

REVIEW

Preleukemia: one name, many meanings

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Definition of preleukemia has evolved. It was first used to describe the myelodysplastic syndrome (MDS) with a propensity to progress to acute myeloid leukemia (AML). Individuals with germline mutations of either *RUNX1*, *CEBPA*, or *GATA2* can also be called as preleukemic because they have a markedly increased incidence of evolution into AML. Also, alkylating chemotherapy or radiation can cause MDS/preleukemia, which nearly always progress to AML. More recently, investigators noted that AML patients who achieved complete morphological remission after chemotherapy often have clonal hematopoiesis predominantly marked by either *DNMT3A*, *TET2* or *IDH1/2* mutations, which were also present at diagnosis of AML. This preleukemic clone represents involvement of an early hematopoietic stem cells, which is resistant to standard therapy. The same clonal hematopoietic mutations have been identified in older 'normal' individuals who have a modest increased risk of developing frank AML. These individuals have occasionally been said, probably inappropriately, to have a preleukemia clone. Our evolving understanding of the term preleukemia has occurred by advancing technology including studies of X chromosome inactivation, cytogenetics and more recently deep nucleotide sequencing.

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PRELEUKEMIA ASSOCIATED WITH MYELODYSPLASTIC SYNDROME

The term 'preleukemia' has undergone a major evolution since it was first used in 1953 by Block *et al.*¹ At that time, the term referred to the heterogeneous group of hematopoietic disorders associated with a block in myeloid differentiation and chronic cytopenias. We now call these disorders, myelodysplastic syndromes (MDS).^{2–9} Prevalence of these abnormalities is markedly increasing as our population ages; about 75 new patients per 100 000 individuals per year over the age of 65 years develop MDS. About 20–30% of these individuals do indeed progress to acute myeloid leukemia (AML); and thus, these individuals might justifiably be known as preleukemic.

In the late 1960s and 1970s, one of the prominent advances in our understanding of MDS/AML was the concept that the MDS/leukemic event occurred in 1 stem cell, which had a hematopoietic growth advantage causing a clonal population of MDS/leukemic cells. This insight was based on the observation of X-inactivation in females.^{10,11} Females have two X chromosomes; between the 4th and 16th cell stage of blastogenesis, each cell stochastically inactivates either its maternal or paternal X chromosome. A number of methods have evolved to determine which X chromosome has been inactivated.^{12,13} The initial approach was pioneered by Ernest Beutler who utilized G6PD isoenzymes.^{14–16} Over the years, additional methods were developed to identify X-inactivation in females, with one of the most versatile taking advantage of 2 features: cytosine methylation of the inactive X chromosome and different numbers of CAG trinucleotides within the promoter region of the androgen receptor, which resides on the X chromosome.¹⁷ This technique allows interrogation of almost all females as opposed to a small population of predominantly African Americans with G6PD

isoenzymes. Philip Fialkow's group showed clonal hematopoiesis occurred in a number of hematopoietic diseases including chronic myelogenous leukemia, acute lymphocytic leukemia and AML.¹⁸ Also, Fialkow noted that in many cases of AML, the clonality occurred in the myeloid compartment and not the lymphoid compartment. MDS, in contrast, appeared to have an identical clonality of both the myeloid and lymphoid compartments, albeit the myeloid compartment appeared to have a major growth advantage.¹⁹ This suggested that the MDS clone initiated in a very early hematopoietic stem cell.

With the era of massively parallel nucleotide sequencing, we have a fairly clear idea of the major mutations that affect patients who have MDS, who evolve from MDS to AML (secondary AML, sAML) and those with *de novo* AML (Table 1).^{4,5,20–28} Nine genes occur more frequently in MDS than *de novo* AML (Table 1) including four spliceosome genes that are mutated in 60–70% of MDS cases, but these latter genes are mutated in fewer than 5–10% of *de novo* AML cases.²⁹ These mutant genes are involved in ineffective dysplastic hematopoiesis, not frank AML.

Mutations that are often observed in *de novo* AML are known as Driver or pan-AML mutations including mutations of *NPM1*, *FLT3*, *IDH1*, *N/KRAS*, *RUNX1*, *CEBPA*, *WT1*, *PTPN11*, *c-KIT*. For example, *NPM1* is rarely mutated in MDS, but occurs in 27% of *de novo* AML cases. Likewise, chromosomal translocations (often a fusion of a transcription factor gene with another gene) occur frequently in *de novo* AML (18%). Examples include fusion of genes such as *RUNX1* with *ETO*, *PML* with *RARA*, *CBFB* with *MYH11*, as well as fusion of *MLL* with various partner genes, which only rarely occur in MDS (4%).³⁰ These Driver mutations produce a major block in differentiation providing a proliferative advantage to this clone of cells.³⁰

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Table 1. Exome sequencing: mutational defects in MDS and sAML versus *de novo* AML

MDS		sAML		De novo AML	
Gene	Mutations (%)	Gene	Mutations (%)	Gene	Mutations (%)
TET2 ^a	33	TET2 ^a	20	FLT3 ^b	28
→ SF3B1 ^a	32	SF3B1 ^a	11	NPM1 ^b	27
ASXL1 ^a	23	ASXL1 ^a	32	DNMT3A	26
→ SRSF2 ^a	17	SRSF2 ^a	20	IDH1/2	10
→ ZRSR2 ^a	8	ZRSR2 ^a	8	RUNX1 ^b	9
EZH2 ^a	5	EZH2 ^a	9	TET2	9
BCOR ^a	4	BCOR ^a	8	NRAS/KRAS ^b	8
→ U2AF1 ^a	8	U2AF1 ^a	16	TP53	8
STAG2 ^a	8	STAG2 ^a	14	CEBPA ^b	6
RUNX1	10	RUNX1 ^b	31	WT1 ^b	6
DNMT3A	13	NF1 ^b	6	PTPN11 ^b	5
IDH2/IDH1	5	NRAS/KRAS ^b	31	KIT ^b	4
TP53	6	TP53	15	U2AF1	4
FLT3	5	IDH1/2	20	KRAS ^b	4
CBL	5	FLT3 ^b	19	SMC1A	4
JAK2	5	DNMT3A	19	SMC3	4
BCOR	3	PTPN11 ^b	5	PHF6	3
NRAS or KRAS	7	CEBPA ^b	3	STAG2	2
MPL	3	NPM1 ^b	5	RAD21	2
NF1	3	SMC3	2	FAM5C	2
ATM	3	CBL	5	EZH2	1

→, mutant spliceosome gene; sAML, secondary AML (MDS → AML). MDS data;⁹ sAML data;³⁰ de novo AML data.²⁶ ^aNotable difference of incidence during MDS and sAML versus de novo AML. ^bDriver (Pan AML) mutations.

Because cases of sAML evolves from MDS, these cells often contained genes mutated in MDS (Table 1).³⁰ Driver (pan-AML) mutations are gained as MDS evolves to sAML.³⁰ Of interest, individuals with sAML achieve complete remission only about 50% of the time and their event-free survival (EFS) is 4.2 months. In contrast, 92% of *de novo* AML patients enter a complete remission, and their EFS is ~15.7 months.³⁰

OLIGOCLONAL HEMATOPOIESIS: NORMAL AGING VERSUS PRELEUKEMIC CLONE

Analysis of X chromosome inactivation discovered skewing of X chromosome inactivation (also known as Lyonization).^{31–34} X-inactivation during blastogenesis usually results in equal inactivation of the maternal and paternal X chromosomes in the female. Skewing represents unequal distribution of cells expressing either the paternal and maternal allele of the female X chromosome. It can occur during early embryogenesis, but rarely deviates > 10%. However, X chromosomal skewing increases with age approaching 20% of those who are 28–32 years old, and 40% of those ≥ 60 years.^{10,12,13,32–34} A study in normal females ≥ 75 of age found that 56% of individuals had skewing of neutrophils and 40% had skewing of T-lymphocytes.³⁴ Thus, older individuals have hematopoietic cells that favor usage of one X allele. This has been explained by unequal exhaustion of hematopoietic stem cells as individuals age. This observation was buttressed by genomically marking murine hematopoietic stem cells and using them for complete hematopoietic transplantation. Multiple clones of hematopoiesis initially developed in these transplanted animals; but as the mice aged, oligoclonal hematopoiesis predominated, suggesting that with age, certain hematopoietic clones can predominate.³⁵ Of note, this phenomenon was not noted in humans who underwent hematopoietic transplantation.³⁶

SNP-Chip analysis of peripheral blood of 50 000 normal individuals showed that copy-number changes in the genome increased with increasing age; > 0.5% of individuals from birth to 50 years of age had copy number alterations; but after age 50 years, the rate rose to about 2–3%.³⁷ These copy-number changes

often occurred in genes that also are abnormal in MDS and AML.³⁷ These individuals with copy-number changes had a 10-fold increased risk of developing MDS or AML.³⁷

Recently, a number of studies using high throughput nucleotide sequencing confirmed what had been suggested by studies of skewing of the inactive X-chromosome in normal elderly individuals. On average, a normal appearing 60-, 70- and 90-year-old individual has a 5%, 10% and 20% incidence, respectively, of clonal hematopoiesis as marked by a mutation.^{38–40} A recent study of a normal population of 50–60-year-old individuals found with exceptionally deep sequencing that clonal hematopoiesis occurred in 95% of individuals as marked by a mutation of either *DNMT3A* or *TET2*.⁴¹ An interesting, unifying study found *TET2* mutations in the blood of ten of 182 elderly 'normal' women who also had chromosomal skewing of the X chromosome. In contrast, 105 normal women of the same age without X-chromosome skewing, had no *TET2* mutations.⁴² Mutation of *DNMT3A* occurs about fivefold more frequently in the normal elderly individuals than mutations of either *TET2*, *IDH1/2*, *AXSL1*, *JAK2*, *SRSF2* or *SF3B1* (to name a few). Many of these mutations of the normal elderly are the same as those in the preleukemic clone identified in complete remission of sAML, or found in the very early hematopoietic stem cells in individuals with either MDS or AML (both to be discussed).^{38–40,43} The base change of these mutations was most commonly cytosine to thymine transition, which is a somatic mutational signature of aging. Usually only one gene and on rare occasions two or three genes are mutated; these additional mutations occur more frequently as the 'normal' individual becomes older.^{38–40} On average, < 20% and more often < 3% of either the peripheral blood or marrow cells are part of the mutant hematopoietic clone in these elderly individuals (also called variant allele frequency (VAF)).

For lack of a better nomenclature, this hematopoietic clone in the elderly has occasionally been called an age-related preleukemic clone; this is usually a misnomer. About 0.5 to 1% of these individuals per year developed MDS/AML (hazard ratio 11.1–12.9).^{38,39} The greater the number of different mutations or the greater the contribution (VAF) of the mutant clone, the greater the

chance to evolve into MDS/AML (for example, if the mutant clone constitutes >10% of the hematopoietic population, these individuals have at least a 1% risk per year of developing AML³⁹). Acquisition of a Driver (pan-AML) mutation(s) is required for development of AML in these individuals with morphologically normal marrow cells. These genes behave as the 'gatekeepers' of leukemogenesis providing, when mutated, the needed stimulus for establishment of frank AML.

Thus as humans age, they often develop a hematopoietic clone carrying a mutation of at least either *DNMT3A*, *ASXL1*, *TET2* or *IDH2*, but only a small minority of these individuals actually develop myeloid malignancies. These Driver mutations provide a competitive advantage to the hematopoietic stem cells compared with normal hematopoiesis. Whether this mutant stem cell pool is more receptive to secondary mutations requires further study.

Analogous phenomenon occur in the lymphoid system. Monoclonal gammopathy of unknown significance (MGUS) features a small accumulation of plasma cells producing a monoclonal immunoglobulin.⁴⁴ The aberrant clone of plasma cells infrequently (1% per year) progresses to multiple myeloma. Likewise, a clonal mature B-cell population can develop in older individuals, which rarely progresses to chronic lymphocytic leukemia.⁴⁵ Incidence of all three of these clonal hematopoietic conditions increase with age. Clonal abnormalities have been noted in other tissue which otherwise appear normal. For example, the normal sun-exposed eyelid frequently has mutations including *NOTCH1*, *NOTCH2*, *NOTCH3*, *FAT1*, *TP53* and *RBM10*; some of these mutations are associated with clonal expansion of these skin cells.⁴⁶ Also, we and others noted years ago that the normal appearing colon and lung near the tumor can harbor genomic alterations (for example, aneuploidy, *RAS* mutations).^{47,48} These too represent a precancerous genomic abnormality.

PRELEUKEMIA: GERMLINE MUTANT HEMATOPOIETIC CLONE

Twins studies have suggested familial clustering of AML,^{49,50} and a large analysis of over 24 000 first-degree relatives of about 7000 patients with AML noted that if the proband develops MDS/AML before the age of 25 years, the first-degree relative has an increased risk of MDS and AML.⁵¹ Individuals with inherited germline mutations of one of several transcription factors (*RUNX1*, *GATA2* or *CEBPA*) have an increased propensity to develop AML over their life.^{52–56} Other inherited germline that can also predispose to MDS includes Diamond–Blackfan anemia (20% risk of MDS/AML), dyskeratosis congenita (disorder of telomere maintenance, 30% risk MDS/AML), Fanconi anemia (40% risk), severe congenital neutropenia (20–40% risk), Shwachman–Diamond syndrome (10–35% risk), mutations of the *SRP72* gene (increased risk of aplastic anemia or MDS), Bloom syndrome (25% risk for MDS/AML), Li-Fraumeni syndrome (p53 mutation) (5–7% risk) and familial monosomy 7.^{57–59} For each of these inherited germline mutation, their progression to frank AML requires the clonal acquisition of additional genetic mutations.⁶⁰

Familial AML with germline mutant *CEBPA* (OMIM 11697) is inherited (autosomal dominant) and has nearly complete penetrance for development of AML.^{54,56} AML can develop anytime from the age of 1 year to old age. The AML is usually associated with biallelic *CEBPA* mutations, usually the first mutation is germline and often at the 5' end of the gene, and the second mutation occurs in the second allele often in the 3' region of the gene. This is similar to what is observed in those with somatic mutations of *CEBPA* (*de novo* AML). *CEBPA* is critical for myeloid proliferation and granulocytic differentiation. Most of these patients have a normal karyotype when they develop AML; and they have a relatively favorable prognosis with 50–60% that may be cured. The AML is often FAB subtype M1 or M2, with aberrant CD7 expression on the blast cells and often a N-RAS mutation present in the blast cells. As expected, siblings of the proband

with the germline *CEBPA* mutation have a 50% risk of also carrying the mutation.

Familial platelet disorder (FPD), (OMIM 601399) with a propensity to AML is caused by a germline mutation of *RUNX1*. The *RUNX1* gene is important for myeloid proliferation and differentiation; and its alteration in FPD is either a deletion, a loss of function missense mutation or occasionally a dominant-negative missense mutation. These individuals often have a low peripheral blood platelet count, a functional platelet disorder and G-CSF hypersensitivity of their myeloid cells.^{52,61} The frequency of developing AML with FPD is about 40%, and AML can occur at any age but more often between 40 and 70 years of age.

AML that develops in FPD individuals do not acquire *NPM1* or *FLT3* mutations, which are frequently found in *de novo* AML. A recent study found that asymptomatic *RUNX1* carriers can harbor additional somatic mutations with a mean VAF varying between 20 and 45%, suggesting that clonal skewed hematopoiesis frequently precedes the development of overt MDS or AML.⁶⁰ All asymptomatic *RUNX1* carriers were less than the age of 50. By the age of 50 years, clonal hematopoiesis was easily found in 80% of *RUNX1* carriers.⁶⁰ One Japanese study reported that these patients had frequent mutations of the *CDC25C* gene when they progressed to AML.⁶² However, this mutation could not be detected by other investigators.^{60,63} This could represent ethnic differences. The number of mutations in the blast cells found at the time of AML varies between 8 and 18. The VAF of these mutations in both the MDS and AML phases of FPD were comparable (approximately 15–45%).^{60,61,64}

Another germline alteration is a heterozygous mutation of the transcription factor *GATA2*. These individuals often have peripheral blood eosinophilia, deficiency of monocytes, B cells and natural killer cells and an increased incidence of non-tuberculosis mycobacteria and other opportunistic infections including papilloma virus.^{53,55,65–67} The syndrome is often called (MonoMAC). These patients in addition can develop leg lymphedema and monosomy 7 (Emberger syndrome). The predilection to develop MDS or AML is about 70% with a median age of 29 years, but AML can develop over a wide time span (0.4 to 78 years). Their MDS phase is often characterized as chronic myelomonocytic leukemia. The progression to AML is associated with an initial cytopenias often followed by monosomy 7 (30%) which appears to occur before an *ASXL1* mutation (29% of cases). Interestingly, this latter mutation occurs only in females, who are often young; it is associated with a bad prognosis.^{68,69} Another frequent secondary mutation is *NRAS*, but other Driver mutations have been noted including *RUNX1*, *STAG2*, *IDH2*, *TP53* and *SETDB1*. Transformation is often to a hypercellular fibrotic AML.

Clearly, more experience is required to determine at what stage these individual with germline preleukemic mutations need aggressive therapy. Monitoring their marrow for the acquisition of additional Driver mutations might prompt initiation of aggressive therapy such as allogeneic hematopoietic stem cell transplant.⁶⁰ If a sibling allotransplant is contemplated, germline DNA sequencing of the donor for the germline mutation is required because the sibling has a 50% chance of also having the germline mutation. The ability to take fibroblasts from patients with germline mutations, induced dedifferentiation to pluripotent stem cells (iPSC), correct the mutation and then induce these cells to differentiate into functional hematopoietic stem cell, has been done.^{70,71} However, to date, full hematopoietic reconstitution *in vivo* has not been possible using the iPSC technique. Furthermore, gene therapy to repair directly the hematopoietic cells is a distant dream with a multitude of barriers because unlike treating sickle cell anemia or thalassemia, every cell must be corrected.

THERAPY-RELATED PRELEUKEMIA

Several types of therapy-(mutagen)-related MDS/AML have been identified. One that almost always has a preleukemic (MDS) phase is associated with exposure to either alkylating agents (85%), radiation or selected carcinogens, such as benzene.⁷²⁻⁷⁴ Most frequently an individual with Hodgkin disease, non-Hodgkin disease, breast cancer, multiple myeloma and so on receives an alkylating agent and approximately 4 years later develops MDS. The incidence of therapy-related preleukemia varies from <1 to 10% depending on type of agent administered and the intensity of the therapy for the initial cancer.^{72,75-78} Their preleukemic marrow often (>70%) has deletion of either the long arm or the entire chromosome 5 and/or 7; or they develop a complex karyotype (≥ 3 abnormalities).^{75,79-81} Some of the frequently mutated genes in this type of preleukemia includes *p53*, *PTPN11* and the ABC family of genes. The *p53* mutation often occurs early in the course of MDS. A second group of mutations are commonly associated with secondary AML (Table 1). In contrast to *de novo* acute leukemia, mutations of *NPM1*, *CEBPA* or *FLT3* are infrequent.^{30,72,82-84} The RAEB and RAEB-t subgroups are frequently linked to therapy-related preleukemia (73%).⁸⁵ These individuals almost always progress to AML with a mean duration of the preleukemic phase of 11.2 months.⁸⁶⁻⁸⁸ They have a poor prognosis (survival with AML < 6-8 months).^{73,75,89} Treatment for these individuals when AML develops has a number of clinical challenges including a greater number of co-morbidities, decreased organ reserve from either previous therapy or primary disease and a higher incidence of unfavorable cytogenetic changes. The key prognostic factors in therapy-related MDS/AML are patient's age, performance status and karyotype. Because of their poor outcome, intensive chemotherapy with allogeneic hematopoietic stem cell transplant should be encouraged.

In summary, the development of unexplained pancytopenia and the finding of specific karyotypic abnormalities (loss of chromosome 5 and/or 7 or their long arms in the marrow) in patients who received chemotherapy and/or radiation therapy for another disease about 4 years earlier is pathognomonic of preleukemia. Evolution to overt leukemia is almost universal if the preleukemic individual survives the complications of hemorrhage and infection. Therapy preleukemia can be viewed as an early phase of therapy AML in which the malignant hematopoietic clone is established and becomes predominant.

The second type of therapy-related AML develops in individuals whose initial malignancy was treated with a topoisomerase II inhibitor (for example, etoposide, doxorubicin, mitoxantrone).^{72,90,91} Typical latency period from therapy to AML is a median of 33 months but can be as short as 12 months. The AML often has a chromosomal translocation especially at 11q23 (MLL gene) or chromosome 21q22 (RUNX1). The morphologic phenotype often is FAB M4 or M5. These individuals usually do not have a MDS (preleukemic) phase. Overall survival of these individuals after development of AML is equal to that of a patient with *de novo* leukemia having the same cytogenetic abnormalities in their AML cells.

PRELEUKEMIA IN MORPHOLOGIC COMPLETE REMISSION AFTER AML CHEMOTHERAPY

During the 1980s and the early 1990s, studies by Phil Fialkow and Claus Bartram noted that about 1/4 of AML patients who achieved complete morphologic remission by chemotherapy, continued to have an abnormal clone as measured by X-inactivation.³¹ This has been buttressed by studies showing strong association between persistent karyotypic markers of AML in first complete morphologic remission. Hence, the term 'preleukemic clone' has been used to describe the loss of the major phenotype of AML (Driver mutations associated with AML blast cells), but where the clonal hematopoiesis persisted, and could evolve into relapse AML. Thus,

the term preleukemia is used in a very different context than utilized in the 1950s and 1960s.

High throughput sequencing of AML samples reinforced the concept that a preleukemic clone can exist at the time of complete morphologic remission after standard aggressive chemotherapy for AML. Individuals whose AML cells at diagnosis had mutations of either *DNMT3A*, *IDH2*, *TET2*, *UAF1* or *SRSF2* often had the same alteration at complete morphologic remission.⁹²⁻⁹⁸ In contrast, more classical Driver (pan-AML) mutations such as *FLT3*, *RUNX1*, *RAS*, *NPM1*, *CEBPA* and *WT1* genes, although present at diagnosis of AML, were no longer found in complete remission. Dr. Ley's group reported that 21/37 individuals whose AML cells carried *DNMT3A*, *TET2* or *IDH1/2* mutations at diagnosis, continued to have the same mutation in >5% of the hematopoietic cells at complete morphologic remission; but the classical Driver mutations (for example, *NPM1*, *FLT3* and *RAS*) found at diagnosis, were not detectable at remission.⁹⁴ Of note, if the individual had a persistence of a mutation at complete remission (day 30 after induction therapy), their event-free survival was 6 months and their overall survival was 10 months as compared with an event-free survival of 18 months and an overall survival of 42 months in individuals with no mutation in the 30-day complete remission marrow.

Another study focused on *DNMT3A* mutations. The investigators noted that the *DNMT3A* mutation was often present in the marrow cells in complete morphologic remission at an allele frequency of 1-50% and could be detected even 8 years after initial complete remission of AML with no other molecular markers of AML. Likewise, the mutations could be found at a lower allele frequency in T and B lymphocytes in the same patients.⁹⁷ The authors concluded that *DNMT3A* mutations precede *NPM1* and *FLT3-ITD* mutations in AML pathogenesis; and the fact that this mutation can be found in B and T cells suggest that it involves a very early hematopoietic stem cell that can differentiate both into lymphoid and myeloid cells.^{93,97}

Dr. Dick's group studied a cohort of individuals whose AML blast cells had mutations of *DNMT3A* and *NPM1* at both diagnosis and relapse.⁹³ In morphologic complete remission, only the *DNMT3A* mutation could be found. In a second part of the study, human bone marrow cells at diagnosis of AML (264 samples) were injected into immunodeficient mice (NSG mice). A total of 37% of samples did not engraft, 40% of samples recapitulated the AML blasts in the mice; but most interestingly, 23% of the samples produced multi-lineage engraftment that appeared normal, but continued to have mutations of either *DNMT3A* or *IDH1/2*.⁹³ This suggests that even at diagnosis of AML, a preleukemic clone exists. Interestingly, the same group also purified different stages of hematopoietic cells at diagnosis of AML whose blast cells were marked with both *DNMT3A* and *NPM1* mutations.⁹³ The very early common hematopoietic progenitors carried the mutation of *DNMT3A*, but not *NPM1*. In contrast, the more mature myeloid progenitor cells (granulocyte-monocyte progenitors), as well as the AML blast cells had both the *DNMT3A* and *NPM1* mutations (Figure 1 summarizes these studies).

A very detailed study focused on *IDH2* found that about 22% of individuals with an *IDH2* mutant allele at diagnosis of sAML continued to have the mutational clone in complete morphologic remission after chemotherapy; but with marrow transplant, these aberrant alleles were destroyed.⁹⁸ In addition, the VAF of the mutant *IDH2* clone exceeded the VAF of the *NPM1* or *FLT3* clones in frank AML, and the *NPM1* and *FLT3* clones disappeared with complete remission. Colony assays of CD34+ hematopoietic stem cells and progenitor cells were done in complete remission and 96% and 50% of the erythroid and granulocyte macrophage colonies, respectively, had only the *IDH2* mutation. Further, colony and single-cell-nucleotide sequencing also confirmed that *DNMT3A*, *TET2* and *ASXL1* were early clonal mutations during evolution to AML.^{99,100}

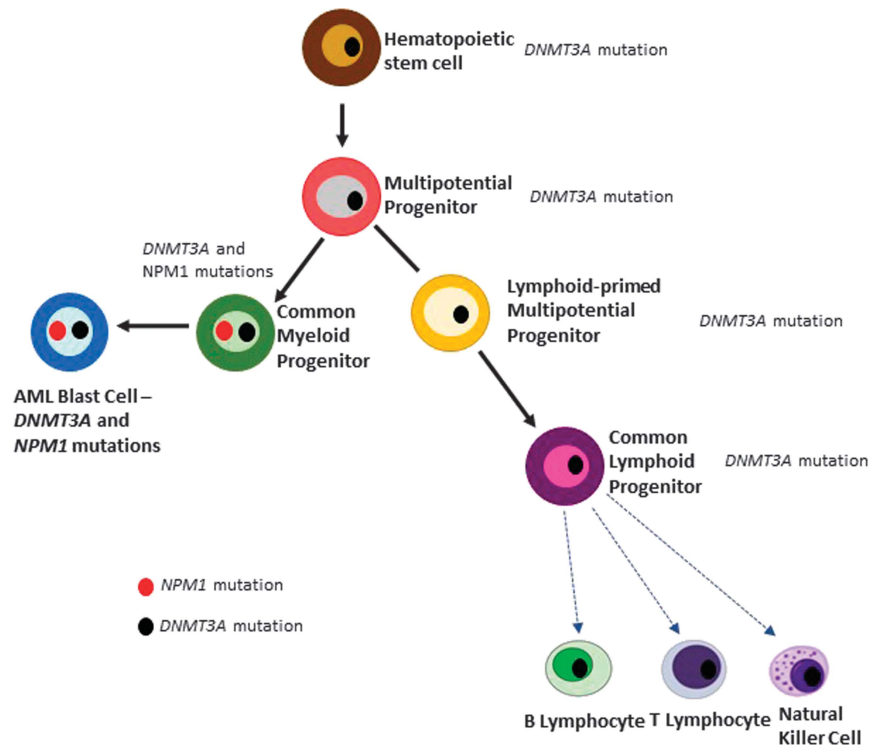


Figure 1. Schematic diagram of the hematopoietic tree with stem cells having a preleukemic mutation (*DNMT3A*) and blast cells acquiring a Driver (pan-AML) mutation (*NPM1* mutation), and retaining the *DNMT3A* mutations. Black dot, *DNMT3A* mutation; Red dot, *NPM1* mutation. Figure summarizes data by John Dick's lab.⁹³

Interestingly, recent studies found a rapid expansion of a pre-existing non-leukemic/preleukemic mutant hematopoietic clone occurred following induction therapy for *de novo* AML.^{101,102} Investigators discovered that at diagnosis of AML, a small, barely detectable, non-leukemic clone was overshadowed by the leukemic clone. After remission inducing chemotherapy, the non-leukemic/preleukemic clone markedly expanded (VAF of 20–40%) compared with the normal hematopoietic population. Thus, the non-leukemic/preleukemic mutant clones had a competitive advantage over the normal hematopoietic cells. These studies highlight the importance to distinguish Driver-related leukemia versus non-leukemic/preleukemic mutant populations when the physician assesses by DNA sequencing the response to induction chemotherapy.

PRELEUKEMIC STEM CELLS AND THERAPEUTIC IMPLICATIONS

A long-lasting preleukemic (MDS) clone often marked by mutations of either *DNMT3A*, *TET2*, *IDH1/2* or a spliceosome gene, can have a competitive growth advantage over the normal hematopoietic cells. These mutations can cause ineffective hematopoiesis. This can be followed by a subclone that develops a major Driver mutation (for example, *NPM1*) or fusion gene, resulting in frank AML with blast cells. These Driver (pan-AML) mutations block differentiation providing these cells with a proliferative advantage and causing life-threatening peripheral blood cytopenias. After ablative chemotherapy accompanied by a complete morphologic remission, the preleukemic clone may persist suggesting that these cells are resistant to standard chemotherapy, but are still able to differentiate to normal-appearing mature blood cells. If frank AML reoccurs, the original or new blastic AML subclone emerges from the preleukemic clone. Event-free survival of individuals with a preleukemic clone at

morphologic complete remission, is inferior to those with no preleukemic clone during complete remission.^{30,94} Perhaps, therapeutic intervention might be considered while these patients are in complete morphologic remission if nucleotide monitoring shows the acquisition of a Driver mutation.¹⁰³ This might prompt hematopoietic transplantation before frank AML develops (Figure 2). Timing will be critical because reports have found that the preleukemic clone may be present for 8–10 years without emergence of relapsed blastic AML. Repeat targeted nucleotide sequencing of the marrow may be no more expensive than cytogenetic examination; and in the future sequencing may supplant cytogenetics in define circumstances.

IDH2 mutant AML cells can generate D-2-hydroxyglucate (D-2-HG) as a marker of the mutation.⁹⁸ The plasma level of this compound is high in AML patients; however, those in complete morphologic remission with a mutant *IDH2* clone may also have elevated serum D-2-HG. Therefore, measuring D-2-HG is not a good surrogate for making clinical decisions.⁹⁸ Specific pharmacologic inhibitors of *IDH2* have been developed. *IDH2* mutation is often in the founder mutant clone; this clone acquires a Driver mutation to develop frank AML. The ability of the *IDH2* inhibitor to eliminate the AML or founder clone requires further study.

Of interest, mathematic modeling noted a good correlation between the lifetime risk of developing cancer and the number of stem cell division that specific tissue undergoes during an individual's lifespan.^{104,105} Mutations are passed to daughter cells only at cell division. Normal cells undergo about 1 mutation per 10^8 cell divisions. An estimated 11 000 to 22 000 early hematopoietic stem cells are present in an individual. This number decreases with age. Indeed, a likely hypothesis is the fewer hematopoietic stem cells that are present as an individual ages, the greater their proliferative pool to maintain normal peripheral blood counts; hence, the greater chance for a mutational

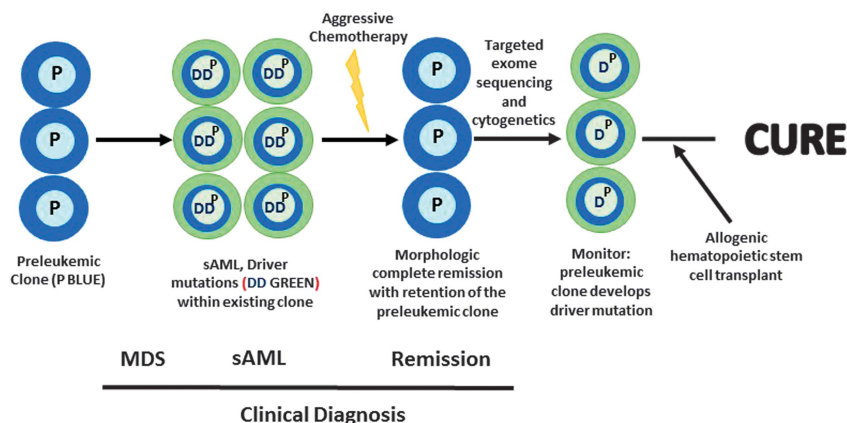


Figure 2. Evolution to MDS → secondary (s) AML (sAML) → remission and potential therapeutic intervention.

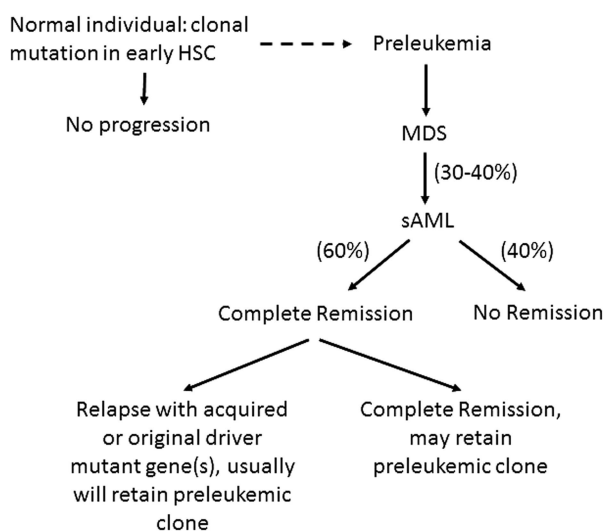


Figure 3. Hematopoiesis: Normal elderly individual ± evolution to sAML. Normal elderly individuals can develop clonal hematopoiesis marked by a mutation in their early hematopoietic stem cells (HSC). Some of these individuals will develop myelodysplastic syndrome (MDS); and with the acquisition of a Driver mutation, they can develop secondary AML (sAML). These patients may go into complete morphologic remission but often retain their preleukemic clone. With relapse, they can either retain the original Driver mutation or acquire a new Driver mutation. Dotted line suggests that some normal elderly individuals with clonal hematopoiesis may develop MDS and sAML.

event.^{104,105} Many of the random mutations that occur in these cells affect non-critical genes (passenger genes) providing no growth advantage to these stem cells. Thus, ‘bad luck’ happens when a mutation occurs in a gene that provides a proliferative advantage to the hematopoietic cell. The resulting clone of cells has a greater chance to acquire additional mutations that might evolve into frank AML.

SUMMARY

In summary, the term ‘preleukemia’ has evolved over the years. Initially in the 1950s and 1960s, preleukemia was viewed as MDS. We now know that the clinical course as well as chromosomal and mutational changes of MDS are often markedly different from those present in *de novo* AML. Individuals with an inherited mutation of *RUNX1*, *GATA2* or *C/EBP* in their germline have a very

high chance of developing AML; and thus have a preleukemic mutation. Also, previous treatment of a primary malignancy with either an alkylating drug or radiation therapy can result in a preleukemia that almost always progresses to AML. As outlined in Figure 3, very early hematopoietic stem cells can develop mutations that results in clonal hematopoiesis especially in those > 60 years of age. In hematopoietically normal older individuals, this clone probably should not be called preleukemic. The incidence of evolution to AML in these individuals requires further study, but may be 0.5 to 1% per year. Quizzically, the mutations associated with MDS are often the same as those observed both in the normal elderly individual as well as the preleukemic clone in AML patients who are in morphologic complete remission. Development of overt AML requires Driver mutations. X-inactivation and DNA-sequencing studies showed that AML patients that obtained a complete morphologic remission can retain a preleukemic clone suggesting that these cells are resistant to normal ablative chemotherapy perhaps because they involve quiescent hematopoietic stem cells not easily killed by our standard chemotherapy. Patients whose leukemia evolves from an ancestral preleukemic clone, often will require therapy that exceeds our standard chemotherapy and perhaps monitoring of the preleukemic clone for acquisition of Driver mutations during remission may help to determine when aggressive therapy should be initiated. In conclusion, preleukemia might be defined as a condition that has modifying mutations in the bone marrow that either cause MDS or cause clonal hematopoietic expansion initially without disease but associated with progression to AML.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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