloid differentiation, mutant-*IDH2* allele burden was not significantly reduced in those patients deriving clinical benefit from IDH2 inhibition [4], and similar to other prodifferentiation agents such as all-trans retinoic acid used in acute promyelocytic leukemia, differentiation syndrome manifesting predominantly as dyspnea, fever, pulmonary infiltrates, and respiratory insufficiency was observed and requires prompt treatment with corticosteroids and supportive care when suspected [38]. The efficacy seen with IDH2 inhibitors in a relapsed and/or refractory AML population led to FDA approval of enasidenib as well as the impetus to perform trials incorporating enasidenib and ivosidenib into upfront intensive induction and/or consolidation regimens (ClinicalTrials.gov Identifier NCT02632708) and less intensive regimens with hypomethylating agents (HMA) (ClinicalTrials.gov Identifier NCT02677922). Less than a year after the approval of enasidenib, ivosidenib followed suit, with approval based on a single-arm phase I dose-escalation and dose-expansion study of 258 IDH1-mutant AML patients having a CR or CR with partial hematologic recovery rate of 30.4% or an overall response rate of 41.6% within the primary efficacy population [5]. It remains to be seen whether the combination of cytotoxic chemotherapy with differentiation therapy will enhance anti-leukemic activity and result in more durable remissions. Given the precedent observed in almost all targeted agents given as monotherapy, it is not surprising that acquired resistance mutations affecting drug binding, similar to what is seen in epidermal growth factor receptor (EGFR) resistance in nonsmall cell lung cancer, has been observed in patients who initially respond to IDH inhibition but then exhibit disease progression [39]. Additionally, the enrichment of co-occurring NRAS mutations in those patients who failed to respond to IDH inhibition is an important finding worthy of further investigation to delineate primary resistance mechanisms to these agents [40].

Liposomal daunorubicin and/or cytarabine (CPX-351)

The reformulation of cytarabine and daunorubicin into liposomes at a fixed molar ratio of 5:1 was based on experimental data demonstrating improved killing of AML cells [41,42].

CPX-351 was then compared against standard 7+3 induction in a randomized phase 2 study in patients above 60 years of age [43]. Even though this trial showed no overall survival benefit in the CPX-351 arm, there was also no significant difference in treatment-related mortality and preplanned subgroup analyzes demonstrated a survival benefit in the CPX-351 arm particularly in secondary AML patients. These findings were confirmed in a phase 3 trial that specifically accrued secondary AML patients who were between 60 and 75 years of age [44]. While the validation of an overall survival benefit within a patient population with a poor prognosis in need of novel therapies is encouraging, additional studies should be aimed at empirically defining genomic subgroups within secondary AML that may not necessarily benefit from cytotoxic chemotherapy. For instance, *TP53*-mutant and complex karyotype-AMLs have long been known to be chemoresistant [45] and are more highly represented in therapy-related myeloid neoplasms (t-MN) [46], which has previously been shown to be due to a preferential outgrowth of a pre-existing *TP53*-mutant clone after exposure to cytotoxic chemotherapy and/or radiation therapy [47]. Further exposure of cytotoxic therapy to a dominant chemoresistant clone could potentially be futile and may instead benefit from novel approaches. The finding that a regimen of 10-day decitabine resulted in higher response rates and mutation clearance among *TP53*-mutant myeloid neoplasms (but unfortunately no effect on overall survival) is a promising signal that should be further validated in multi-institutional randomized trials [48].

Despite the recent surge of AML-directed therapies, most of the leukemia-associated mutations are not yet actionable. Precision medicine is predominantly framed within a construct in which a mutation is paired with its respective targeted therapy, a construct inspired during the postimatinib era that pervades all of oncology beyond leukemia therapeutics but unfortunately is bottlenecked by drug development [49]. Compounded by mutational heterogeneity and the dynamic process of AML clonal evolution, it becomes apparent that complementary treatment paradigms are needed in order to achieve deeper remissions and disease eradication in AML patients. Recent trials incorporating the addition of the BH3 mimetic venetoclax to low-intensity regimens such as HMA or low-dose cytarabine (LDAC) have illustrated the promise of seemingly mutation-agnostic targeted therapies. Already FDA-approved for use in the setting of relapsed or refractory chronic lymphocytic leukemia (CLL) [50], DiNardo et al. demonstrated the tolerability and efficacy of venetoclax combination in the upfront [51] or relapsed setting [52] for AML, with a striking 61% of patients achieving either a complete remission (CR) or CR with incomplete count recovery in treatment-naïve elderly patients deemed unfit for standard induction therapy [51]. These data provided support for the FDA to grant breakthrough designation to venetoclax for use in combination with HMA or LDAC for this patient population. Elegant preclinical studies led by Letai and colleagues [53] have already established proof-of-principle that functional precision medicine approaches employing assays, such as dynamic BH3 profiling to assess the extent to which cancer cell lines or even primary patient samples are "primed" to undergo apoptosis, have predictive power in identifying AML patients–irrespective of their mutational profile–who could benefit from therapies such as venetoclax or who may not necessarily respond to chemotherapy [54]. Functional readouts such as these may enhance the reach of precision medicine by identifying broader pools of patients who can be matched to the most efficacious drug or drug combinations.

Epigenetic determinants of AML

Epigenetic dysregulation plays a critical role in the pathogenesis of a number of disease states, particularly in cancer. The Cancer Genome Atlas-led efforts resulted in the deconvolution of 200 AML genomes revealing that a sizeable proportion of these AMLs had mutations in epigenetic regulators, 44% of which were found in DNA methylation-related genes, and 43% of which encoded chromatin modifiers or cohesin-complex genes [55]. The potential reversibility of leukemic epigenetic states, together with the high representation of recurrent AML-associated somatic mutations in epigenetic regulators, make these processes attractive therapeutic targets. Recent developments in drugging chromatin in cancer, with an emphasis on hematologic malignancies, have previously been reviewed [56]. In this section, we highlight the underlying biology of key epigenetic drivers of AML and novel epigenetic mechanisms employed by AML cells to maintain a leukemogenic state.

Mutations in the de novo *DNA methyltransferase 3A* (*DNMT3A*) are present in approximately one-third of cytogenetically normal AML cases [57]. These mutations confer adverse risk and have been shown to promote anthracycline-resistance [58]. The $DNMT3A^{R882H}$ mutation located within the catalytic site is the most frequently occurring mutation in AML and was shown to have dominant-negative activity, blocking wild-type *DNMT3A* from forming homo-tetramers and thereby reducing DNA methylation activity by approximately 80% in vitro [59]. As a consequence, DNMT3A-mutant AMLs exhibit focal areas of DNA hypomethylation not only within the leukemic compartment but also within non-leukemic hematopoietic cells harboring the mutation, suggesting that the $DNMT3A^{R882H}$-dependent hypomethylation phenotype plays a role in leukemia initiation [60]. When AML is driven by *DNMT3A* initiating mutations, they frequently cooperate with *NPM1* and *FLT3*. Studies aimed at delineating clonal hierarchy have shown that the *DNMT3A* mutation, unlike *NPM1* or *FLT3* is present in all leukemic clones [61]. This feature of *DNMT3A* makes it an attractive therapeutic target, based on the concept that therapies targeting vulnerabilities present within the founding clone and thus shared among all clones would be most effective at eradicating disease, as illustrated by the remarkable success of tyrosine kinase inhibitors and all-trans retinoic acid in treating chronic myelogenous leukemia and acute promyelocytic leukemia, respectively. However, it remains to be seen whether inhibition of mutant DNMT3A or disrupting the interaction between wild-type and mutant DNMT3A to normalize *de novo* DNA methylation has anti-leukemic activity. Additionally, recent studies assessing the importance of molecular minimal residual disease (MRD) detection at time of CR have demonstrated that CH-associated mutations, in particular *DNMT3A*, *TET2*, and *ASXL1* are not adversely prognostic, in contrast to MRD detection of other mutations that are independently prognostic for higher rates of relapse and inferior overall survival [62-64]. These findings suggest that it may not be absolutely necessary to eradicate DNMT3A mutant allele burden provided that the mutation is being propagated through CH and not through a leukemic clone.

In addition to DNA methylation, a role for RNA methylation as an epigenetic dependency in AML has been identified. N^6-methyladenosine (m^6A) is an abundant nucleotide modification placed in mRNA through the catalytic activity of the METTL3-METTL14 RNA methyltransferase complex [65]. Two independent groups demonstrated that genetic

perturbation of METTL3 induced myeloid differentiation and cell death in AML cells [66,67]. METTL3-dependent m^6A modification of mRNA results in enhanced translation of target proteins that are implicated in AML pathogenesis, including c-MYC and BCL-2. Furthermore, a prior study revealed another dependency of AML cells on the RNA demethylase fat mass- and obesity-associated protein [65]. Additional studies are required to determine precisely how the balance in RNA methylation status of critical target genes acts as a determinant in AML pathogenesis. Regardless, the critical role of RNA methylation in AML biology represents another therapeutic opportunity to dismantle the epigenetic state of leukemia cells.

Conclusions

Next-generation sequencing technologies will continue to grow and gain applicability to an increasing number of clinical contexts in AML. These settings include AML diagnostics where prognostic subgroups will continue to be refined with higher granularity data and deeper biological understanding of AML pathogenesis to guide treatment decisions for clinicians. As functional consequences of specific mutations become elucidated, higher order interactions dictated by temporal associations of mutation acquisition, cell of origin, and cell type-specific chromatin state will also shed mechanistic insights on the molecular underpinnings of the disease. Despite the relatively low mutational burden of AML when compared to other malignancies such as melanoma or lung cancer, diversity in genetic, epigenetic, and clonal organization all contribute to the immense complexity of this heterogeneous group of hematologic malignancies. The rise of single-cell sequencing technologies and application directly to primary AML patient samples will invariably help to deconvolute these important biological questions. In addition, the definition of MRD will also continue to evolve as technology improves, the limits of detection of MRD are pushed, and the consequence of co-existing CH is understood. The more important question of how clinicians should appropriately manage patients with MRD will trail these discoveries, and ultimately be defined by practice-changing trials that offer management guidelines at various intervals throughout a patient’s treatment course. Finally, the integration of novel targeted agents also hold promise for achieving durable remissions, as precision medicine in AML broadens its reach beyond patients with a small subset of targetable mutations. It is clear there is still much work to be done, but there is tremendous excitement that persistent focus on the essential genetic and epigenetic determinants of AML will lead to novel therapies ushered through informed clinical trials that ultimately improve outcomes for AML patients.

Acknowledgments

S.F.C. is supported by a Young Investigator Award from the Conquer Cancer Foundation, a Career Development Program Fellow Award from the Leukemia & Lymphoma Society, a Momentum Fellowship Award from The Mark Foundation for Cancer Research, and a Truth 365 Young Investigator Award. The authors are also supported in part through the NIH/NCI Cancer Center Support Grant P30 CA008748.

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Table

FDA-approved therapies for AML since 2017.

Drug	Class	Target	Patient Population	Combination or monotherapy
Midostaurin	Small molecule inhibitor	FLT3 tyrosine kinase activity	Newly diagnosed FLT3 mutation-positive AML	Combination with standard daunorubicin/ cytarabine induction and cytarabine consolidation
Ivosidenib/enasidenib	Small molecule inhibitor	Mutant IDH	Relapsed/refractory IDH-mutant AML	Monotherapy
Gemtuzumab ozogamicin	Monoclonal antibody-drug conjugate	CD33	Newly diagnosed or relapsed/refractory CD33-positive AML	Combination with standard daunorubicin/ cytarabine induction for newly diagnosed AML; monotherapy for relapsed/refractory AML
CPX-351	Liposomal daunorubicin and cytarabine (fixed molar ratio)	Undefined	Newly diagnosed therapy-related AML or AML with myelodysplasia-related changes	Monotherapy