Review Article

Leukemic stem cells and advances in hematopoietic stem cell transplantation for acute myeloid leukemia: a narrative review of clinical trials

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Objective: The purpose of this literature review is to summarize and provide a brief overview of our current understanding of acute myeloid leukemia (AML) and the role of stem cell transplantation (SCT) in its management.

Background: AML is a malignant hematological disorder that is characterized by the uncontrolled proliferation of myeloid blood cells. This disease has been associated with various risk factors such as ionizing radiation, cigarette smoke, pesticides/herbicides, and chemotherapy. SCT remains the most beneficial treatment for medically fit AML patients due to superior survival outcomes.

Methods: A thorough search was conducted on PubMed, Scopus, ClinicalTrials.gov, Embase and Web of Science using related keywords. Current articles on the uses of stem cell therapy in AML patients were selected.

Conclusions: Long term exposure to ionizing radiation and other harmful substances such as benzene, cigarette smoke and chemotherapeutic drugs plays an important role in AML carcinogenesis. Mutations in certain genes (e.g., *ASXL1*, *RUNX1*, *KIT*, *TP53*, *BCR-ABL1*) seem to accelerate the process as they affect normal cellular proliferation and cell death. These events may give rise to a small subpopulation of leukemic stem cells (LSC) which continuously sustain tumor development and growth. Patients who are deemed to be medically "fit" should receive an allogenic hematopoietic stem cell transplantation (allo-HSCT) due to improved overall survival (OS) (~50%) and decreased relapsed risk (32% *vs.* 59%). Several studies have revealed that the medically "unfit" may benefit from more conventional agents such as azacytidine, decitabine, venetoclax or sorafenib.

Keywords: Cancer stem cells (CSC); stem cell transplantation (SCT); acute myeloid leukemia (AML); malignant hematology

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Introduction and background

Acute myeloid leukemia (AML) is an aggressive hematological malignancy that is characterized by the uncontrolled proliferation of myeloid cells within the bone marrow and peripheral blood (1). Currently, there are two classifications systems that can used to for AML. The French-American-British (FAB) is an outdated classification system no longer used in clinical practice that recognizes

eight different AML subtypes: undifferentiated AML (M0), AML with minimal maturation (M1), AML with maturation (M2), acute promyelocytic leukemia (APL), acute myelomonocytic leukemia (M4), acute monocytic leukemia (M5), acute erythroid leukemia (M6) and acute megakaryocytic leukemia (M7) (2). In contrast, there are six distinct AML subgroups in the World Health Organization (WHO) classification system: AML with recurrent genetic abnormalities, AML with myelodysplasia-related changes (MRC), therapy-related myeloid neoplasms (t-MN), AML not otherwise specified (NOS), myeloid sarcoma and myeloid proliferations related to Down syndrome (DS) (3). It is estimated that AML represents 15–20% of all acute leukemia cases in children and 80% in adults (4).

In the United-States, the median age of diagnosis is 68 years with an age-adjusted incidence of 4.3 per 100,000 annually (5). According to the Surveillance, Epidemiology, and End Results (SEER) database, this cancer has a predilection for Caucasian males and is responsible for 1.9% of all cancer-related deaths (6). Based on 2016–2018 data, it is estimated that 0.5% of men and women will be diagnosed with AML at some point during their lives (6). Unfortunately, AML carries a 5-year relative survival of 29.5% as most patients die around the age of 73 years (6).

Long-term exposure to ionizing radiation and certain chemicals such as benzene, cigarette smoke, pesticides and herbicides increases the risk of acquiring AML (7). These cytotoxic substances have been observed to directly damage multiple genes by altering the molecular structure of cellular deoxyribonucleic acid (DNA). This subsequently promotes carcinogenesis by altering the function of several proteins responsible for cell-cycle arrest and apoptosis (8). Alkylating agents (e.g., cyclophosphamide and doxorubicin) and topoisomerase II inhibitors (e.g., etoposide and teniposide) may also induce AML by altering the structure of different chromosomes (9). The presentation of AML is dependent on the following factors: (I) extent of blood cell shortage, (II) quantity of leukemic cells and (III) affected organs (10). Signs and symptoms often include anorexia, unintentional weight loss, fatigue, dyspnea, recurrent infections, and persistent bleeds. If a patient is suspected of having AML, an initial work-up consisting of a complete blood count (CBC) and peripheral smear should be obtained (11). Although flow cytometry remains the preferred method for immunophenotyping AML, immunohistochemistry performed on a bone marrow aspirate/biopsy can also aid in obtaining a final diagnosis (11,12). Detecting specific gene rearrangements such as t(11q23), t(6;11)(q27;q23), t(9;11) (p22;q23), t(2;11)(11;17)(q37;q11q23; q11), t(11;17)(q23;q25) and t(11;19)(q23;p13.1) with the help of fluorescence *in situ* hybridization (FISH) is useful for risk stratification and directing therapy (13,14).

AML is a hematological malignancy which remains difficult to treat due to its aggressive and rapidly progressive nature. Nonetheless, stem cell transplantation (SCT) remains an important treatment strategy in medically "fit" patients with AML due to high cure rates (15). The purpose of this literature review is to describe the clinical significance of cancer stem cells (CSC) in the development of AML and the potential role of SCT in treating this disease. We present the following article in accordance with the Narrative Review reporting checklist (available at https://sci.amegroups.com/article/view/10.21037/sci-2022-044/rc).

Methods

A thorough search was performed on the PubMed, Scopus, ClinicalTrials.gov, Embase and Web of Science databases. The initial query was performed on March 2nd 2022 and required keywords such as "AML", "AML pathogenesis", "AML prognosis" "AML AND stem cell transplant" and "AML AND autologous stem cell transplant OR allogenic stem cell transplant". Articles were only included if they were written in English, published data discussing the etiology and pathogenesis of AML and/or published data discussing the use of SCT in AML (*Table 1*). A total of 15 clinical trials were included for this literature review.

Etiology and pathogenesis

Benzene and other known occupational exposures

Benzene is a leukemogenic chemical which is associated with AML (16). According to Khalade *et al.*, benzene increased the risk of AML in a dose-response pattern (17). Low benzene exposure [<40 parts per million (ppm)-years] was linked to an increased risk of 1.64, while medium (40–99.9 ppm-years) and high exposure (>100 ppm-years) increased the risk by 1.90 and 2.62, respectively (17). It is hypothesized that benzene accelerates the formation of hematological malignancies by altering the function of lymphocytes and destabilizing the supportive stromal and endothelial cells within the stem cell hematopoietic stem cell (HSC) niche (18).

Cigarette smoking has also been observed to increase the risk of acquiring AML (19). Per Colamesta *et al.*, the risk of developing AML was at its highest in the "current smoker"

Table 1 The search strategy summary

Items	Specification
Date of search	March 2nd 2022
Databases and other sources searched	PubMed, Scopus, ClinicalTrials.gov, Embase and Web of Science
Search terms used	AML, AML pathogenesis, AML prognosis, AML AND stem cell transplant, AML AND autologous stem cell transplant, AML AND autologous stem cell transplant OR allogenic stem cell transplant
Timeframe	1990–2022
Inclusion and exclusion criteria	Inclusion criteria: papers in English only (focus on clinical trials), data discussing the etiology and pathogenesis of AML and/or discussing the use of stem cell transplantation in AML. Exclusion criteria: case reports, papers not in English
Selection process	Primary author and second author screened manuscripts during initial screen. During second screen, remaining manuscripts were only chosen if they strictly met inclusion criteria. Prior to analysis and paper development, the selected manuscript was sent to the final author for final approval. Paper was then thoroughly edited by final author for most up-to-date data

AML, acute myeloid leukemia.

group [odds ratio (OR): 1.36 and relative risk (RR): 1.52] and slightly decreased in the "former smoker" group (OR: 1.21 and RR: 1.45) (19). Cigarette smoke contains various carcinogens that directly alter the physical structure of various chromosomes (e.g., -7 or 7q-, -Y, +13), which in turn promotes excessive CpG methylation, telomere shortening and microsatellite shortening (20,21). Affected genes include bcl-2, C-CBL, CD95, HOXA9 and TNFα (21). Pesticides/herbicides also have deleterious effects on human health and have been shown to accelerate the development of AML (22,23).

Ionizing radiation

Radiation induced leukemogenesis is a complex phenomenon which involves the accumulation of irreparable genetic mutations in cells. Such genetic aberrancies hinder the cell's innate ability to repair radiation-induced DNA damage through base excision repair (BER), mismatch mediated repair (MMR), homologous recombination (HR) and non-homologous end joining (NHEJ) (24). Murine models exposed to radiation were found to harbor several genes such as *N-ras*, *Flt3-ITD* and *Sfpi1/PU.1* (25-27). It is estimated that most cases of radiation-induced AML occur 3–10 years after radiation exposure (28).

Chemotherapeutic agents

Therapy-related AML (t-AML) is hypothesized to be a direct consequence of accumulating genetic mutations induced by chemotherapy (29). Although most cases of t-AML are linked to alkylating agents or topoisomerase II inhibitors, medications such as fludarabine and azathioprine have also been observed to contribute (9,29-31). T-AML patients who have received alkylating agents are prone to harbor mutations in chromosome 5 and/or 7 with most abnormalities being monosomy of chromosome 7, deletion of the long arm of chromosome 5 and monosomy of chromosome 5 (29,32). T-AML secondary to alkylating agents such as melphalan and cyclophosphamide typically occur 5–7 years after chemotherapy (29,33). In contrast, t-AML secondary to topoisomerase II inhibitors tends to appear within 2–3 years (29). Most topoisomerase II t-AML cases are characterized by chromosomal translocations involving chromosomes 11 (11q23) and 22 (21q22) (34). Affected genes include AML1, CBFB, NUP98 and PML/RARA (29).

Diagnosis

It is crucial for potential AML patients to undergo prompt cytogenetic and molecular testing for risk stratification and treatment modality purposes. AML is characterized by the presence of numerous blast cells (e.g., myeloblasts, monoblasts, and megakaryoblasts) in the peripheral blood and/or bone marrow (35). While most cases of AML require a blast count of ≥20% on the peripheral smear or within the bone marrow to make a final diagnosis, certain forms of the

disease harboring t(15:17). t(8;21), inv(16) or t(16;16) can be diagnosed at a lower blast count (35-37). Blast cells that stain positively for myeloperoxidase (MPO) or Sudan black B (SBB) would indicate a malignancy of myeloid origin, whereas blasts that stain positively for nonspecific esterase (NSE) would favor a cancer of monoblastic origin (35). Immunophenotyping may also be used to identify lineage involvement. Detecting hematopoietic differentiation antigens such as CD34, CD38 and HLD-DR with the help of flow cytometry aids in confirming a diagnosis of AML (35). Khalidi *et al.* observed that CD45 (97.2%), CD33 (95.3%) and CD13 (94.3%) were the commonly expressed antigens in AML (38). Other markers like CD20, CD7, CD19, CD2, CD3, CD5 and CD10 can also be detected on certain leukemic cells (38).

It is estimated that approximately 55% of all AML cases may be attributed to recurrent karyotype abnormalities (39). These chromosomal aberrations determine prognosis in AML and can be detected using conventional cytogenetic analysis (40). If the results of cytogenetic analysis are inconclusive, a fluorescence in situ hybridization (FISH) should be obtained because of its high prognostic utility in determining common gene rearrangements such as RUNX1-RUNX1T1, CBFB-MYH11, MLL and EVI1 gene fusions (35,41).

Prognostic factors

European LeukemiaNet (ELN) has developed guidelines regarding treatment of AML. ELN published an updated genetic risk stratification in 2022 to standardize reporting of genetic abnormalities in association with outcomes. Risk categories were made based on genetic prognostic factors (I) favorable, (II) intermediate, and (III) adverse. In-frame CEBPA mutations in the leucine zipper region, NPM1 gene mutations without FLT3-ITD, CBFB:MYH11 gene mutation involving inv(16)(p13.1q22) or t(16;16)(p13.1;q22), RUNX1-RUNX1T1 gene mutation involving t(8;21)(q22;q22.1) were among factors associated with a favorable outcome. FLT3-ITD allelic ratio (regardless of presence or absence of NPM1 gene mutation), MLLT3:KMT2A gene mutation involving t(9;11)(p21.3;q23.3), and other cytogenic and/or molecular abnormalities not classified into favorable or adverse risk categories were examples of factors associated with an intermediate outcome. TP53 gene mutations, NPM1 gene mutations with adverse-risk cytogenetic abnormalities, genetic mutations related to myelodysplasia (e.g., ASXL1, RUNx1, BCOR, EZH2, SF3B1, SRSF2, STAG2, U2AF1, ZRSR2), MECOM gene mutation t(3q26.2;v), KAT6A::CREBBP gene fusion t(8;16)(p11;p13), prior history of hematologic disease, complex karyotype (3 or more unrelated chromosomal abnormalities with no other class-defining recurrent genetic abnormalities, excluding hyperdiploid karyotypes with 3 or more trisomies or polysomies without structural abnormalities, and monosomal karyotypes (2 or more distinct monosomies, excluding loss of X or Y, or 1 single autosomal monosomy with 1 or more structural chromosomal abnormalities, excluding core-binding factor AML) were among factors associated with a poorer outcome and are stratified as adverse risk (42).

Changes made regarding the AML genetic risk stratification in the updated 2022 ELN guidelines compared to 2017 include (42):

(I) FLT3-ITD is now considered intermediate-risk, regardless of NPM1 mutation. (Previously, mutated NPM1 without FLT3-ITD or with FLT3-ITD(low) was categorized as favorable (low-risk); wild-type NPM1 and FLT3-ITD(high) was categorized as adverse (high-risk); wild-type NPM1 without FLT3-ITD or with FLT3-ITD(low) without adverse-risk genetic lesions was categorized as intermediate-risk.)

(II) t(3q26.2;v)/MECOM(EVI1)-rearranged is now considered adverse (high-risk).

(III) Mutated BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, ZRSR2 are now considered adverse (high-risk).

(IV) Biallelic mutated CEBPA in the favorable (low-risk) group has now been replaced by bZIP in-frame mutated CEBPA. (In-frame mutations of the CEBPA basic leucine zipper region have been shown to have a favorable outcome, regardless of biallelic or monoallelic status).

(V) AML with myelodysplasia-related gene mutations is now considered adverse (high-risk).

(VI) Hyperdiploid karyotypes with multiple trisomies or polysomies are now removed from adverse (high-risk) group (no longer considered a complex karyotype).

In a study of 599 cases <60 years of age, estimated 5-year overall survival (OS) among the risk categories (favorable/low-risk, intermediate, poor/high-risk) was 64%, 42%, and 20% respectively; in a study of 517 cases 60 years of age or older, estimated 5-year OS was 37%, 16%, and

6% respectively (43). Induction therapy with cytarabine and anthracyclines to achieve complete remission (CR) with a blast count of <5% in the bone marrow followed by consolidation therapy with high-dose cytarabine and HSCT (allogeneic preferred over autologous) is associated with move favorable outcomes (42). Of note, relapse-free survival (RFS) was found to be higher in several students for patients receiving HSCT (autologous or allogeneic) that achieved first CR (P<0.001), while OS was no different (P<0.12). Regarding allo-HSCT, patients noted as low-risk (favorable prognostic factors) showed a 10-year OS at 85% with matched-related donor transplantation versus 70% without (P<0.26); intermediate-risk category showed 10-year OS at 40% with match-related donor transplantation versus 70% without (P<0.03); high-risk (poor prognostic factors) showed 10-year OS at 50% with match-related donor transplantation versus 15% without (P<0.02) (44).

Scoring systems

In 1997, Greenberg *et al.* developed the IPSS (International Prognostic Scoring System) for evaluating prognosis in MDS. Risk scores were based on bone marrow blast percentage, cytogenic subgroup, and number of cytopenias, stratifying patients into 1 of 4 categories: (I) low (<0.5), (II) intermediate-1 (0.5–1.0), (III) intermediate-2 (1.5–2.0), and (IV) high (2.5 or higher). Bone marrow blast %: <0.5% score 0, 5–10% score 0.5, 11–20% score 1.5, 21–30% score 2.0. Karyotype good score 0, intermediate score 0.5, poor/complex score 1. Cytopenias 0/1 score 0, 2/3 score 0.5. Based on this Scoring System, 3-year OS was determined to be 5% for High-Risk, 20% for Intermediate-2-Risk, 60% Intermediate-1-Risk, 75% Low-Risk (P<0.001); 5-year OS was determined to be 0% for High-Risk, 5% for Intermediate-2-Risk, 35% for Intermediate-1-Risk, 60% for Low-Risk (P<0.001) (43).

In 2019, Patkar *et al.* used single molecule molecular inversion probes to develop a genomics-based scoring system for AML outcomes targeting 50 genes implicated in the pathogenesis of myeloid malignancies. Genetic testing was performed using cytogenetics (conventional karyotyping and/or FISH), panel-based NGS, and a machine learning-based genetic score. The top six variables per case were assigned an individualized score then summed for a total score. Each variable was assigned –1 if the results predicted an unfavorable outcome or +1 if favorable outcomes were predicted. Patients were categorized into 1 of 2 categories based on overall genetic risk: (I) favorable and (II) poor. Favorable genetic risk scored 4 or higher; poor genetic risk scored 3 or less. Poor genetic risk cases showed 40-month OS of 45% (P<0.01) compared to favorable genetic risk cases with 40-month OS of 75% (P<0.01) (44).

In 2020, Nakamura *et al.* developed an updated system analyzing AML outcomes in patients >65 years of age who received conventional chemotherapy based on JALSG GML 200 Protocol. Patients were categorized into 1 or 3 categories based on prognostic factors: (I) favorable, (II) intermediate, (III) poor. Median OS was 10.3 months for all patients, 19.3 months for patients who achieved CR after induction therapy, with a total patient 3-year survival rate of 20.9%. A multivariate analysis then identified several significant poor prognostic factors to develop a scoring system, assigning a score of 1 to each of the following categories: (I) low performance status [hazard ratio (HR) 3.93, 95% confidence interval (CI), 1.81–8.49, P<0.001], (II) age 70 and older (HR 3.38, 95% CI, 1.81–8.49, P<0.001), (III) ELN adverse risk criteria (HR 3.16, 95% CI, 1.83–5.47, P<0.001), (IV) previous MDS diagnosis (HR 2.36, 95% CI, 1.29–4.30, P<0.001). A min/max scoring system of 0–4 was made. Based on this system, 1-year OS was 85.7% for score 0, 59.4% for score 1, 5.9% for score 2, with 0% survival in cases scored 3–4. 5-year OS was 70% for score 0, 18% for score 1, with 0% survival in cases scored 2–4 (45).

Leukemic stem cells (LSC)

CSC hypothesis

Also known as “tumor initiating cells” or “tumorigenic cells”, CSC are hypothesized to be the source of various solid and hematological malignancies (46). These abnormal pluripotent stem cells self-renew and give rise to different progenitor cells which aides tumorigenesis, metastasis, and therapy resistance (46-48). It is believed that CSCs originate from non-neoplastic progenitor cells which acquire non-repairable somatic mutations and differentiated daughter cells that reacquire stem cell-like properties via the epithelial-to-mesenchymal transition (EMT) (49,50). The inherent ability of CSCs to self-renew is tightly controlled by the Wnt/Beta-catenin, Hedgehog, Janus kinase (JAK)/signal transducer and activator of transcription (STAT) and phosphatidylinositide 3-kinase (PI3K) signalling pathways (51). In CSCs, the TNF-α, TGF-β and IL-1/6 pro-inflammatory cytokines have been observed to modulate EMT by controlling the expression of Snail, Twist and Zeb and other master transcription

factors such as STAT3, Smads and NF-kb (50,52).

LSCs in AML

First discovered in AML in the 1990s, LSC represent a small subpopulation of abnormal leukemia cells which sustain tumorigenesis by their various self-renewal properties (51). These anomalous cells maintain their quiescence by remaining hidden within the bone marrow, which shields them from chemotherapy, corruptive reactive oxygen species and the anaerobic metabolism (53). It is hypothesized that healthy HSC can give rise to new leukemic clones after acquiring sufficient genetic mutations (54). At times, LSCs can be difficult to differentiate from normal HSCs as they both express the CD34+/CD38- surface immunophenotypes, but Velten *et al.* developed a clonal tracking approach utilizing MutaSeq and mitoClone which aided in mapping the oncogenic mutations creating a clear separation between healthy HSCs, pre-LSCs, and cancerous LSCs (55). With the help of DNA-sequencing, the study identified cells carrying pre-malignant mutations and malignant mutations such as *CEBPA* and *NPM1*. Recent studies have demonstrated that LSCs are prone to express various surface markers such as CD32, CD44, CD47, CD123, TIM3, CD45RA and CD96 (53). Haubner *et al.* analyzed the expression profile of LSCs in AML patients (n=356) and discovered that CD123 (97.0%), CD244 (96.7%), CD33 (96.4%), TIM3 (87.3%) and CLL1 (80.1%) were the most common surface markers (56). The overexpression of CD244 and TIM3 on LSCs correlated with the proliferative and self-renewal capabilities of LSCs.

Mutational patterns

Several chromosomal rearrangements and gene mutations contribute to the variability and severity of AML by causing clonal transformation of HSCs (4). It is important to note while 45% of patients diagnosed with AML have a normal karyotype, 55% of AML patients have at least 1 cytogenetic abnormality. The 2001 Gilliland two-hits model categorized genetic contributions to AML into 3 categories: (I) class I mutations with proliferative and survival advantages, (II) class II mutations that alter cell differentiation and apoptosis, and (III) without classification, which promote epigenetic modifications. Under the Gilliland hypothesis, AML results from having at least 2 applicable gene mutations. Class I mutations include: FLT3, KIT, RAS, PNPN11, JAK2, CBL; class II mutations: NPMI1, CEBPA, PML-RARA, RUNX1- RUNX1T1, CBFB-MYH11, MLL; without classification mutations: DNMT3a, TET2, IDH1 and IDH2, ASXL1, WT1. Identifying these gene mutations can help determine the type and prognosis of AML as well as the targeted treatment of AML (4).

A 2016 study identified 76 genes with 5,234 driver mutations contributing to AML in 1,540 patients, 86% of which had more than 1 mutation (57). Three broad categories of AML gene mutations were made: mutations in (I) chromatin-encoding genes, RNA-splicing regulator genes, or both; (II) TP53, chromosomal aneuploidies or both; (III) IDH2 mutations. Mutations in chromatin-encoding genes, RNA-splicing regulator genes, or both accounted for 18% of the cohort; mutations in TP53, chromosomal aneuploidies, or both accounted for 13%; IDH2 mutations for 1%. Of these 3 categories, 80% of AML cases were classified into a single subgroup, while 4% fell into 2 or more categories, particularly the first two categories.

Subgroups of AML were then identified based on their corresponding mutations: 27% AML with NPM1 mutations; 18% AML with mutated chromatin, RNA-splicing genes, or both; 13% AML with TP53 mutations, chromosomal aneuploidy, or both; 11% AML with driver mutations but no detected class-defining lesions; 5% AML with inv(16), t(16;16), CBFB-MYH11; 4% AML with t(15;17), PML-RARA; 4% AML with t(8;21), RUNX1-RUNX1T1; 4% AML with no detected driver mutations; 4% AML meeting criteria for at least 2 genomic subgroups; 3% AML with MLL fusion genes, t(x;11); 1% AML with inv(3), t(3;3); 1% AML with IDH2 mutations and no other class-defining lesions, 1% AML with t(6;9), DEK-NUP214 (57).

This same 2016 study identified which driver mutations have the strongest effect on OS in AML, listed below in order of frequency (%) (*Table 2*).

The most frequent mutations were: NPM1 (28%); FLT3 (22%); RUNX1 (9%). The mutations with the highest HR were: inv(3), GATA2, MECOM(EVI1) (HR 2.9); TP53 (HR 1.7); FLT3, BRAF, SRSF2, complex karyotype (HR 1.4) (57).

FTL3 (class I) mutations, most commonly as internal tandem duplications (ITD) between exons 14 and 15, result in mutation in receptor tyrosine kinase, an essential component of hematopoiesis and cell proliferation, differentiation, and apoptosis. FLT3-ITD mutations have been found in AML patients also with normal karyotype as well as mutations related to: translocations t(15;17) and t(6;9), NPM1, DNMT3a. Missense FLT3 mutations, commonly in exon 20 of the activation loop of the tyrosine

Table 2 Driver mutations that affect the overall survival of AML patients

Driver mutation	Frequency	HR	95% CI	P value (<)
NPM1	28%	0.7	0.6–0.9	0.0004
FLT3	22%	1.4	1.2.1–7	0.0010
Complex Karyotype	10%	1.4	1.2.1–7	0.0010
RUNX1	9%	1.1	0.9–1.3	0.5000
-5/5q	7%	1.3	1.1–1.5	0.0007
TP53	6%	1.7	1.4–2.2	0.0010
SRSF2	6%	1.4	1.1–1.7	0.0030
-7	6%	1.3	1.1–1.5	0.0030
NPM1-FLT3-DNMT3a	6%	1.5	1.2–1.9	0.0002
-17/17p	5%	1.3	1.1–1.5	0.0030
CEBPA	5%	0.6	0.4–0.7	0.0010
ASXL1	5%	1.3	1.0–1.6	0.0040
Inv(16), CBFB-MYH11	4%	0.3	0.2–0.4	0.0010
t(15;17), PML-RARA	4%	0.3	0.2–0.4	0.0010
t(8;21), RUNX1-RUNX1T1	4%	0.7	0.4–1.0	0.0030
+21	3%	1.3	1.1–1.6	0.0010
-9q	3%	1.2	1.1–1.5	0.0100
DNMT3a-IDH2	3%	1.4	1.1–1.8	0.0070
NPM1-FLT	3%	0.7	0.6–0.9	0.0090
IDH2	3%	0.8	0.6–1.0	0.0070
+22	2%	1.2	1.1–1.4	0.0080
T(x;11), not MLLT3-MLL	2%	1.4	1.0–2.1	0.0600
Inv(3), GATA1,MECOM (EVI 1)	1%	2.9	1.8–4.7	0.0010
BRAF	1%	1.4	1.1–1.7	0.0090
+13	1%	1.3	1.1–1.5	0.0040
MLL-FLT3	1%	1.4	1.2–1.8	0.0005
STAG2-IDH2	1%	0.8	0.6–0.9	0.0100
DNMT3a-RAD21	1%	0.7	0.5–0.9	0.0008
ZRSR2	1%	1.3	1.0–1.7	0.0400
t(9;11), MLLT3-MLL	1%	0.8	0.4–1.4	0.5000

AML, acute myeloid leukemia; HR, hazard ratio; CI, confidence interval.

kinase domain, result in a point mutation that substitutes aspartate with tyrosine, interfering with ATP binding (4).

NPM1 (class I) mutations are most commonly in exon 12 but also noted cases in exons 9 and 11, most at the C-terminus of the protein nucleotide position 960 in 95% of cases. Mutation resulting in loss of NPM1 forms in leucine-rich abnormal proteins within the nucleoli that interfere with ribosomal processes important for DNA repair, DNA transcription regulation, histone and nucleosome assembly (4).

Fusion of RARA (transcription factor) PML (tumor

suppressor) genes result in abnormal regulation of cell proliferation, differentiation, and apoptosis by forming the fusion product PML-RARA (class II) mutation. AML M3 (acute promyelocytic leukemia) is highly associated with PML-RARA in t(15;17). Since this fusion product results in binding of retinoic acid to trigger conformational change and transcription activation for differentiation into promyelocytes, treatment with trans-retinoic acid has been found to be effective by promoting an alternative pathway for promyelocytic transcription and differentiation. RUNX1-RUNX1T1 (class II) mutations are associated with AML in patients with t(8;21); CBFB-MYH11 (class II) mutations with inv(16) and t(16;16); MLL (class II) with t(9;11) (4).

Conversely, DNMT3a (class II) mutations have not been found in patients with t(15;17), inv(16), or t(8;21). Instead, DNMT3a mutations are more associated with advanced age, with up to 25% arising from *de novo* mutations. DNMT3a (class II) mutations result in DNA methylation of promoting factors which regulate tumor growth and have phenotypical similarities with AML M4 (acute myelomonocytic leukemia) and AML M5 (acute monocytic leukemia) (4).

IDH1 and IDH2 (without classification) mutations result in abnormal proteins that affect the tricarboxylic acid (TCA) cycle, leading to leukemogenesis by transforming the intermediate product alpha-ketoglutarate to 2-hydroxyglutarate, forming accumulation of 2-hydroxyglutarate, an oncometabolite that competitively inhibits TET2, a subset of the TET gene that mediates DNA demethylation. Up to 23% of AML cases are identified to have TET2 (class II) mutations and are associated with a poor prognosis (4).

Bone marrow microenvironment

The bone marrow microenvironment is a complex system containing numerous cell types which may be categorized as hematopoietic and non-hematopoietic. Studies evaluating HSC niches have attempted to identify and localize the interactions between these cell types which influence both normal hematopoiesis and leukemic transformation (58). HSCs are found adjacent to the sinusoids and are strictly influenced by the production of growth and differentiation factors such as stem cell factor (SCF) and c-x-c motif chemokine ligand-12 (CXCL-12) produced by the adjacent endothelial and mesenchymal stromal cells (58). The categorization of this niche and the changes that occur upon malignant transformation have led to unique perspectives in our understanding of potential therapeutic targets.

The bone marrow niche perspective supports the hypothesis that LSCs survive immunoregulation by their direct and indirect manipulation of these relationships (59). Wang *et al.* summarized the effects of this bone marrow niche on leukemogenesis and found that leukemic cells integrate into the vascular endothelium and seize growth factors to reinforce their own neoplastic proliferation (60). AML cells adhere to the bone marrow endothelium via the very late antigen-4 (VLA-4) and fibronectin (FN) association (61). Matsunaga *et al.* demonstrated that leukemic cells which produced high amounts of VLA-4 acquire resistance to apoptosis via its interaction to FN and subsequent activation of the phosphatidylinositol-3-kinase (PI-3K)/AKT/Bcl-2 signaling pathway (61). This mechanism of leukemogenesis was eliminated when antibodies to VLA-4 were introduced to the murine model.

Rationale behind HSCT in AML

Allo-HSCT has been considered the most effective therapy to prevent relapse in AML with greater than 3,000 annual transplants in the United States (62). Tumor genetics have a significant impact on which patients will relapse after first remission, and therefore aid in identification of patients who have potential to benefit from allo-HSCT. Early studies comparing transplant versus consolidative chemotherapy were hindered by the genetic diversity between transplant and non-transplant groups which made generalization to a broader population a significant issue (63). Meta-analyses attempted to control for this genetic randomization and found improved relapse free survival (RFS) and OS in patients with related donor matched transplant (HR 0.80, 95% CI: 0.74–0.86; OS 0.90, 95% CI: 0.82–0.97). This survival benefit was most significant in the subset of patients with intermediate and high-risk cytogenetics (64).

Patients with relapsed/refractory AML (R/R AML) have poor OS of less than 20% (62). The factors associated with an improved outcome include younger age at diagnosis, favorable cytogenetics, late relapse (>1-year), and no prior receipt of allo-HSCT (62). Most R/R AML patients do not receive allo-HSCT as they fail to achieve a second remission. Despite these poor outcomes, allo-HSCT may still be offered as the only available treatment for these patients.

Allo-HSCT is a powerful treatment modality with the potential to cure AML. Many newly diagnosed AML patients respond to induction therapy; however, relapse

after induction is common, ranging from 35% (favorable risk category) to 80% (adverse risk category patients) (65). Allo-HSCT decreases relapse risk (32%) compared to chemotherapy (59%) at four years, albeit with significant treatment-related mortality (TRM) (25% *vs.* 4%) (66).

Patients who achieve first CR, defined as bone marrow blasts <5%, no Auer rods, no extramedullary disease, and full recovery of neutrophils and platelets without growth factor support with induction therapy have three options for post-remission therapy including (I) allo-HSCT, (II) intense chemotherapy (IC) or (III) high-intensity chemotherapy with the autologous rescue (11). A dynamic assessment of stratifying factors predicting disease relapse and TRM helps guide appropriate post remission therapy. Patient's demographics, disease type, cytogenetic abnormalities, and measurable residual disease (MRD) status before transplant guides allo-HSCT as a potential treatment modality.

Indications and eligibility criteria for HSCT in AML

Several factors are considered when determining the clinical benefit of stem cell transplant in AML. In general, younger patients without comorbid conditions or active infections who have good psychosocial support, an HLA-matched donor and with disease in remission or previously responsive to therapy have superior outcomes. In contrast, older patients with significant comorbid conditions who have required aggressive chemotherapy and/or have relapsed or refractory disease with poor social support have been shown to display increased morbidity and mortality (67).

While eligibility criteria vary by institution, most patients are assessed by some standardized factors. These factors include overall health/performance status, prior therapies, age, and stage of disease. Performance status is typically estimated by proven risk scores including the Karnofsky performance score (KPS) (68). The hematopoietic stem cell transplant comorbidity index (HCT-CI) provides valuable prognostic information and predicts survival after allo-HSCT in patients with hematologic malignancies (69). This tool was designed in the late 1990s and assesses seventeen different categories of comorbid conditions including cardiopulmonary, gastrointestinal, metabolic, rheumatologic, and hepatorenal function. In both allo-HSCT and autologous hematopoietic stem cell transplantation (auto-HSCT), even after age adjustment, HCT-CI index scores of greater than or equal to 3 are associated with higher non-relapse related mortality (NRM) and lower OS (69). These patients may benefit more from nonmyeloablative/reduced intensity regimens and their poor score may ultimately dictate the type of regimen which may be offered.

Role of allo-HSCT in AML

As randomization of AML patients needing allo-HSCT is not ethical, many donors versus no-donor studies using myeloablative conditioning (MAC) and HLA matched sibling donors from the 1990s have shown improved OS and disease-free survival (DFS) among suitable AML patients (70).

Basara *et al.* evaluated the role of allo-HSCT in AML patients. Seven hundred and eight AML patients were enrolled between 1997 to 2006. All patients were less than 60 years of age. Forty-seven patients received allo-HSCT. Adverse cytogenetic risk factors were found in 138 (19.5%) patients. Among them, 77 (56%) achieved CR with induction therapy. The median follow-up in living patients was 19-months. The OS at 2-years was better in the allo-HSCT group compared to the no-donor group (52% *vs.* 24%). The allo-HSCT group also decreased relapse incidence of 39% compared to 77% in no-donor patients at the cost of increased TRM by 15% compared to the 5% in no-donor group (71).

Suciu *et al.* evaluated 1,136 young AML patients (15–46 years) from 1993 to 1999 in the European Organization for Research and Treatment of Cancer Leukemia Group and Gruppo Italiano Malattie Ematologiche dell' Adulto (EORTC-LG/GIMEMA) AML-10 trial. CR was achieved in 822 (72.4%). There were 293 patients with HLA-identical sibling donors (donor group) available, whereas 441 had no family donor. Patients were treated with allo-HSCT or auto-HSCT depending upon cytogenetic risks. The hazard ratio (HR) of the DFS between the donor and the no-donor group was 1.21 for good cytogenetics risk and 0.58 for adverse cytogenetics risk category. The Relapse/OS HR was 0.83/1.41 in favorable cytogenetics, 0.84/1.14 in intermediate, and 0.42/0.62 in the adverse group suggesting better outcomes of adverse risk AML patients with allo-HSCT (72).

Sakamaki *et al.* compared the role of allo-HSCT with chemotherapy in patients with intermediate or poor-risk AML who achieved remission in a multi-center JALSG AML 97 trial. Patients were enrolled between 1997 to 2001. The CR was achieved in 392 patients after induction therapy. The HLA-identical sibling was found in

73 patients (donor group). Patients with no HLA identical sibling were no-donor group (n=92). Patient ages ranged from 15–50 years. Among poor risk (n=19) and intermediate risk (n=162), 14 patients had no HLA typing available. Of 154 patients, 73 had matched sibling donors (donor group), and 79 had no matched sibling donor (no-donor) available. Conditioning was performed per participating facility protocol. In the donor group, 52 (76%) patients received allo-HSCT. Thirty-eight patients (52%) had allo-HSCT after CR at a median of 159 days (43–314 days). The 8-year relapse was better in the donor group *vs.* the no-donor group (52% *vs.* 77%). The OS between donor and no-donor group was 46% *vs.* 29% but did not reach statistical significance (73).

Jourdan *et al.* retrospectively evaluated the role of allo-HSCT in young AML patients who achieved remission with MAC. The conditioning regimen was cyclophosphamide (Cy) and total body irradiation (TBI) or Busulfan (Bu) with Cy. The median duration from CR to allo-HSCT was 60 days (11–284 days). The non-relapsed mortality (NRM) in the donor group was 37% *vs.* 11% in the no-donor group. The relapse rate (RR) was 11% in the donor group and 23% in the no-donor group (74).

Keating *et al.* compared HLA identical sibling allo-HSCT to chemotherapy in phase III AML 8A clinical trial. All 831 patients were 46 years old or younger and enrolled in the trial from 1986 to 1993. The donor group (received HLA-matched sibling allo-HSCT) had 295 participants. The conditioning regimen was cyclophosphamide and TBI. Cyclosporine alone (30%) or methotrexate (41%) was used for graft *vs.* host disease (GVHD) prevention (75). The six-year OS of allo-HSCT was 8% more than the no-donor group (51% *vs.* 43%). The OS at six years for patients who received allo-HSCT in CR was 46% in the donor group and 33% in the no-donor group. The RR at six years was 42% in the donor group and 63% in the no-donor group (75).

Cornelissen *et al.*, the Dutch-Belgian Hemato-Oncology Cooperative Group and the Swiss Group for Clinical Cancer Research (HOVON-SAKK) performed donor versus no-donor comparison in AML patients. Two thousand two hundred eighty-seven patients were evaluated. Among them, 326 patients (32%) underwent allo-HSCT. Patients with allo-HSCT had a cumulative incidence of relapse (CIR) of 32% compared to 59% in no-donor. Treatment-related mortality was 21% in allo-HSCT compared to 4% in the no-donor group. The DFS was better in the donor group at 48% versus 37% in the no-donor group (66).

Schetelig *et al.* conducted Study Alliance Leukemia (SAL) AML 2003 trial that evaluated 1,179 patients who achieved the first remission and had intermediate or high-risk AML underwent matched allo-HSCT. In contrast, patients with complex karyotype, FLT ITD allelic ratio of 0.8, underwent matched unrelated allo-HSCT. The conditioning regimen for all patients was total body radiation with cyclophosphamide or fludarabine. This study showed similar outcomes in patients with a matched unrelated donor versus HLA identical sibling donors. The five-year OS and EFS after allo-HSCT for patients with intermediate-risk AML was 63% and 62%, compared to 28% and 27% in high-risk AML (76).

Jentzsch *et al.* evaluated the role of allo-HSCT in secondary/treatment-related AML (s/t AML). A total of 644 patients were analyzed with 228 diagnosed as s/t AML and 416 with *de novo* AML. Patients were significantly older in the s/t AML group, with a median age of 62.1 *vs.* 56 years. Among 178 patients who received allo-HSCT for s/t AML there was significantly higher NRM and shorter OS when compared to *de novo* AML patients; however, CIR was similar in both groups (77).

Allo-HSCT efficacy in AML patients is well established; however, eligibility criteria for allo-HSCT in AML is evolving and remains an active field of research. It is estimated that patients with a relapse risk of ≤40% should be treated with intense chemotherapy (IC) with/without auto-HSCT rescue at first CR, compared to patients with a relapse risk of ≥40% who benefit more from allo-HSCT. Potential allo-HSCT candidates are evaluated for TRM with an HCT-specific comorbidity index (HCT-CI) score system. A score of ≥3 and age ≤60 years or a score ≥2 and age ≥60 years are not ideal candidates for allo-HSCT (78).

Role of cytogenetics risk category on allo-HSCT

The enhanced knowledge of the molecular architecture of AML helps define disease biology and potential molecular targets for novel therapies. These cytogenetic and mutational changes are pivotal in determining candidacy for allo-HSCT (79). Per ELN 2022 criteria, tests/procedures for a patient with AML include: tests to establish diagnosis (CBC with differential count, bone marrow aspirate, bone marrow trephine biopsy, immunophenotyping by flow cytometry); genetic analyses (cytogenetics to screen for gene mutations and gene rearrangements); medical history (demographics and medical history, detailed family history, patient bleeding history, analysis of comorbidities) (42).

Allo-HSCT is recommended for patients with a relapse

probability without allo-HSCT of >35–40%. Per 2022 ELN criteria, allo-HSCT is considered for patients: (I) with primary refractory disease; (II) in second CR (or incomplete complete response or incomplete count recovery); (III) With major cytoreduction but still active disease after salvage therapy. Genetic analysis upon diagnosis of AML is key for not only estimating outcome measures [early death (ED), OS, RFS, CIR, cumulative incidence of death (CID)], but for also estimating the RR for patients eligible for allo-HSCT as a salvage regimen. AML patients with favorable (low-risk) factors are only recommended allo-HSCT in CR1 in cases of inadequate clearance of MRD. AML patients with intermediate-risk and poor (high-risk) factors are more likely to be recommended for allo-HSCT, after careful consideration for other factors such as age, co-morbidities, social support, patient goals, and donor source. For eligible candidates, allo-HSCT is one of the best treatment options for AML patients not responding to initial induction of chemotherapy or experiencing relapse of disease (42).

Role of minimal residual disease (MRD) in allo-HSCT

The prognostic implication of MRD in AML patients undergoing HSCT is an active field of research. Multiple MRD measuring techniques, including polymerase chain reaction (PCR), multiparametric flow cytometry (MFC), or next-generation sequencing (NGS), are currently under clinical use; however, no head-to-head study has compared efficacy between these techniques.

Ustun *et al.* evaluated 203 AML patients with MAC given to 80 patients and RIC to 123 patients. Of these, fifty-two patients in MAC and 96 in the RIC arm achieved CR1. The pretransplant flow cytometry found residual disease in 25 patients (15 in MAC and 10 in RIC). In MRD + RIC arm (n=10), the hazard of DFS was 2.9 compared to 1.0 in MRD-RIC patients (n=113). In the MRD + MAC arm (n=15), the hazard was 1.1 compared to MRD-MAC arm (n=65) (80).

Araki *et al.* retrospectively analyzed 359 patients who underwent MAC allo-HSCT from peripheral blood or bone marrow. Patients with MRD positive status at the time of transplantation had a RR of 67% compared to 22% in MRD negative AML at three years (81).

Jourdan *et al.* conducted CBF 2006 trial and evaluated 198 CBF AML t(8;21), inv (16)/t(16;16) patients. The median age was 42 years old (18–60 years old). The KIT mutation was found in 40 patients. The MRD levels were monitored serially for RUNX1-RUNX1T1 or CBFBMYH11 transcripts by real-time quantitative PCR The CIR in unmutated RTK (FLT# and KIT mutations) patients with 3 log MRD reduction was 18%. In contrast, for patients with unmutated RTK without 3 log reductions, CIR was 45% which suggests that MRD status is an important predictor of relapse after allo-HSCT (79).

Ineligible elderly patients

Hypomethylating agents (HMA) such as azacitidine and decitabine have been established as an optimal treatment option for elderly patients (age 65 or more) or patients with significant comorbidities and poor performance status. One large phase 3 multicenter trial by Dombret *et al.* analyzed 488 patients who did not qualify for induction therapy or allogeneic stem cell transplant (82). All patients had newly diagnosed AML with greater than 30% blast cells. Patients were divided into two cohorts, azacitidine (n=241, median age 75; range, 64–91) *vs.* conventional therapy (n=247, median age 75; range, 65–89). Conventional therapy included best supportive care, standard induction chemotherapy, or low dose ara-c. This study was significant for median OS of 10.4 months in the azacitidine group compared to 6.5 months in conventional induction therapy. One-year survival was also significantly better with azacitidine (46.5% compared to 34.2% for conventional induction therapy). The safety profile was also better with azacitidine. Even though almost all patients (99.2% azacitidine and 100% conventional) had some treatment-related adverse events (TRAEs), azacitidine had a better or comparable grade 3/4 TRAE profile (69.9% azacitidine *vs.* 66.8% of conventional induction group requiring hospitalization due to TRAEs).

Newer agents have been added to azacitidine and proven beneficial in the elderly population who do not qualify for induction therapy. DiNardo *et al.* conducted a clinical trial of azacitidine and venetoclax in 431 previously untreated elder AML patients who did not qualify for intensive induction therapies (83). A total of 286 patients received azacitidine and venetoclax (median age 75; 49–91), while 145 received azacitidine and placebo. The OS in the treatment group was 14.7 months compared to 9.6 months in the placebo group. CR was 36.7%, while combined CR/CRi was 66.4% in the venetoclax/azacitidine group while CR was 17.9%, and CR/CRi was 28.3% in the placebo/azacitidine group. The treatment group had slightly

higher TRAEs (any grade nausea 44% *vs.* 33%, grade 3/4 thrombocytopenia 45% *vs.* 38%, and grade 3/4 neutropenia 42% *vs.* 28%, respectively for the treatment group and placebo group). Incidence of serious TRAEs was 83% in the treatment group and 73% in the placebo group. This study demonstrated superior efficacy and acceptable safety profile for patients in this age group.

Morsia *et al.* reported one study with 86 patients treated at Mayo Clinic evaluating venetoclax addition to standard HMA therapy (84). This study consisted of 44 treatment-naive patients (median age 73.5; 37–91) and 42 relapsed/refractory (median age 64.5; 18–79). In the treatment naïve group, 42.9% patients had complex karyotype, and 50% (n=22/44) achieved CR/CRi (complete response with incomplete hematological recovery). On the contrary, among relapse/refractory group, 42.9% had a complex karyotype and 33.3% (n=14/42) achieved CR/CRi. Patients who had prior exposure to HMA also demonstrated CR/CRi of 35.7%. Median OS was not reached in patients who achieved CR/CRi in both groups by the time of this publication. It is of note that mutations in TP53, FLT3, IDH and NPM1 demonstrated similar response rates.

Sorafenib has also been found to have survival benefit when combined with HMAs in some studies in the elder population. Ohanian *et al.* evaluated this combination in 27 patients with AML (median age 74; 61–86) who had FLT3 mutation and had treatment naïve (85). The ORR was 78% with 26% (n=7) achieving a complete response, and 44% (n=12) with an incomplete response. The median OS was 8.3 months. This combination proved to be well tolerated by this fragile population as most TRAEs were mild with only 26% of patients experiencing grade 3/4 infections and 26% with grade 3/4 neutropenic fever.

Conclusions

The CSC hypothesis proposes that CSCs originate from non-neoplastic progenitor cells that acquired genetic mutations to allow for carcinogenesis. The identification of these mutational patterns may help not only in estimating patient outcomes based on AML subtype and prognostic factors; it holds potential for correcting or preventing these mutations with therapies such as HSCT.

It is important to identify AML prognostic factors, pre- and post- treatment, to measure the expected clinical outcome of the disease. Favorable outcomes are found in AML patients with certain gene mutations (e.g., NPM2 in the absence of FLT3-ITD, biallelic CEBPA, CBFB-MYH11), translocations t(8;21) and t(15;17), and inversion of chromosome 16. Unfavorable outcomes persist in patients with advanced age, high WBC count >100 k at time of diagnosis, certain gene mutations (e.g., ASXL1, RUNX1, KIT, TP53, BCR-ABL1), history of prior allo-HSCT, and presence of leukemic cells in the CNS.

Newer scoring systems integrate single molecule molecular inversion probes to develop a genomics-based scoring system using cytogenetics, panel-based NGS, and a machine learning-based genetic score; this scoring system stratifies patients into favorable and poor genetic risk categories based on a sum of favorable and unfavorable variables. While models developed by Nakamura *et al.* for AML patients aged >65 who received conventional chemotherapy, categorizes patients as favorable, intermediate, and poor based on prognostic factors such as low performance status, advanced age, prior diagnosis of MDS.

Many newly diagnosed AML patients respond to induction therapy; however, post-induction relapse is common, ranging from 35% to 80%. Allo-HSCT decreases relapse risk (32%) compared to chemotherapy (59%) at four years, albeit this treatment comes with significant treatment-related mortality (25% *vs.* 4%). However, the risk/benefit of HSCT for treatment of AML must be considered on a case-by-case basis. Medically fit AML patients have shown to have better outcomes with HSCT, whereas AML patients with unfavorable outcomes have a higher risk of morbidity and mortality. For such patients, HMAs such as azacytidine and decitabine remain the optimal treatment option.

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Footnote

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