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Update on Non-M3 Acute Myeloid Leukemia — Etiology, Classification, Risk Stratification, Emergencies, Complications, Disease in Special Circumstances and Current and Future Therapeutics

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Abstract

Acute myeloid leukemia (AML) is a heterogeneous condition characterized by clonal proliferation of myeloid precursors and accumulation of leukemic blasts in the bone marrow (BM), ultimately resulting in failure of the BM. It accounts for approximately 80% of cases of acute leukemia in adults. AML has several life-threatening complications.

After establishing the diagnosis of AML, classifying the disease into the appropriate subtype, stratifying the risk group and determining fitness of the patient for chemotherapy, induction treatment is usually commenced. For elderly individuals and those unfit for chemotherapy, several alternative therapeutic options are available. After achieving complete remission of the disease, the patient will either receive consolidation therapy or will be subjected to hematopoietic stem cell transplantation (HSCT). Autologous and allogeneic forms of HSCT have their own indications, inclusion as well as exclusion criteria. The recent advancements in the diagnostics and therapeutics have facilitated the introduction of personalized therapy in patients with AML. There are several targeted therapies for AML and their clinical use is increasing with time. Evaluation of minimal residual disease and determination of drug resistance are vital tools to improve the outcome of AML therapy.

Keywords: Acute myeloid leukemia, induction chemotherapy, hematopoietic stem cell transplantation, drug resistance and residual disease

1. Introduction

AML is a heterogeneous condition, or group of disorders, at both phenotypic and molecular levels with a variety of distinct genetic alterations that give rise to the disease [1]. It is characterized by clonal cells that exhibit maturation defect corresponding to the stages in hematopoietic differentiation [2]. Leukemic stem cells (LSCs) play a major role in the maintenance of AML, while the bone marrow (BM) microenvironment permits leukemogenesis as well as disease progression [2,3]. Various environmental exposures such as exposure to chemicals and radiation, and various infections in addition to hereditary factors can predispose vulnerable individuals to develop AML [2,4,5]. The two-hit hypothesis of leukemogenesis is characterized by two types of genetic mutations that evolve following certain environmental exposures [2].

AML accounts for approximately 80% of cases of acute leukemia in adults [6]. It is characterized by clonal proliferation of myeloid precursors and accumulation of leukemic blasts in the BM, ultimately resulting in various cytopenias due to BM failure [6-8]. After establishing the diagnosis of AML, the disease is classified into the appropriate type and cytotoxic chemotherapy is commenced in order to control the disease and restore BM function [6,8,9]. Currently, various induction regimens are available for AML patients belonging to various age groups [9-11]. Once the disease is controlled, patients usually receive post-remission therapy (PRT) and the choice of treatment depends on several factors including: age of the patient, performance status, comorbid medical conditions, availability of donors for HSCT and experience of the medical institution, particularly if stem cell therapy is considered [10-12].

This review on AML will cover the following aspects: insights into the pathogenesis of AML; complications of AML with particular attention to various infections; diagnosis, classification and risk stratification; AML in special situations such as: old age, pregnancy, Philadelphia chromosome positivity and therapy-related AML (t-AML). The available and future AML therapeutics will cover the following: induction, consolidation and maintenance therapies; drug resistance; treatment of relapsed and refractory disease; various forms of HSCTs; immunotherapies; and newly evolving targeted therapies in AML.

2. Etiology and pathogenesis of AML

The existence of cancer stem cells (CSCs) was established about two decades ago, following demonstration that only a small fraction of leukemic cells from AML patients were able to propagate the disease in xenografts [1]. AML appears to be maintained by LSCs or leukemia-initiating cells that are more immature than the majority of circulating leukemic cells and are capable of self-renewal [2]. Thus, the heterogeneity of AML extends to LSC compartment at both cellular and molecular levels [1]. Further identification of LSC-specific markers paves the way for novel therapeutic platforms, although no single marker has been found to be uniform for LSCs [1]. However, the following surface markers are expressed by LSCs: (1) strongly positive CD13, CD33, CD123, CD44, CD45, CD96 and TIM3, (2) positive CD25, CD32, CD45 RA and CLL-1, and (3) weakly positive CD34, CD38 and CD90 [reviewed in 1].

The two-hit hypothesis of leukemogenesis implies that AML is a consequence of at least 2 mutations: (1) class I mutations that confer a proliferative advantage, and (2) class II mutations that impair hematopoietic differentiation [2]. These leukemic mutations may occur following the exposure to: cytotoxic chemotherapy, ionizing radiation, chemical compounds and infection with retroviruses. Additionally, certain familial disorders are associated with increased incidence of AML [2].

The BM is a dynamic network of growth factors, cytokines and stromal cells that provide a permissive environment of leukemogenesis and tumor progression. The BM stroma and leukemic blasts promote angiogenesis which is enhanced in AML [3]. Vascular endothelial growth factor (VEGF), basic fibroblast growth factor and angioproteins are the main proangiogenic mediators in acute leukemia. Also, high expression of CXC chemokine ligand 4 (CXCR4) by leukemic blasts and activation of CXCR4-CXCR14 axis are involved in the disruption of normal hematopoiesis and progression of leukemia [3]. Tumor microenvironment has a major role in cancer progression and resistance to treatment and, recently, it has been receiving particular attention reflecting its vital importance in cancer progression. The interaction between leukemic cells, BM microenvironment stromal cells and soluble mediators plays a critical role in blast survival, disease progression and resistance to chemotherapy [3].

Hematopoietic stem cells (HSCs) are a unique population of somatic stem cells that are capable of self-renewal and differentiating into myeloid and lymphoid lineages [4]. The accumulation of genetic mutations and cytogenetic abnormalities, within partially differentiated cells belonging to the myeloid lineage, following the exposure to benzene and cytotoxic anticancer agents can give rise to malignancies such as AML [4]. However, the etiology of AML is multifactorial and the following can predispose to the development of AML: (1) exposure to ionizing radiation and chemicals, (2) long-term exposure to benzene, (3) exposure to cytotoxic chemotherapy in t-AML, (4) infection with retroviruses, (5) familial forms of AML, (6) secondary to myelodysplastic syndrome (MDS), (7) secondary to congenital disorders of DNA repair such as Fanconi anemia, and (8) secondary to chronic myeloproliferative neoplasms (MPNs) such as: polycythemia rubra vera, chronic myeloid leukemia (CML), essential thrombocythemia and primary myelofibrosis [2,4,5]. Thus, AML is a heterogeneous disease in terms of the underlying chromosomal or molecular aberrations [13]. However, despite genetic heterogeneity, there is increasing evidence for common molecular and biological mechanisms in AML [13].

3. Diagnosis and classification of AML

AML refers to a group of hematopoietic neoplasms involving cells committed to the myeloid line of cellular development [7]. It is characterized by a clonal proliferation of myeloid precursors and reduced capacity to differentiate into more mature cellular elements [7]. AML should be suspected in any patient presenting with varying combinations of the following: (1) manifestations of anemia such as fatigue, dyspnea, dizziness and pallor; thrombocytopenia such as bruising and excessive bleeding; and neutropenia or neutrophils dysfunction such as

various infectious complications, (2) marked reduction in red blood cells, platelets and mature neutrophils on complete blood count, and (3) the presence of leukemic blasts on peripheral blood, BM and other tissues [6]. AML is diagnosed by BM biopsy using morphologic, cytochemical, immunophenotypic and cytogenetic analyses and molecular assays. Blasts should account for at least 20% of the total cellularity of the BM biopsy sample, except in leukemia with certain cytogenetic abnormalities and myeloid sarcoma which are diagnostic of AML regardless of the proportion of blast cells [6]. The blast forms must be identified as cells belonging to the myeloid, not the lymphoid, lineage. AML blasts express the following surface markers: CD13, CD32, CD33, CD34, CD38, CD64, CD11b, CD4 dim and HLA-DR [6]. After establishing the diagnosis of AML, the disease should be classified into the appropriate subtype according to the World Health Organization (WHO) classification scheme and/or the French-American-British (FAB) classification as shown in Tables 1 and 2 [6,14,15]. The subtype of AML is essential for prognostic scoring and therapeutic interventions [6]. The WHO classification system of AML is based on: morphology, immunophenotyping, genetics and clinical grounds and thus the 4 main subgroups of AML include: (1) AML with recurrent genetic mutations that accounts for 11% of all cases of AML, (2) AML with MDS-related features (6%), (3) t-AML and therapy-related MDS (t-MDS) (2%), and (4) AML, not otherwise specified, accounting for 81% of all cases as shown in Table 1 [6,8,14].

(1) AML with recurrent genetic abnormalities:
(a) AML with t(8,21) (q22, q22) RUNX1-RUNX1T1 (CBFA-ETO)
(b) AML with inv 16 (p3q22) or t(16,16) (p3q22) CBFB-MYH11
(c) APL with t(15,17) (q22,q12) PML-RARA
(d) AML with t(9,11) (q22,q23) MLLT3-MLL and other balanced translocations of 11q23 (MLL)
(e) AML with t (6,9) (p23,q34) DEK-NUP214
(f) AML with inv(3) (q21q26.2) or t(3,3)(q21,q26.2) RPNI-EV11
(g) AML (megakaryoblastic) with t(1,22)(p13,q13) RBM15-MKL1
(h) AML with mutated NPM (provisional entity)
(i) AML with mutated CEBPA (provisional entity)
(2) AML with myelodysplasia-related changes
(3) Therapy-related myeloid neoplasms; t-MDS and t-AML
(4) AML; NOS; not otherwise specified:
(a) AML with minimal differentiation
(b) AML without maturation
(c) AML with maturation
(d) Acute myelomonocytic leukemia
(e) Acute monoblastic/monocytic leukemia

(f) Acute erythroid leukemia (erythroid/myeloid and pure erythroleukemia)
(g) Acute megakaryoblastic leukemia
(h) Acute basophilic leukemia
(i) Acute panmyelosis with myelofibrosis
(5) Myeloid Sarcoma
(6) Myeloid proliferation related to Down syndrome (+21);
- Transient abnormal myelopoiesis
- Myeloid leukemia associated with Down syndrome
(7) Blastic plasmacytoid dendritic cell neoplasms
WHO: World Health Organization

Table 1. WHO classification of AML related tumors

FAB subtype	Morphological classification	Percentage of all AML cases
AML - M ₀	Undifferentiated acute myeloid leukemia	5%
AML - M ₁	Acute myeloid leukemia with minimal maturation	15%
AML - M ₂	Acute myeloid leukemia with maturation	25%
AML - M ₃	Acute promyelocytic leukemia	10%
AML - M ₄	Acute myelomonocytic leukemia	20%
AML - M ₄ e05	Acute myelomonocytic leukemia with eosinophilia	5%
AML - M ₅	Acute monocytic leukemia	10%
AML - M ₆	Acute erythroid leukemia	5%
AML - M ₇	Acute megakaryocytic leukemia	5%
FAB: French – American - British		
AML: acute myeloid leukemia		

Table 2. FAB classification of acute myeloid leukemia

Several cytogenetic abnormalities and genetic mutations have been reported in patients with AML as shown in Tables 3 and 4 [12,15]. In AML patients with normal cytogenetics, certain mutational abnormalities are present and these molecular mutations have their impact not only on the response to chemotherapeutic regimens, but also on the overall prognosis of patients as shown in Tables 5 and 6 [12,16-22]. According to their cytogenetic abnormalities and their molecular profiles, patients with AML are stratified into 4 risk groups, namely favorable, intermediate I, intermediate II or unfavorable as shown in Table 6 [12,21,22].

Translocation or chromosomal Abnormality	Oncofusion protein involved	Frequency of occurrence in AML
t (8,21)	AML1 - ETO	10%
t (15,17)	PML - RARA	10%
inv (16)	CBF - MYH11	5%
der (11q 23)	MLL - fusions	4%
t (9,22)	BCR - ABL	2%
t (6,9)	DEK - CAN	<1%
t (1,22)	OTT - MAL	<1%
t (8,16)	MOZ - CBP	<1%
t (7,11)	NUP 98 - HOX A9	<1%
t (12,22)	MN1 - TEL	<1%
inv (3)	RPN - EV11	<1%
t (16,21)	FUS - ERG	<1%

Table 3. Chromosomal abnormalities and their frequencies in AML

Category	Frequency	Examples	
Transcription factor fusions	18%	- PML / RARA - CBFB / MYH11 - RUNX1 / RUNX1T1 - PICALM – MLLT10	
NPM1 mutations	27%	-	
Tumor suppresser genes	16%	- TP 53 - WT1 - PHF 6	
DNA methylation	44%	- DNMT3A - DNMT3B - DNMT1	- TET1 - TET2 - IDH1 - IDH2
Activated signals	59%	- FLT3 - KIT - Other tyrosine kinases - Serin-theonine kinases - PTP: protein tyrosine phosphatases	- KRAS - NRAS
Myeloid transcription factors	22%	- RUNX1 - CEBPA - Other myeloid transcription factors	
Chromatin modifiers	30%	- MLL fusions - MLL - PTD - NUP 98 – NSD1	- ASXL1 - EZH2 - KDM6A
Cohesion complex	13%	-	
Spliceosome complex	14%	-	

Table 4. Gene mutations in acute myeloid leukemia

Genetic mutation	Chromosome involved	Frequency in CN - AML	Prognostic impact	
NPM1	5q35	45-60%	Favorable	* Higher CR rates * Better OS, EFS and DFS
CEBPA	19q13.1	10-15%	Favorable	* Better OS, EFS and DFS
IDH1	2q33	7.6-13.6%	Unfavorable	* Worse DFS * High risk of relapse
IDH2	15p26	8.7-19%	Unfavorable	* Shorter OS and lower remission rates * High frequency of induction failure
WT-1	11q13	10-13%	Unfavorable	* Shorter OS and DFS * Lower CR rates and higher relapse rates
FLT-ITD	13q12	25-35%	Unfavorable	* Worse OS and DFS
MLL-PTD	11q23	11%	Unfavorable	* Worse median survival and RFS * Shorter duration of remission
DNMT3A	2p23.3	15-36.1%	Unfavorable in young and older patients	* Worse OS, EFS and risk free survival * Lower CR rates
TET2	4 q 24	7 - 34%	Unfavorable in young and older patients	* Worse OS and EFS * Lower CR rates * Poorer prognosis in favorable risk CN-AML
ASX1 -1	20 q11	5.3 - 17.2%	Unfavorable	* Worse OS and EFS * Poorer prognosis in favorable risk CN-AML
RUNX 1	8 q 22	6.3 - 13.2%	Unfavorable	
EZH 2	-	Occasional in AML	Unfavorable	* Carries poor prognosis in AML secondary to: MDS, CMML and primary myelofibrosis.
BAALC	8 q 22.3	19 - 50%	Unfavorable	* Worse OS and DFS * Greater disease resistance to treatment
MN1	22 q 12.1	50%	Unfavorable	* Worse OS and risk free survival * High relapse rates and poor response to therapy
ERG-1	21 q22.3	25 - 37%	Unfavorable	* Worse OS and higher relapse rates

Genetic mutation	Chromosome involved	Frequency in CN - AML	Prognostic impact	
NRAS	1 p13	9.1 - 13%	Neutral	*May be favorable when other genetic aberrations are considered * May sensitize to cytarabine
CKIT	4 q12	17%	Unfavorable	* Poor prognosis
MIR - 181q	9q33.3	-		** Favorable outcome
MIR - 191	3p 21.31	-		** Poor prognosis
MIR-199a	19p13.2	-		** Poor prognosis

OS: overall survival

MDS: myelodysplastic syndrome

EFS: event free survival

CMML: chronic myelomonocytic leukemia

DFS: disease free survival

CR: complete remission

RFS: relapse free survival

Table 5. Genetic mutations in AML with normal cytogenetics and their impact on prognosis

Risk category	percentage	Subsets and examples	
Favorable	27%	- t(8,21) (q22,q22) - inv 16 (p13.1,q22) or t(16,16) (p13.1,q22) - Mutated NPM ₁ without FLT ₃ - ITD (CN - AML) - Mutated CEBPA (CN-AML)	RUNX1 - RUNX 1T1 (AML-ETO) CBFB - MYH 11
Intermediate I	31%	- Mutated NPM ₁ and FLT ₃ - ITD - Wild-type NPM1 and FLT3 - ITD - Wild-type NPM ₁ without FLT ₃ - ITD	(CN - AML) (CN - AML) (CN-AML)
Intermediate II	19%	- t (9,11) (p22, q23) - Cytogenetic abnormality not classified as favorable or adverse	MLL T3 - MLL
Unfavorable	23%	- inv 3 (q21, q 26.2) or t (3,3) (q21, q 26.2) - t (6,9) (p23, q34) - t (v,11) (v1q233) - -5 or del (5q) ; -7, abnormal ((17p) ; complex karyotype	RPN ₁ - EV11 DEK - NUP 214 (CAN) MLL - rearranged

Table 6. Risk stratification of AML according to cytogenetics and molecular genetics

After establishing the diagnosis of AML and classifying the disease into the appropriate subtype, all patients should undergo a thorough evaluation in order to determine their fitness for the treatment [23,24]. Particular attention should be given to older patients who are likely to have comorbid medical conditions and decreased performance status which may limit their chances to have the standard therapies [23,24]. When compared to younger individuals, older adults with AML are likely to develop more complications related to chemotherapy and thus have diminished survival rates [23]. The pre-treatment assessment of older patients with AML includes specific investigations to evaluate their physical function and their comorbid conditions [23]. Patients with age-related chronic cardiac, pulmonary, renal, and hepatic disorders in addition to diabetes mellitus suffer greater toxicity of chemotherapy and radiotherapy. However, therapeutic decisions should be individualized and tailored according to the conditions of each patient taking into consideration: the age, performance status, comorbidity index and cytogenetic as well as molecular profiles [23].

4. Complications of AML

AML has numerous complications and these include: (1) anemia [25], (2) infectious complications including neutropenic colitis [25-27], (3) bleeding diathesis due to thrombocytopenia and disseminated intravascular coagulation [25], (4) leukostasis and hyperleukocytosis [25,26,28], (5) metabolic and electrolytic disturbances that include: lactic acidosis, hyperphosphatemia, hypokalemia, hyperuricemia, hypercalcemia, hypocalcemia, hyperkalemia, and tumor lysis syndrome following the administration of chemotherapy [25,26,29,30], (6) venous thromboembolism [25,26], (7) extramedullary involvement including: myeloid sarcomas, central nervous system (CNS), ocular involvement as well as skin and joint involvement [25,26], (8) acute pulmonary failure and pericardial effusions [25], and (9) oral complications including mucositis, mouth ulcerations and gingival hypertrophy [25].

4.1. Infectious complications in patients with AML

Despite the significant advances in supportive care, infectious complications remain a significant cause of morbidity and mortality in patients with leukemia [31]. The risk factors for infectious complications in patients with acute leukemia include: (1) impaired cellular and humoral immunity caused by the underlying disease, (2) the utilization of more intensive chemotherapeutic regimens in induction and salvage therapies, (3) the incorporation of monoclonal antibodies, (4) the use of consolidation and maintenance strategies, (5) profound neutropenia, (6) severe mucositis, (7) the increased use of indwelling vascular catheters, and (8) the degree of hemorrhagic diathesis of skin and mucosal tissues [31,32]. However, studies have shown that: (1) the addition of cladribine to standard induction chemotherapy in patients with newly diagnosed AML, aimed at increasing the response rate to chemotherapy, has no impact on the incidence and spectrum of infectious complications, and (2) in AML patients younger than 65 years, fludarabine-based induction chemotherapy is not associated with an increase in the incidence of infections, particularly invasive fungal infections (IFIs), compared to conventional regimens that are commonly used in AML induction treatment [32,33]. The

early utilization of empirical antimicrobial therapy and the maintenance of proper hygiene are the most effective strategies to combat infectious complications and to reduce mortality in hospitalized AML patients, while delayed recognition of infections and late administration of antimicrobial therapy not only increase morbidity and mortality but also potentially increase the economic burden associated with infections in patient with leukemia [31,34].

Neutropenic fever is an important cause of mortality and morbidity in patients with AML receiving intensive chemotherapy [35]. The implementation of empirical antibacterial therapy in patients with febrile neutropenia has led to a dramatic reduction in infection-associated mortality in patients with hematological malignancies [31]. Careful selection of antibiotics and early institution of antifungal treatment in addition to consideration of endemic infections such as tuberculosis may help in reducing morbidity and mortality during AML treatment [35]. However, the emergence of multidrug-resistant microorganisms is a real concern [31]. Therefore, medical institutions should evaluate antibacterial resistance routinely and regularly in order to select appropriate empirical antibiotic therapy [35]. Additionally, timely diagnosis and early initiation of appropriate antimicrobial therapy are crucial in combating infections in these immunocompromised hosts [31].

Gram-negative bacterial infections are still among the most important causes of mortality during neutropenia in patients with hematological malignancies, especially when related to *Pseudomonas aeruginosa* [36]. Due to its potent anti-pseudomonas activity, ceftazidime represents one of the antibiotics of choice for the empirical therapy of bacterial infections in high-risk febrile neutropenic patients. In these immunocompromised patients, blood concentrations of the drug 4 - 5 times the minimum inhibitory concentration may be required for maximal bacterial efficacy with ceftazidime [36].

Fluoroquinolone prophylaxis is frequently used in high-risk neutropenic patients world-wide. In particular, levofloxacin prophylaxis during neutropenia in high-risk patients was shown to be effective in preventing infectious complications in a study published in 2005 by GIMEMA group and was confirmed by a Cochrane systemic review in 2012 [37]. In a retrospective study performed in Italy that included 81 patients with AML diagnosed and treated between 2001 and 2007, the following results were obtained: Gram-positive bacterial infections predominated during the induction phase of chemotherapy, while Gram-negative bacterial infections predominated during the consolidation phase of chemotherapy, and a high rate of bacterial resistance to levofloxacin was encountered during the consolidation therapy [37]. Therefore, constant monitoring for fluoroquinolone resistance among Gram-negative bacterial isolates is recommended to preserve the efficacy of levofloxacin prophylaxis [37]. In a randomized prospective study, which included 95 patients with AML, conducted in South Korea between March and July 1999, it was found that antibiotic prophylaxis did not reduce the incidence of infections or infection-associated deaths in patients with AML on intensive chemotherapy. The authors recommended that routine use of antibiotic prophylaxis should be reconsidered taking into consideration the increased incidence of Gram-positive bacterial infections, and the high level of resistance to fluoroquinolones and macrolides [38].

In patients with AML receiving cytotoxic chemotherapy, sepsis remains a serious complication of neutropenia as it is associated with significant morbidity and mortality [34,39]. Risk factors

for bloodstream infections (BSIs) in patients with AML include: (1) intensive chemotherapeutic regimens, (2) prolonged neutropenia, (3) neutropenic enterocolitis, (4) lower respiratory tract infections, (5) uncontrolled hematological malignancy, (6) cellulitis, (7) mucositis, (8) central venous catheter (CVC) infections, and (9) IFIs [40,41]. The expression of Toll-like receptors has been found to be an independent risk factor for the development of sepsis in patients with AML following the administration of intensive induction chemotherapy. Toll-like receptors play an important role in host defense against microorganisms [39].

In a French multicenter study that included 459 younger patients with AML: 1369 febrile neutropenic episodes were encountered, no identifiable cause for fever was found in 23% of patients, and clinically and microbiologically documented infections were identified in 77% of patients [42]. BSIs were reported in 314 episodes (29% of patients), Gram-positive organisms were cultured in 129 episodes (12%) and Gram-negative organisms were cultured in 144 episodes (14%). Pulmonary infections were documented in 14% of episodes while IFIs were documented in 11% of episodes of febrile neutropenia [42]. In another study that included 129 febrile episodes experienced by 42 patients with AML receiving chemotherapy and antibiotic prophylaxis; non-infectious sources of fever accounted for 17.8% of febrile episodes, Gram-positive microorganisms accounted for 75.8% of BSIs, while Gram-negative bacteria accounted for 12.1% of BSIs [41].

Despite the broad use of primary antifungal prophylaxis (PAP), IFIs particularly mold infections remain an important cause of treatment failure in patients with acute leukemia and a leading cause of morbidity and mortality in patients with AML, predominantly during the remission-induction phase of chemotherapy [43-45]. The incidence of IFIs has increased dramatically over the past few decades [43]. In the USA, the incidence of fungal sepsis has doubled between 1979 and 2000 due to the following reasons: (1) the extended survival of patients with acute leukemia, (2) the recent advances in supportive care, (3) the improvement in controlling bacterial infections, (4) the use of more intensive chemotherapy, (5) the utilization of potent immunosuppressive agents, and (6) the increase in the performance of HSCT [43]. The risk factors for IFIs in patients with acute leukemia include: (1) the primary disease such as AML, (2) cytotoxic chemotherapy including high-dose cytarabine and fludarabine, (3) indwelling devices including CVCs, (4) colonization with *Candida* species, (5) neutropenia, (6) old age, (7) use of total body irradiation (TBI) in pre-transplant conditioning therapy, (8) HSCT, particularly allogeneic grafts and mismatched donors, (9) graft versus host disease (GVHD) and (10) corticosteroid therapy [43]. Patients with acute leukemia can be stratified into 3 risk categories for IFIs: low risk, intermediate risk and high risk depending on a variety of factors that include: (1) host factors: age, fitness and comorbid conditions, (2) factors related to the primary disease: responsive or refractory to chemotherapy, and (3) other factors including the use of aggressive cytotoxic chemotherapy and immunosuppressive treatment, organ function and prior fungal infection or exposure to antifungal therapy [45].

In patients with AML, treatment of IFIs faces the following challenges: (1) the changing epidemiology of fungal infections, (2) early and correct diagnosis of fungal infections is usually difficult, and (3) monotherapy with antifungal agents is often unsuccessful [46]. The management of IFIs in patients with acute leukemia is further complicated by the recent increase in

the frequency of infections by non-*Aspergillus* molds such as zygomycosis and the emergence of drug-resistant fungal pathogens [43]. Therefore, despite the recent and rapid expansion in antifungal armamentarium over the past few decades, the mortality associated with IFIs in patients with acute leukemia is still high [43]. Early diagnosis of IFIs in neutropenic patients has the potential to increase antifungal therapeutic response [47]. In patients with AML, the following chemotherapy-independent factors influence the onset of invasive mold infections: (1) hospital-independent exposure to infectious agents, (2) comorbidities, and (3) personal habits [48]. The recognition of these risk factors at the time of hospital admission helps to define the risk category of the patient and improve targeted prophylactic strategies [48]. The gold standard diagnostic tests are histopathological demonstration of organisms in tissue specimens and growth of fungal agents in culture media [47]. Blood cultures are positive in 50% of patients with invasive *Candida* or *Fusarium* infections, but are rarely positive in patients having invasive aspergillosis. Unfortunately, obtaining specimens for histopathology or culture may be difficult [47]. The glutacell serum β -glucan detection assay is highly sensitive and specific as a diagnostic test for IFIs in patients with AML [47]. Advancements in the diagnostic techniques are the following: (1) non-culture-based serum biomarkers such as β -glucan and *Aspergillus* galactomannan, (2) molecular tests such as polymerase chain reaction (PCR) for fungal DNA, and (3) high resolution radiological imaging such as computed axial tomography (CAT) scans that have improved the early detection of fungal infections and facilitated prompt pre-emptive antifungal therapy [43].

In patients with acute leukemia, the following factors influence decisions regarding the initiation of antifungal therapy and the choice of antifungal treatment: (1) the net state of immunosuppression, (2) the risk stratification of the primary disease such as AML, (3) the disease status such as high risk, relapsed or refractory AML, (4) the concomitant comorbidities, (5) the presence or absence of organ dysfunction, (6) the local fungal epidemiological patterns, (7) the pharmacological profile of antifungal agents, (8) exposure to pathogenic fungi, (9) drug-drug interaction, (10) the overall cost, (11) the presence of CVCs, (12) old age, and (13) neutropenia [43-45]. The following immunocompromised patients are at risk of invasive aspergillosis: (1) patients with AML during remission induction, (2) recipients of HSCT, and (3) patients having severe and prolonged immunosuppression [43-45,49]. The risk factors for invasive candidiasis include: (1) hematological malignancy such as AML, (2) extremes of age: < 1 month and > 65 years, (3) neutropenia, and (4) recent abdominal surgery [43-45,50]. In patients with leukemia having documented or presumed IFIs, the following factors influence the decision making of antifungal therapy: (1) the presence of active leukemia and plans for HSCT, (2) the type of chemotherapy: induction, consolidation or palliative, (3) certainty of diagnosis of fungal infection, (4) the type of fungus, (5) the site of infection, (6) prior antifungal exposure, (7) refractory IFI or previous lines of antifungal therapies, (8) concomitant infection such as cytomegalovirus or bacteria and their treatments, (9) risk of nephrotoxicity, (10) liver dysfunction, (11) infected CVCs, (12) ability to take oral medications, (13) interactions between antifungal agents and other medications, (14) compliance of the patients, (15) outpatient versus inpatient treatment, and (16) preference of the patient and the ability to cover medication costs [31,43-46,50].

The recent advances in the diagnostic modalities such as *Aspergillus* galactomannan test, 1,3- β -D-glucan test and PCR for fungal DNA, and antifungal therapeutics have facilitated an early diagnosis of IFIs and improved the response to treatment, ultimately resulting in improved outcome [50,51]. Strategies that improve survival of patients with AML having mold infections include: the preemptive initiation of antifungal therapy at the first sign of invasive aspergillosis on CAT scans, and antifungal prophylaxis with posaconazole and other drugs [49,51].

In patients with AML, management of fungal infections, particularly IFIs, requires an individualized treatment plan and a multimodal approach that includes: the use of more effective antifungal agents such as combination therapies or high-dose treatments, and the use of agents to enhance the immune response of the host [46]. Strategies that are utilized to enhance the immunity of cancer patients having opportunistic infections include: donor granulocyte transfusions, growth factors such as granulocyte-colony stimulating factor (G-CSF), and interferon- δ [46].

In patients with IFIs, early initiation of antifungal therapy has a profound impact on mortality rates, but reliable diagnostic tests are lacking [49]. In patients with febrile neutropenia suspected to have fungal infection, empirical therapy includes caspofungin as the first choice and liposomal amphotericin-B (ambisome) as the second choice [49]. In AML patients undergoing induction chemotherapy, 200 mg of prophylactic oral fluconazole twice daily is safe and well tolerated and results in trends towards reduced incidences of lung infiltrates and hepatosplenic candidiasis [51]. In the setting of consolidation therapy for AML, fluconazole is the most cost-effective approach to antifungal prophylaxis compared to posaconazole or voriconazole [52]. During the induction phase of AML, posaconazole was found to reduce the incidence of proven or probable breakthrough IFI, particularly aspergillosis, in a real life setting [53]. Hence, in patients at risk of invasive aspergillosis, posaconazole prophylaxis is recommended [49]. In patients with AML subjected to remission-induction chemotherapy, echinocandin-based primary antifungal prophylaxis has been associated with a higher risk of breakthrough IFIs than patients receiving azoles [44,54]. Isavuconazole is a novel, broad-spectrum, triazole antifungal agent which has recently been proven to be safe and tolerable at doses of 200-400 mg daily when used as prophylaxis in immunocompromised patients at high-risk of fungal infections [55]. In patients with AML receiving induction chemotherapy, a single high dose of ambisome [15 mg/kg] is safe and feasible when used as antifungal prophylaxis [56]. The mortality rate of IFIs in neutropenic individuals having *Candida* infection may reach 50% and those having aspergillosis, fusariosis and trichosporonosis may reach 100% [47].

4.2. Emergencies in AML

The following emergencies can be encountered in patients with AML: (1) tumor-lysis syndrome that occurs mainly in patients presenting with high white blood cell (WBC) count and high tumor load. It manifests with hyperkalemia, hyperuricemia, hypocalcemia, and hyperphosphatemia. It requires urgent treatment with aggressive hydration, correction of electrolytic abnormalities and reduction of elevated uric acid levels [26,29,30]. (2) Hyperleukocytosis and leukostasis which can predispose to thromboembolic manifestations, paradoxical bleeding as well as respiratory distress. Management can be in the form of leukapheresis if the WBC

is more than 50,000 in symptomatic patients or more than 100,000 in asymptomatic patients as well as early initiation of chemotherapy [26,28]. (3) Neutropenic complications that include: febrile neutropenia; bacteremia, fungemia and septic shock; IFIs; and enterocolitis which manifests as fever, abdominal pain and thick bowel wall. Neutropenic colitis can be treated by: bowel rest, intravenous fluids, total parenteral nutrition in addition to intravenous antimicrobials with appropriate anaerobic cover [26,28]. (4) Transfusion-related acute GVHD which is a rare complication, but mortality rate may exceed 95%. It occurs 1-4 weeks after blood transfusion and manifests with fever, skin rash, diarrhea and hepatitis or elevated bilirubin and liver enzymes. This rare complication can be managed with corticosteroids and other immunosuppressive therapies but can be prevented by irradiation, leukodepletion and leukofiltration of blood products [26]. (5) Leukemic meningitis, which requires specific interventions including intrathecal chemotherapy [26]. (6) Cytarabine-induced cerebellar toxicity that requires replacement of cytarabine by another cytotoxic agent. (7) Finally, life-threatening hemorrhagic tendency [26].

4.3. AML in old age

The management of older patients with AML is a real therapeutic challenge because of the following reasons: (1) these patients are more likely to have comorbid medical conditions that limit their treatment options, (2) their clinical condition and their performance status may not allow the application of intensive chemotherapeutic regimens, (3) their disease is more likely to be resistant to chemotherapy, and (4) they have more frequent unfavorable AML subtypes [10]. Once AML is diagnosed in older subjects, physicians should make a therapeutic plan and try to adhere to it as much as possible, although therapeutic modifications may become justified under certain circumstances [11]. The same therapeutic principles that apply in younger adults can be followed provided the clinical circumstances of the patients allow the delivery of intensive induction chemotherapy [11]. The molecular features that characterize the risk of AML in middle-aged adults also apply to older patients with AML although the incidence of unfavorable genotype is significantly higher in older patients [11]. Numerous new drugs including targeted therapies and monoclonal antibodies are emerging from the development pipeline. Some of these drugs may be more tolerable and more efficacious in elderly patients than the standard induction regimens [11]. Otherwise healthy older patients with AML having good performance status can be subjected to standard induction chemotherapeutic regimens that include anthracyclines and cytarabine [10]. For older patients with: indolent AML, high comorbidity index, poor performance state and unfavorable risk disease, it is justifiable to offer them supportive care alone and/or less intensive chemotherapy rather than the standard induction chemotherapy which is associated with high treatment-related mortality (TRM) [10]. These patients can be offered: (1) transfusion of blood components, (2) various antimicrobials to treat their infectious complications, and (3) less intensive therapies such as: hydroxyurea, decitabine and low-dose cytarabine [10]. Although growth factors, such as G-CSF, may be beneficial in patients having neutropenia and sepsis, randomized trials have found no benefit to the routine use of G-CSF during remission induction in older patients with AML [10]. Older patients with AML should be encouraged to participate in well-designed clinical trials in order to draw conclusions on the safety and efficacy of the agents used in this

age group [10,11]. Also, older patients with AML achieving complete remission (CR) of their disease, but are not eligible for standard myeloablative HSCT, should be offered either a reduced intensity conditioning (RIC)-allogeneic HSCT or a clinical trial for post-induction or maintenance therapy [10,11].

The available therapeutic options for older patients with AML include: (1) standard induction therapy with 7+3 regimen, (2) hypomethylating agents such as azacitidine and decitabine, (3) nucleoside analogs such as clofarabine and sapacitabine, (4) immunomodulatory agents such as lenalidomide, (5) farnesyl transferase inhibitors such as tipifarnib, (6) monoclonal antibodies such as gemtuzumab ozogamicin (GO, myelotarg), (7) low-dose cytarabine, and (8) the best supportive care with blood product transfusions, antimicrobials as needed and oral cytotoxic drugs such as hydroxyurea [10,11,57,58].

In general, the median age for AML is in the 6th decade of life, but in certain countries like Sweden, most patients with AML are on the old side and the median age for AML is 71 years [59]. However, survival in patients with AML decreases with age, most patients with AML who are younger than 75 - 80 years, in certain geographic locations, tolerate and benefit from induction chemotherapy [59]. As AML induction chemotherapy is cost-effective in elderly individuals, all AML patients younger than 80 years without specific contraindications should be considered candidates for remission induction [60].

In AML patients older than 60 years of age, doubling the dose of daunorubicin in the induction phase of chemotherapy has been associated with a more rapid response and a higher response rate than the conventional dose without having significant adverse toxic effects [61]. Additionally, decitabine is well tolerated in older patients with AML who are medically unfit to have intensive chemotherapy, but myelosuppression related to the use of decitabine is the major toxicity encountered [62]. Interestingly, the response rate to decitabine and the overall survival (OS) are not adversely influenced by poor risk cytogenetics and preceding MDS [62].

In a study in patients with AML older than 60 years of age, the addition of bortezomib at doses of 1.3 mg/m² to the standard induction therapy, 7+3 regimen, and consolidation therapy with intermediate dose cytarabine has been shown to be associated with encouraging remission rates. Additionally, that study showed effectiveness and tolerability of bortezomib [63]. Also, in older AML patients, who are medically fit, the farnesyl transferase inhibitor tipifarnib, can be safely administered at a dose of 600 mg twice daily for 10 days in combination with the standard 7+3 induction regimen with severe gastrointestinal toxicity as the chief limiting factor [64].

Clofarabine, a nucleoside analog, may be an appropriate alternative to intensive induction regimens in certain subsets of older patients with newly diagnosed AML, particularly those with decreased performance status and history of cardiovascular disease [65]. Clofarabine has been shown to be an active drug in the treatment of older patients with AML as a single agent or in combination with other drugs [66]. It produces similar responses and potentially decreased mortality when compared to the traditional 7+3 induction chemotherapy [66]. Clofarabine use in combination with DNA methyltransferase inhibitors such as decitabine is a promising approach in older patients with AML who are not eligible for intensive chemo-

therapy [66]. The use of clofarabine therapy in the induction treatment of older AML patients, who are not suitable for intensive chemotherapy, has produced superior CR rates compared to low-dose cytarabine, but survival may be either similar to or even superior to low-dose cytarabine [67,68]. Sapacitabine, a novel oral nucleoside analog, has shown promising clinical efficacy and favorable toxicity once used in treating AML in older subjects [69].

GO is an anti-CD33 monoclonal antibody which has been shown to be effective in the treatment of older patients with CD33 positive AML in first relapse and it has acceptable toxicity. Unfortunately, the duration of remission is relatively short [57]. Also, in patients with CD33 positive in relapsed or refractory setting, the combination of: GO, intermediate-dose cytarabine and mitoxantrone has been shown to be an effective salvage regimen with 2-year rates of overall survival, event-free survival (EFS) and disease-free survival (DFS) of 41%, 33% and 53% respectively [70]. Once GO is used in combination with induction therapies in older AML patients, it reduces the risk of relapse and improves survival with slight increase in toxicity as revealed by a meta-analysis that included 2228 patients in the United Kingdom [71]. A randomized phase II study has shown that the addition of GO to the 7+3 induction regimen did not show significant superiority over the standard 7+3 regimen. In fact, death during the induction phase of chemotherapy was higher in the GO group due to venoocclusive disease (VOD) [72]. However, the addition of GO to: fludarabine, cytarabine and idarubicin induction therapy in CD33-positive AML in patients younger than 65 years has shown higher CR rates and lower mortality rates during the induction phase of chemotherapy [73].

5. Philadelphia chromosome positive AML

Studies have shown that the incidence of Philadelphia chromosome positivity in de novo AML is extremely rare and that Philadelphia chromosome positive AML accounts for only 0.3-3.0% of all newly diagnosed cases of de novo AML [74-77]. However, Philadelphia chromosome positive AML has to be distinguished from: CML in blast cell crisis, mixed phenotype leukemia and secondary AML with positive Philadelphia chromosome [75,76,78].

The following features have been reported in Philadelphia chromosome positive de novo AML: (1) lack of splenomegaly, (2) lack of history of abnormal hemogram, (3) lack of history of chronic phase CML, (4) lack of significant peripheral blood basophilia, (5) frequent expression of lymphoid markers, (6) lower cellularity and lower myeloid/erythroid ratio on BM examination, (7) presence of monosomy 7, inversion 16 and chromosome 10 deletion, (8) predominance of p210 rather than p190 fusion protein, and (9) return to normal karyotype after induction chemotherapy [75-78]. On the contrary, CML in myeloid blast cell crisis presents in patients with history of an abnormal hemogram and chronic phase CML in addition to splenomegaly on physical examination. Also, trisomy 8, trisomy 19 and iso-chromosome 17q as well as return to chronic phase CML after induction therapy are in favor of CML in myeloid blast cell crisis [75-79].

The available therapeutic interventions in patients with Philadelphia chromosome positive AML include: induction chemotherapy using various chemotherapeutic regimens, tyrosine

kinase inhibitors (TKIs) such as imatinib and dasatinib as well as allogeneic HSCT with its graft versus leukemia (GVL) effect which constitute the only potentially curative therapeutic modality [74,75,80]. Prognosis of Philadelphia chromosome-positive AML is usually poor as the disease is aggressive, relapse rates are high and durations of responses are rather short. Additionally, responses to anthracyclines and cytarabine are poor and the median survival is limited to 6-9 months [74,75,77,80]. However, complete molecular responses have been reported after conventional chemotherapy and imatinib and tetraploidy have been reported in a patient with Philadelphia chromosome-positive AML [74,81]. As the disease is insufficiently studied, further epidemiological studies and large-scale registries may contribute not only to a better understanding of the disease but also to more efficacious therapeutic interventions [75,76,80].

5.1. Therapy-related AML

Therapy-related myeloid neoplasms (t-MNs) account for approximately 10-20% of all cases of: AML, MDS, MSD/MPNs [82]. The incidence of t-MNs among patients treated with cytotoxic chemotherapy varies according to the underlying disease, specific agents used, timing of exposure and doses of chemotherapeutic agents used [82,83]. The median age for t-MNs is 61 years, although they can develop at any age [82]. The risk of t-MNs associated with the use of alkylating agents and radiation appears to increase with age, while the risk of t-MNs associated with the use of topoisomerase II inhibitors appears to be constant across all ages [82]. The latency period between the first exposure to cytotoxic therapy and the development of t-MNS ranges from 1 to 10 years [82,83]. Patients usually present with complications of pancytopenia and they should have thorough clinical and laboratory evaluation [82,83]. However, therapeutic recommendations depend on: (1) age of the patient, (2) comorbidities, (3) performance status, (4) status of the primary disease, (5) presence of complications from the primary disease, and (6) clonal abnormalities present in t-AML cells [83].

t-AML is an important disease entity to be studied for several reasons: (1) it represents the most serious complication of current cancer treatment, (2) it is directly induced by chemotherapeutic agents and irradiation, (3) t-AMLs presenting with the same chromosomal abnormalities and genetic mutations that are present in de novo AML are particularly suited for studies on leukemogenesis and leukemia biology, (4) t-AML may be preceded by a transient phase of t-MDS and cytopenias and this stage is suited for further studies and comparison with de novo MDS transforming into AML, (5) there is still no consensus on the therapeutic management of t-AML, and (6) t-AML stimulates scientists to study the safety of various therapeutic modalities that are used in the treatment of various cancers in order to replace these treatments by safer ones in the future [83].

t-AML can occur at any age and it represents 5-14% of all newly diagnosed cases of AML [83]. The primary disease in t-AML can be: (1) a hematological malignancy such as acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, Hodgkin's disease, non-Hodgkin lymphoma, multiple myeloma or acute promyelocytic leukemia, (2) a solid tumor such as small cell lung cancer, germ cell tumor and cancers of ovaries, breast, testis or prostate, and (3) an autoimmune disease such as rheumatoid arthritis, systemic lupus erythematosus, multiple

sclerosis, ulcerative colitis and Wegener's granulomatosis [83,84]. The use of the following drugs and chemotherapeutic agents is associated with the development of t-AML: (1) alkylating agents such as: busulfan, melphalan, chlorambucil, cyclophosphamide, procarbazine as well as mitomycin-C, (2) topoisomerase II inhibitors such as: etoposide, mitoxantrone, doxorubicin and dactinomycin, (3) antimetabolites such as methotrexate, 6-mercaptopurine in addition to fludarabine, (4) antimicrotubules such as vincristine and paclitaxel, (5) growth factors such as G-CSF, and (6) immunomodulators such as azathioprine [82-92].

Studies have shown that the use of alkylating agents is associated with the development of t-MDS and unbalanced chromosomal aberrations such as: 5q-, -5, 7q-, -7, 17p13 (TP53) and unarranged mixed lineage leukemia (MLL) gene mutation, while the use of topoisomerase II inhibitors is associated with the evolution of balanced chromosomal aberrations such as: 11q23, 21q22, 16q22, rearranged MLL, AML_t, CBFB and retinoic acid receptor-alpha (RARA) [89]. A study that included 761 patients analyzed between 1976 and 1993 and 5098 patients reported in literature between 1974 and 2001 revealed that: exposure to radiation was associated with the development of t-MDS and 5q- chromosomal abnormality, exposure to alkylating agents was associated with the evolution of t-MDS and t-AML with monosomy 7, while exposure to topoisomerase II inhibitors was associated with the development of t-AML and t11q23 [86]. The cytogenetic abnormalities and the genetic mutations that have been reported in patients with t-AML are included in Table 7 [83,86,88,89]. The genetic mutations that are involved in t-AML can be classified into: (1) class I mutations that can be sub-classified into: (a) tyrosine kinases such as FMS-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD) point mutations, c-Kit point mutations and cFMS and Janus Kinase 2 (JAK-2) point mutations, and (b) genes in the RAS/BRAF pathway such as: KRAS or NRAS point mutations and BRAF and PTPN11 point mutations, and (2) class II mutations that can be divided further into: (a) transcription factors that include: AML1/CBFB chimerically rearranged, AML1 point mutations, MLL chimerically rearranged, MLL-ITD, chimerically rearranged RARA and EV11 in addition to CEBPA and nucleophosmin-1 (NPM1) point mutations, and (b) tumor suppressor genes such as P53 point mutations [87].

Clinical data obtained from 4 major cohorts on t-AML that included a total of 395 patient revealed the following: the mean age for the development of t-AML was 53.5 - 58 years, the median latency period to develop t-AML was 48 - 55 months, the mean WBC count at presentation was 6.7 - 27.4 x10⁹/L, CR rates ranged between 23.8 and 63.0% and the median OS was 7 - 12 months [83].

The diagnosis of t-AML identifies a group of high-risk patients with multiple and varied poor prognostic features [84,90]. The spectrum of cytogenetic abnormalities in t-AML is similar to de novo AML, but the frequency of unfavorable cytogenetics such as complex karyotype or deletion or loss of chromosomes 5 and 7 is higher in t-AML. In t-AML survival varies according to cytogenetic risk group with better outcomes observed in patients with favorable-risk karyotype [84,90].

Abnormal response to DNA damage is a common finding in t-AML. TP53 aberrations are one of the most common mutations in t-AML and are usually associated with complex karyotypes. AML having complex karyotype accounts for 70% of cases of AML and has a high degree of

Cytogenetic abnormalities	(%)	Mutational abnormalities		(%)	
t (8,21)	6.4%	TP53	18-25%	TET 2	9%
t (15,17)	5.4%	WT1	17%	IDH1/IDH2	3-12%
inv (16) , t (16,16)	14%	NPM1	12-16%	EV 11	0.2-1.0%
11 q 23	12.9%	KRAS	-	RUN X-1	2.9%
t (9,11)	-	NRAS	11-12%	AML 1	
inv(3), t (3,3)	-	BRAF	6%		
17 p deletion	-				
t (11,19)	-	FLT3-ITD	7-12%	PML-RARA	2.3%
- 7, 7q-	-				
- 5, 5q-	-	FLT3-TKD	2-2.5%	C-KIT	1-4%
- 17	-	CEBPA	0-6%	C-FMS	-
- 21	-	DNMT3A	16%	JAK 2	Rare
- 18	-	MLLT3-MLL	4-12%	CBF β - NYH 11	1.8%
t (1,3)	-				
Normal cytogenetics	14%				
Complex cytogenetics	26.39%	MLL-PTD	2-4%	PTPN 11	4%

Table 7. Cytogenetic and mutational abnormalities encountered in therapy-related AML

genomic complexity and is associated with interior OS [93]. The most common chromosomal translocation associated with topoisomerase-II inhibitor-induced t-AML is t(9,11) (p22, q23). MLL gene located on chromosome 11 at band q23 is the gene most commonly involved in secondary acute leukemias (ALL and AML) related to the use of topoisomerase II inhibitors [92]. t-AML having t(8,21) shares many features, including morphologic and immunophenotypic features as well as the characteristic AML-ETO (RUNX1-RUNX1T1) fusion, with de novo AML having t(8,21) (q22,q22), but affected individuals have a relatively worse outcome [94]. The poor prognosis and the rather aggressive behavior of t(8,21) t-AML may be explained by: (1) the older age of patients, and (2) the presence of active primary cancer at the time of diagnosis of t-AML with t(8,21) [94]. In t-AML, cytogenetic profile is an important prognostic parameter, while the age of patient and the WBC count at presentation have no impact on OS [89]. In general, survival and prognosis of t-AML is often poor despite prompt diagnosis and early institution of therapy [83]. CR rates of t-AML range between 24 and 63% and are interior to those of de novo AML (65-80%) [83]. However, within certain cytogenetically defined subgroups, the prognosis of t-AML does not differ significantly from their de novo AML counterparts [83,84,90]. Standard chemotherapy and allogeneic HSCT as well as experimental therapies are used in the treatment of t-AML [83,84,90]. Several studies have shown that

therapeutic outcomes of t-AML patients after induction phase of treatment are not different from those of de novo AML [83]. In patients older than 60 years of age having t-AML, significantly greater relapse rates, mainly due to lower doses of chemotherapeutic agents used compared to those given to younger patients, have been encountered [83]. However, intensive induction chemotherapy should not be withheld in patients with t-AML provided they are fit to receive it [83,84,90]. Novel HSCT strategies using RIC regimens as well as targeted therapies await clinical evaluation in patients with t-AML and these may prove to have a positive impact on the prognosis of affected individuals [83]. Studies have shown that allogeneic HSCT can cure some patients with t-AML. Finally, patients with t-AML should be enrolled in front-line chemotherapeutic trials that are appropriate for patients with de novo AML with similar disease characteristics [84,90].

6. Prognosis in AML

The response to treatment and the OS of AML patients are very variable [7,8,83]. A number of prognostic factors related to the patient and the tumor characteristics have been described and they include age, performance status, comorbid medical conditions, cytogenetic profile, molecular or genetic profile, history of MDS or chronic MPNs, and history of receiving cytotoxic chemotherapy or ionizing radiation. Amongst these prognostic factors, the following have a direct effect on treatment outcome: age at diagnosis, performance status and karyotype [7,8,83,84].

The clinical role of gene mutational analysis, gene expression profiling, and micro-RNA profiling remains uncertain at this time, although a number of mutations and changes in the levels of certain proteins have been shown to have prognostic impact and will likely become part of the routine characterization of AML in the near future [7,8,83,84].

6.1. Induction therapy for AML

Once the diagnosis of AML is established, induction chemotherapy will be commenced in order to rapidly restore normal BM function [9]. The goal of the initial intensive course of combination chemotherapy is to obtain a CR [9,95]. Induction chemotherapy aims to reduce the total body leukemia cell population from 10^{12} to below the cytologically detectable level of approximately 10^9 cells [9,95]. A series of pre-1985 studies performed by the Cancer and Leukemia Group-B [CALG-B] established 7+3 regimen, which is composed of cytarabine and daunorubicin, as the standard of care in induction therapy of AML [96]. However, various induction regimens of chemotherapy are available for younger and older AML patients [9,96-100]. In younger patients with AML, the following induction chemotherapeutic regimens are used: (1) the classical or standard 7+3 regimen, which produces remission rate of 60-80%, comprises continuous intravenous (IV) infusion of 100-200 mg/m² of cytarabine for 7 days and 60-90 mg/m² IV push of daunorubicin daily for 3 days, (2) high-dose cytarabine (HiDAC) + daunorubicin regimen, which produces remission rate of 90%, comprises 1-3 g/m² of cytarabine IV infusion over 3 hours twice daily for 6 days, and (3) cytarabine + idarubicin regimen, which

produces remission rate of 88%, is composed of 100-200 mg/m² of cytarabine daily by continuous IV infusion for 7 days and 12-13 mg/m² IV push of idarubicin daily for 3 days [9,96,97].

Throughout the years, many studies have been performed with the intent to improve on the outcome of 7+3 induction regimen for AML and the following are examples of these attempts: (1) replacement of daunorubicin by idarubicin, (2) replacement of daunorubicin by mitoxantrone, (3) escalation of daunorubicin dose, (4) addition of etoposide to the 7+3 regimen, (5) addition of thioguanine to the 7+3 regimen, (6) replacement of daunorubicin by amsacrine and gemtuzumab ozogamicin, (7) the use of HiDAC alone or in combination with fludarabine and/or G-CSF as induction therapy, and (8) incorporation of new agents such as cladarabine, clofarabine, lomustine, AC-220 and sorafenib [96,97,100].

Age is an important factor for the treatment outcomes demonstrated in numerous studies [7,9-11,23,24,58-69,71,73]. In patients with AML, younger than 46 years of age, HiDAC (3 g/m² IV twice daily for 4 days) in addition to daunorubicin and etoposide produces higher remission and survival rates compared to a standard dose cytarabine [100]. In AML patients who are ≥ 50 years of age, idarubicin has produced superior long-term outcome compared to high-dose daunorubicin in a French study that included 727 patients with AML [98]. In older patients with AML, the following factors can predict a better long-term outcome: favorable-risk AML, idarubicin treatment rather than daunorubicin, and belonging to a younger age as studies have shown that the younger the age of the patient the better the response to chemotherapy [98]. In patients with AML who are ineligible for intensive chemotherapy, volasertib (adenosine triphosphate competitive kinase inhibitor) and low-dose cytarabine produce responses across all AML genetic subgroups, improve survival and have clinically manageable safety profile [101]. In AML patients who are ≥ 60 years of age, the following induction therapies have been used: (1) single dose daunorubicin; 45 or 90 mg / m² IV or liposomal daunorubicin, (2) hypomethylating agents such as azacytidine or decitabine, (3) immunomodulatory agents such as lenalidomide, (4) clofarabine, and (5) other agents such as: plerixafor, flavoperidol, *Diphtheria* toxin linked to interleukin-3 (IL-3) and all trans-retinoic acid (ATRA) [99]. Recent studies have shown that doubling the dose of daunorubicin (90 mg/m² instead of 45mg/m²) produces significantly higher CR rates in selected groups of AML patients, regardless of the age. Patients with favorable- or intermediate-risk cytogenetics have achieved better responses than patients with unfavorable karyotype or high-risk disease [96]. The following circumstances require unique therapeutic implications: Philadelphia chromosome positive AML, mixed-phenotype leukemia, AML with extramedullary involvement, t-AML, and AML in pregnancy [9].

Seven to ten days after completion of induction chemotherapy, response to chemotherapy is usually evaluated by a BM aspiration and trephine biopsy [9]. After achieving CR₁ of AML, PRT starts with either consolidation chemotherapy or HSCT in transplant-eligible patients [9]. Based on a landmark CALG-B study, the standard consolidation therapy for AML in CR₁ is 4 cycles of HiDAC (3 g/m² IV Q 12 hourly on days 1,3 and 5) [97]. Improving the quality of remission may reduce the risk of relapse in patients with AML [97]. Several emerging prognostic factors may enable a more personalized approach to post-induction therapy that takes into consideration the category of patients who should be offered allogeneic HSCT in CR1 [97].

6.2. Response to treatment in AML

Definition of CR in AML according to the criteria developed by the International Working Group includes: (1) Normal values for absolute neutrophil count $>1000 \times 10^9/L$ and platelet count $>100 \times 10^9/L$ in addition to independence from red blood cell transfusions. (2) BM biopsy that reveals no clusters or collections of blasts. Also, extramedullary leukemia such as CNS and soft tissue involvement must be absent. (3) BM aspiration reveals normal maturation of all cellular components; namely erythrocytic, granulocytic and megakaryotic series. However, there is no requirement for BM cellularity. (4) Less than 5% blasts in the BM and none can have a leukemia phenotype such as Auer rods. (5) The absence of a previously detected clonal cytogenetic abnormality, that is, complete cytogenetic remission (CRc) which confirms the morphologic diagnosis of CR but is not a criterion for CR in AML. The conversion from an abnormal karyotype at the time of first CR is an important prognostic indicator, supporting the use of CRc as a criterion for CR in AML [95,102].

A substantial burden of leukemia cells persist undetected referred to as minimal residual disease (MRD) leading to relapse within weeks to months if no further PRT is administered [95]. Response criteria in AML patients can be categorized as follows: (1) CR, (2) CR with incomplete recovery, (3) morphologic leukemia-free status, (4) partial remission, (5) cytogenetic remission, and (6) molecular remission. Treatment failure in AML can be classified as follows: (1) resistant or refractory disease, (2) disease relapse, (3) death in aplasia, and (4) death from an indeterminate cause [8]. The following methods are used in detecting MRD: (1) multi-parameter flow cytometry (MFC), (2) quantitative, real time, polymerase chain reaction (Q-RT-PCR), and (3) gene expression analysis [95,102-104]. The evaluation of MRD in AML has the following advantages: (1) high-resolution determination of response to treatment, (2) allowing target-driven titration of dose as well as duration of therapy, (3) stratification of the risk of relapse after induction to allow triage of the optimal consolidation therapy, (4) determination of prognosis after completion of standard treatment, (5) sparing toxicity and cost of HSCT in patients with low risk of relapse, and (6) assignment to maintenance treatment after completion of standard therapy [102]. In patients with AML, assessment of MRD by flow cytometry after induction and consolidation chemotherapy has been shown to provide independent prognostic information. Early flow cytometry MRD assessment can improve the current risk stratification approaches by the prediction of relapse-free survival (RFS) in AML and may facilitate the adaptation of PRT for patients at high-risk of relapse [103]. MRD by flow cytometry offers high sensitivity [up to 10^{-4}] and is applicable in more than 90% of all AML cases. The individual choice of PRT requires a comprehensive knowledge of the risk profiles of patients in order to avoid overtreatment or undertreatment. MRD levels constitute a prognostic factor that combines disease-specific as well as patient-specific characteristics thus reflecting sensitivity to chemotherapy [103]. Also, MRD before myeloablative HSCT is associated with an adverse outcome in AML in CR1. The negative impact of pre-transplant MRD is similar for AML in CR1 and CR2 as even minute levels ($\leq 0.1\%$) are associated with adverse outcome [105].

Early blast clearance in AML is an essential indicator of response to chemotherapy [106]. Day 16 blasts represent a highly independent and sensitive prognostic factor and may be used for stratification of therapy early enough before the second course of chemotherapy, that is, salvage chemotherapy, re-induction or double induction. Day 16 blasts allow refinement but not replacement of the most important system for a prognostically based classification of patients with AML which is grouping according to karyotypic abnormalities [106]. Monitoring of early reduction of the leukemia cell burden may be further improved by techniques that are more sensitive and more reproducible than cytomorphology such as immunophenotyping using MFC. The prognostic significance of day 16 blasts is independent of pre-therapeutic parameters and predicts outcome even in patients achieving a CR [106]. Response rates and subsequent therapeutic recommendations for AML patients classified according to their prognostic groups are shown in Table 8 [22].

Prognostic Group	CR rate	Relapse rate	Response and non-relapse mortality	Induction	Post-remission	HSCT comorbidity index
Favorable	> 80-90%	35-40%	* Rapid CR * No MRD < 10-15%	- 3+7 regimen - Consider: FLAG + Idarubicin if age < 60-65 years	- Ara -C at 1.0-1.5g/m ² < 1% daily for 6 doses, total of 2 cycles - Possibly preceded by 1 course of: FLAG + idarubicin	
Intermediate I	50-80%	50-60%	- Rapid CR - No MRD < 20-25%	- 3+7 regimen - Consider: FLAG + Idarubicin if age < 60-65 years or clinical trial	- HSCT from MSD if risk of NRM < 20-25% - If not candidate for HSCT: FLAG + Ida then Ara-C (as above) or clinical trial	≥ 2%
Intermediate II	40-80%	70-80%	- slow CR - CRp-CRi - MRD < 40%	- 3+7 regimen - Consider: FLAG + Idarubicin if age < 60-65 years - Clinical trial combining chemotherapy and FLT3 inhibitors	- HSCT from MSD or MUD if risk of NRM < 30%, otherwise as intermediate I. - If FLT3 positive, consider FLT3 inhibitors post HSCT.	≥ 3-4%
Unfavorable	< 50%	> 90%	- Slow CR - CRp-CRi - MRD < 40%	Clinical trial	- HSCT from MSD or MUD if risk of NRM < 40%	≥ 5%

Prognostic Group	CR rate	Relapse rate	Response and non-relapse mortality	Induction	Post-remission	HSCT comorbidity index
					- Consider post-HSCT trial	
					- If not candidate for HSCT, consider clinical trial	
Ara-C: cytarabine						
MSD: matched sibling donors						
HSCT: hematopoietic stem cell transplantation						
CR: complete remission						
MUD: matched unrelated donor						
FLAG: fludarabine, cytarabine, growth factors						
MRD: minimal residual disease						
NRM: non-relapse mortality						
AML: acute myeloid leukemia						

Table 8. Response rates and therapy recommendations for AML classified according to prognostic group

6.3. Drug resistance in AML

For the management of AML, the following general options are available: (1) standard chemotherapy, (2) investigational therapy, and (3) best supportive care without intensive chemotherapy [107]. Initial treatment for remission induction is still based on cytarabine and anthracyclines (7+3 regimen) which were introduced into the treatment of AML more than 40 years ago [108]. Given the natural history of AML and the uncertainty about the outcome of investigational therapy, 7+3 regimen is still the preferable standard treatment for most of the patients. The principal predictor of response to standard therapy is the duration of first CR [107].

Therapeutic resistance, defined as either failure to achieve initial CR or relapse after achievement of CR, remains the main problem in adult AML. It is widely appreciated that the likelihood of resistance to chemotherapy differs significantly from one individual to another [109]. Resistance to chemotherapy, not TRM, is the chief cause of treatment failure in AML. Drug resistance occurs in 71% of AML patients younger than 56 years, 61% of patients between 66 and 75 years of age and 54% of AML patients older than 75 years [107]. In an analysis that included 4601 AML patients, the following factors were independently associated with failure to achieve remission: (1) age of the patient, (2) performance status, (3) the WBC count at presentation, (4) secondary disease, (5) cytogenetic risk, and (6) presence of certain genetic mutations such as FLT3-ITD. Another study revealed that the

presence of multidrug-resistant (MDR) phenotype such as MDR-gene-1 (*mdr-1*) is associated with drug resistance in AML [109,110].

The ability to accurately forecast drug resistance could have a tremendous impact on the management of AML and evaluation of new drugs [109]. Unfortunately, our ability to predict chemotherapeutic resistance on the basis of the routinely available clinical covariates, even with the inclusion of commonly used molecular data such as FLT3 and NPM1 is relatively limited [109]. In case of failure to respond to 7+3 regimen, alternative salvage therapies include: (1) fludarabine, cytarabine and G-CSF (FLAG) regimen, (2) mitoxantrone and etoposide, or (3) investigational agents [107]. In general, management of drug resistance in AML can be summarized as follows: (1) in case of lower drug resistance and lower TRM, current therapy should be intensified, (2) in case of higher drug resistance and lower TRM, new high-intensity therapy needs to be added, (3) in case of lower drug resistance and higher TRM, new low-intensity therapy, such as ATRA or azacytidine needs to be added, and (4) in case of higher drug resistance and higher TRM, new low intensity therapy should be used [107]. Chemomodulation of sequential HiDAC and idarubicin by fludarabine improves the anti-leukemic efficacy but only at a modest rate [108]. Given the efficacy of chemomodulation of cytarabine, even in a relapsed setting, incorporation of fludarabine into first-line therapy of AML seems warranted and may help to improve the outcome of AML patients [108]. The addition of cladribine to daunorubicin and cytarabine induction regimen increases the anti-leukemic effect or potency of 7+3 regimen resulting in higher CR rates without causing additional toxicity [111]. The combination of azacytidine and lenalidomide improves the outcome of patients with high-risk MDS or AML. This regimen is well tolerated and is not associated with major toxicities apart from neutropenia and thrombocytopenia [112].

Valspodar (PSC833) can not only reverse MDR in patients with hematologic malignancies but alters the pharmacokinetics of concomitant anticancer agents [110]. A phase I study has shown that valspodar and MEC (mitoxantrone, etoposide and cytarabine) combination is safe and effective when administered early in relapsed or refractory AML [110].

A previous phase II study from the Borden laboratory had shown that ribavirin produced initial responses, including remissions in patients who had relapsed after cytarabine treatment, but all patients eventually developed drug resistance and relapsed [113]. A new study from the same group has shown that resistance to cytarabine and ribavirin is due to the sonic hedgehog pathway transcription factor glioma-associated oncogene [113]. However, the best modality to overcome drug resistance in AML patients is allogeneic HSCT, but unfortunately this therapy of choice can only be performed in a minority of patients [108].

7. PRT in AML

The preferred type of PRT in patients with AML in CR₁ is a subject of continued debate, especially in patients at high risk of non-relapse mortality (NRM) including patients above 40 years of age [114]. PRT is applied for the prevention of relapse and may include the following therapeutic options: (1) consolidation chemotherapy with HiDAC, (2) autologous HSCT, and

(3) allogeneic HSCT [12,114,115]. HiDAC at 2-3 g/m² IV-twice daily on days 1, 3 and 5 remains the standard of care for intensive PRT in AML [12,96,97,115]. Identification of the best therapeutic option for each patient regarding the best PRT, allogeneic HSCT versus intensive chemotherapy, should weigh the risk of relapse against the risk of NRM [12]. MRD assessment should be performed at regular intervals or once needed in patients receiving PRT in order to adjust therapeutic options before overt relapse [12,102,115,116].

In individuals at high risk of relapse and after completion of consolidation chemotherapy, maintenance therapy that includes the following agents can be administered: (1) azacytidine and decitabine, (2) lenalidomide, (3) dasatinib and imatinib, (4) bortezomib, (5) panobinostat, (6) midostaurin, (7) sorafenib, (8) AC-220, and (9) IL-2 [116-119]. In recent years, maintenance therapy with demethylating agents and TKIs is under evaluation in clinical trials. The combination of intensive chemotherapy and targeted therapies including TKIs or demethylating agents is currently under clinical evaluation as PRT in AML patients [12,119].

Allogeneic HSCT is preferred over chemotherapy as PRT in patients with intermediate- and poor- risk AML aged 40-60 years, whereas autologous HSCT remains a treatment option to be considered in patients with intermediate-risk disease [114]. Although allogeneic HSCT offers the most effective anti-leukemic therapy, increased NRM may compromise its favorable effects. RIC-allogeneic HSCT in patients above 40 years of age reduces NRM while maintaining the GVL effect. Given the potent GVL effect and the limited toxicity profile associated with the use of RIC-allogeneic HSCT, further evaluation of this form of HSCT in AML patients younger than 40 years is warranted [114]. In AML patients at high-risk of relapse, allogeneic HSCT with grafts obtained from matched-related donor (MRD) or matched-unrelated donor (MUD) is still the gold standard of care, while in patients at low-risk of relapse, autologous HSCT has shown promising results [12].

7.1. PRT for AML in younger adults

Young adult and adolescent patients (15 - 35 years) deserve special attention due to the specific medical and social needs. Approximately 60 - 80% of patients newly diagnosed with AML achieve CR with intensive chemotherapy. Without additional cytotoxic chemotherapy, virtually all of these patients will eventually relapse within 4 - 8 months [115,119]. However, the choice of PRT in these patients depends on a number of factors that include: (1) the expected rate of relapse with consolidation chemotherapy alone, influenced by karyotype, (2) the expected mortality and morbidity associated with each option as determined by age and comorbidities of patients, and (3) the available salvage therapy in case of disease relapse [115]. Although it is essential to maintain CR in AML patients responding to chemotherapy, maintenance therapy is not yet a standard AML treatment as it is still under evaluation [119]. According to risk stratification, the recommended PRT is as follows: (1) favorable cytogenetics such as t(8,21), inv(16) and t(16,16): 3 or more cycles of consolidation chemotherapy with HiDAC rather than standard dose cytarabine, alternative chemotherapeutic agents or early HSCT, (2) intermediate risk cytogenetics including normal karyotype: decision on option either HSCT or chemotherapy should be based on individual patient features such as age, comorbidities, initial WBC count and MRD, patient preference, HSCT donor availability and

access to specific clinical trials, and (3) unfavorable cytogenetics: allogeneic HSCT is preferred over conventional chemotherapy and autologous HSCT [115].

8. Treatment of relapsed / refractory AML

Relapse after achieving CR is one of the most important obstacles in improving the outcome of patients with AML [120]. Approximately 70-75% of AML patients younger than 65 years will achieve CR if treated with standard induction chemotherapy [121]. TRM associated with induction chemotherapy has dropped to less than 5% with modern supportive care [121]. The prognostic scores and the risk factors for relapse in AML include: (1) adverse cytogenetics, (2) age at relapse, worst in patients more than 45 years old, (3) FLT3 mutation status, and (4) duration of first CR, worst if ≤ 6 months [122]. Also, adenine-adenine polymorphism in the cytotoxic T-lymphocyte antigen 4 (CTLA4) gene polymorphisms have higher relapse rates than adenine-guanine polymorphisms [122].

Therapeutic options that are available for patients having relapsed or refractory AML include: (1) salvage therapies that include various combinations of chemotherapeutic agents as shown in Table 9 [119,123], (2) chemotherapy and donor lymphocyte infusion (DLI) [123,124], (3) myeloablative allogeneic HSCT, RIC-allogeneic HSCT and second allogeneic HSCT [119,122,123], (4) novel and targeted therapies including: (a) immunomodulatory agents such as lenalidomide, (b) aminopeptidase inhibitors such as tosedostat, (c) purine analogs such as clofarabine, (d) mTOR inhibitors such as sirolimus and everolimus, (e) hypomethylating agents such as azacytidine and decitabine, (f) histone deacetylase (HDAC) inhibitors such as vorinostat, (g) farnesyl transferase inhibitors such as tipifarnib, (h) FLT3 inhibitors such as quizartinib, sorafenib and lestaurtinib, (i) CXCR4 antagonists such as plerixafor, (j) combination of azacytidine and sorafenib, and (k) monoclonal antibodies such as GO, lintuzumab, avastin and bevacizumab [122,123,125,126], (5) appropriate clinical trials, and (6) palliative care and supportive measures [122].

The following will improve the outcome of relapse: (1) improved understanding of the biology of AML, (2) molecular characterization of the subtype of AML, and (3) the development of new targeted and novel therapies [34]. Improvements in the outcome of patients with relapsed AML can be attributed to the following: (1) improvement in supportive care such as blood products and antimicrobials, (2) availability of better salvage therapies, (3) improvement in HSCT techniques, and (4) the introduction of new targeted therapies [120].

Despite the availability of multiple novel agents, prognosis of patients with relapsed AML remains poor [119,125]. The personalized approach is promising but will bring about new challenges for treating physicians [125]. Molecular tests may contribute to future personalized therapy, ultimately resulting in improved outcome [125]. In patients with relapsed/refractory AML, the dose of lenalidomide escalated to 50 mg/day for 21 days, every 4 weeks is safe, active and has low toxicity [126]. The immunomodulatory effect of lenalidomide can bring AML relapsing after HSCT into CR2 [126]. More than 20% of patients with newly diagnosed AML who fail induction therapy can still be cured, particularly if they are fit to have allogeneic HSCT

Regimen	Main adverse effects	Response rates
Reinduction with cytarabine and daunorubicin	Fever, gastrointestinal (GIT) upset, arrhythmias and daunorubicin-induced reactions	Complete remission (CR): 50%
HiDAC High dose cytarabine	Fever, GIT upset, cerebellar toxicity, chemical keratitis and conjunctivitis	CR: 35-40%
HAM High dose cytarabine and mitoxantrone	Fever, infections, stomatitis, arrhythmias and heart failure	Higher response rates than HiDAC alone
High dose cytarabine and etoposide	Fever, anaphylaxis and peripheral neuropathy	Similar responses to HiDAC alone
Mitoxantrone and etoposide	Fever, infections, heart failure, arrhythmias and Stomatitis	CR: 40%
MEC Mitoxantrone, etoposide and cytarabine	Fever, infections, hepatic dysfunction, arrhythmias and heart failure	Higher CR rates in patients < 60 years and those with unfavorable risk
FLAG; fludarabine, cytarabine and G-CSF (granulocyte monocyte colony stimulating factor)	Mucositis and infections	CR: 45-55%
CLAG Cladribine, cytarabine and G-CSF	Fever, infections, mucositis and GIT upset	CR: 50%
Cyclophosphamide and high dose etoposide	Fever, infection, hemorrhagic cystitis, mucositis and liver dysfunction.	CR: 42%

Table 9. Chemotherapeutic regimens that are used in the treatment of relapsed / refractory AML

[121]. Thus, early human leukocyte antigen (HLA)-typing and donor identification are important components of the initial therapy of AML [121].

In patients with AML receiving allogeneic HSCT, relapse remains the main cause of treatment failure and is associated with poor prognosis [124]. Most treatments are of limited utility in AML patients relapsing after HSCT [124]. Re-induction chemotherapy can induce CR2 in 30 - 40% of AML patients relapsing following allogeneic HSCT, but remissions are usually of short duration and most of these patients ultimately relapse and die [124]. DLI alone may induce remission in 15-20% of patients, but long-term remissions are rare, while chemotherapy and DLI could induce durable remissions in a considerable proportion of patients with relapsed AML following HSCT [124]. Isolated extra-medullary relapse (EMR) is common after DLI and chemotherapy and it requires particular attention in future studies. Second allogeneic HSCT is associated with long-term overall survival of 10-35% and TRM of 40-50% [124].

8.1. Allogeneic HSCT for AML

Allogeneic HSCT is a potentially curative therapeutic option for many patients with AML [127-130]. Currently, AML is the most common indication for allogeneic HSCT [128,131,132]. Over the last decade, safety, effectiveness and the number of allogeneic HSCT procedures performed for patients with AML have increased substantially [128]. The ability to select candidates who benefit from allogeneic HSCT has improved due to an increased understanding of the biology of AML and implementation of risk stratification of AML based on cytogenetic and molecular markers [128,133].

In adult patients with AML, allogeneic HSCT is indicated in the following situations: (1) AML refractory to induction chemotherapy; all patients with AML who failed initial induction treatment and are: younger than 65 years, having available donor and an acceptable comorbidity index should be subjected to allogeneic HSCT, (2) AML in CR2; all patients who are younger than 75 years of age, having appropriate donor and a comorbidity index of ≤ 5 should be offered HSCT, (3) AML patients relapsing after induction or consolidation chemotherapy, (4) all AML patients with intermediate- or high-risk disease such as complex cytogenetics, monosomal karyotype or Philadelphia chromosome, (5) AML secondary to MDS or chronic MPNs, (6) t-AML not having favorable cytogenetics, and (7) AML with extramedullary disease such as CNS involvement [127,128,134-137]. The curative effect of HSCT results from both: (1) the radiation and/or chemotherapy used in the conditioning regimen administered prior to HSCT, and (2) the GVL effect obtained from the donor immune system [128]. The conditioning therapies administered before stem cell infusions can be divided into the following types: (1) conventional intensity conditioning (CIC) or standard myeloablative preparatory regimens which cause prolonged and irreversible pancytopenia and require stem cell rescue, otherwise leading to death, (2) minimally intensive conditioning (MIC) that cause cytopenias, but do not require stem cell rescue, and (3) RIC regimens which include all other conditioning therapies that do not qualify for either CIC or MIC [138]. In the latter form of conditioning therapies, peripheral blood counts may recover only after many weeks and these regimens require stem cell support to be clinically useful [138]. In patients with AML, the following conditioning therapies have commonly been used: (1) cyclophosphamide and total body irradiation, (2) busulfan and cyclophosphamide, (3) total lymphoid irradiation and anti-thymocyte globulin (ATG), (4) fludarabine, busulfan or treosulfan, and (5) clofarabine and busulfan [131,136]. However, myeloablative conditioning therapies, such as TBI and cyclophosphamide or busulfan and cyclophosphamide, cannot be administered to patients who are more than 50 years of age or have comorbidities because of high rate of TRM and toxicity [136].

Allogeneic HSCT has been established as an effective consolidation treatment in AML patients in first or subsequent remissions [134]. Allogeneic HSCT may be restricted to AML patients at a relatively high-risk of relapse such as intermediate and poor risk cytogenetics [134]. One of the main reasons for adopting allogeneic HSCT in the treatment of AML is the proven curative potential of GVL effect associated with allografting [131]. Unfortunately, GVL effect and GVHD are intimately linked [131]. Although allogeneic HSCT can effectively prevent relapse of AML, TRM associated with allogeneic HSCT may compromise that beneficial effect [130,134]. A significant reduction in TRM has been achieved during the last 3 decades and the ongoing developments may add to that improvement [134].

Achieving a cure for AML even in younger adult patients with de novo AML remains a challenge [139]. Despite being a curative therapeutic option for younger patients with AML in CR1, allogeneic HSCT with myeloablative conditioning therapy carries significant toxicity that limits its utilization. Alternative therapeutic options include intensive consolidation chemotherapy and autologous HSCT [139]. The potentially fatal complications of allogeneic HSCT include: (1) GVHD, (2) opportunistic infections, (3) VOD of the liver, (4) interstitial pneumonitis, and (5) organ failure [134]. Compared with non-allogeneic HSCT therapies, allografting has significant RFS and overall survival benefit for intermediate- and poor-risk AML, but not for good risk AML in CR1 according to a meta-analysis that was based on 24 prospective clinical trials which included 6007 AML patients [139]. Studies have shown that the factors determining the outcome of allogeneic HSCT in AML patients include: (1) age of the patient, (2) comorbidity index (3) number of induction cycles of chemotherapy administered to achieve morphological remission, (4) the type of consolidation chemotherapy given, (5) the cytogenetic risk group: favorable, intermediate or unfavorable, (6) pre-transplant karyotype, (7) pre-HSCT peripheral blood count recovery, (8) status of MRD prior to HSCT, and (9) the preparative conditioning therapy; myeloablative versus non-myeloablative [134,135,140]. The following developments or changes in the clinical management of patients with AML subjected to allogeneic HSCT have contributed to the improved survival: (1) improved prevention and treatment of GVHD by methotrexate, cyclosporine-A, mycophenolate mofetil as well as other immunosuppressive therapies, (2) transplantation or infusion of higher doses of hematopoietic progenitor cells, (3) molecular monitoring of viruses such as cytomegalovirus as well as Epstein-Barr virus and subsequent pre-emptive therapy in patients with viral infections, (4) improved detection and treatment of fungal infections, (5) introduction of high-resolution HLA-typing and the increased number of pre-transplant and procedure-related factors, which allow risk assessment and thereby may guide transplant policies that affect transplantation outcome, (6) the application of new techniques that assess the risk of relapse after HSCT such as MRD and chimerism, (7) the recent progress in transplant procedures such as: the use of alternative donors, RIC-allogeneic HSCT with its new conditioning therapies, and novel therapies in maintenance therapy after HSCT to prevent relapse of AML, and (8) improvement in supportive care, treatment of bacterial infections, provision of safer blood products and appropriate utilization of intensive care facilities [128,129,133,134].

In patients with AML, CNS involvement is rare and is associated with poor prognosis, hence allogeneic HSCT is indicated in AML patients with CNS disease [137]. In adults with AML having CNS involvement and subjected to allogeneic HSCT, the independent risk factors for survival include chronic GVHD, disease status and cytogenetic risk category [137].

Allogeneic HSCT in AML patients with monosomal karyotype particularly (-5/5q-) in CR₁ is associated with a significant reduction in relapse rate and an improvement in survival [141,142]. The role of allogeneic HSCT in AML patients with abnormal 17p is questionable as it is associated with poor outcome and a 2 year EFS of only 12% [142]. Hierarchical classification of adverse-risk karyotypes, according to distinct genetic lesions, is effective prognostically in detecting the outcome of allogeneic HSCT in AML patients [142]. Patients with AML who harbor FLT3-ITD genetic mutations carry a poor prognosis. Although allogeneic HSCT may

improve the outcomes of these patients, relapses occur frequently [143]. Two management interventions may improve the outcome of allogeneic HSCT in patients with AML having FLT3-ITDs: (1) early detection of MRD following HSCT may allow early intervention, and (2) maintenance therapy with sorafenib and other agents may prevent or delay relapse [143,144].

In older patients with AML, allogeneic HSCT is a feasible therapeutic option and can provide approximately 40% survival at 2 years in appropriately selected patients [145]. Although increasing age is associated with poorer survival, higher comorbidities and poor performance status have more negative impact than age per se [145]. The increasing number and experience with alternative donors will facilitate allogeneic HSCT and make it a more achievable therapeutic option for elderly AML patients [119,145]. Also, the advent of RIC regimens made allogeneic HSCT an available treatment option with curative intent for older AML patients [114,146]. Recent studies on the role of allogeneic HSCT in AML patients have revealed: (1) little impact of age on the outcome of HSCT, and (2) greater impact of the following on HSCT outcome: (a) the recipient health status; comorbidity index and performance status, (b) the AML disease status; CR1 or CR2 at the time of transplantation, and (c) the associated chromosomal aberrations; favorable versus intermediate or unfavorable cytogenetics [146]. RIC-allogeneic HSCT has resulted in better survival among older patients with AML than autologous HSCT or conventional chemotherapy [146]. Despite the acceptable outcomes of alternative donors and the reports of equivalent outcomes of MRD and MUD allografts, grafts obtained from HLA-identical siblings continue to be associated with the most favorable outcomes after allogeneic HSCT [119,146].

Strategies that can speed up immune reconstitution following allogeneic HSCT for AML include: (1) infusion of genetically modified lymphocytes, (2) in vivo T-cell depletion, (3) rapamycin to promote T-regulatory cell expansion in vivo, and (4) cyclophosphamide, on day 3 after stem cell infusion, to reduce alloreactive lymphocytes [136]. There are new strategies to enhance and maintain the GVL effect of DLIs while minimizing GVHD and these strategies include: (1) pre-emptive DLI before overt relapse, (2) co-stimulation with cytokines or dendritic cells (DCs), and (3) the use of leukemia-specific antibodies and other immunotherapies including: (a) monoclonal antibodies such as GO, (b) hypomethylating agents such as azacytidine and decitabine, (c) proteasome inhibitors such as bortezomib, (d) immunomodulatory agents such as lenalidomide, and (e) HDAC inhibitors [130,147].

The main causes of treatment failure in allogeneic HSCT for AML are disease relapse and treatment toxicity [130,140,147,148]. Relapse after allogeneic HSCT in AML patients remains a major obstacle to survival as it accounts for 20-50% of the primary causes of death [147]. Factors that predict an increased risk of relapse in AML patients subjected to allogeneic HSCT include: (1) disease status at transplant, (2) adverse cytogenetics at diagnosis, and (3) increased intensity of post-transplant immunosuppression [136]. In AML patients relapsing following allogeneic HSCT, the following treatment options are available: (1) intensive chemotherapy and/or DLI, (2) second allogeneic HSCT after withdrawal of immunosuppression or (3) the best supportive care [147,148]. When relapse is the most common concern for treatment failure associated with allogeneic HSCT, a female donor for a male recipient may be beneficial in decreasing the incidence of relapse rate following HSCT [149]. However, AML relapse

following allogeneic HSCT predicts a poor survival [148]. Patients who relapse ≥ 6 months after initial allogeneic HSCT have better survival and may benefit from intensive chemotherapy or a second allograft with or without DLI [148]. The use of alternative donor HSCT is increasing as the transplantation-eligible population ages and as sibling donors become less available [150]. A recently published study that included 414 patients with AML, treated at 2 institutions in the USA and France, evaluated the donor source of stem cells on the outcome of allogeneic HSCT [MRD: 187 patients; MUD: 76 patients; and umbilical cord blood (UCB): 151 patients] revealed that: (1) the 6-year overall survival was similar across all donor types, and (2) the TRM was also similar across all donor types. The data obtained from this study support the use of alternative donors as a graft source with myeloablative or RIC allogeneic HSCT for patients with AML when a sibling donor is not available [150].

In younger patients with high-risk AML, allogeneic HSCT has a significant positive impact on the outcome. In this group of patients, allografts from MRD or MUD yield similar results [151]. In AML patients relapsing after autologous HSCT, allogeneic HSCT using MUD is a feasible option that results in 20% long-term leukemia-free survival [152]. In patients with AML in CR1, without available identical donor, the possible transplantation options are: (1) haploidentical HSCT using T-cell depleted grafts, and (2) autologous HSCT but the choice depends on the experience of the transplant center [153]. Survival of older patients with AML undergoing allogeneic HSCT with grafts obtained from younger unrelated donors has improved compared to allografts obtained from older related donors [132]. UCB-HSCT has provided an alternative transplantation option for patients with high-risk AML lacking MRD and MUD, but it is associated with high risk of relapse which limits its use in this category of patients [154]. Risk factors for extramedullary relapse in patients with AML receiving haploidentical allogeneic HSCT include failure to achieve CR prior to HSCT and absence of chronic GVHD after allografts [155]. The GVL effect may help to prevent and eradicate EMR following allogeneic HSCT [155]. Haploidentical donors can safely extend transplantation options for AML patients without HLA matched siblings or unrelated donors [156,157]. HLA-haploidentical HSCT is an effective and immediate therapeutic modality for high-risk AML patients who lack matched donors [158]. The use of fractionated 800 cGy-TBI-based conditioning therapy and unmanipulated peripheral blood stem cell grafts seems feasible and can result in favorable outcomes for adult patients with AML in CR undergoing haploidentical HSCT [159]. However, relapse remains a leading cause of death in high-risk AML patients subjected to haploidentical HSCT [158]. Achievement of CR before transplantation and recombinant human G-CSF priming of conditioning therapy can improve the outcome of patients with high-risk AML receiving haploidentical allografts [158,160].

8.2. RIC-allogeneic HSCT for AML

The frequency of fatal toxicities in the course of myeloablative allogeneic HSCT for AML patients increases with age [138]. Unfortunately, the vast majority of patients with AML, who would benefit from an allograft, are older and therefore usually ineligible for such therapy [138]. RIC-allogeneic HSCT was introduced more than a decade ago for patients who were older, had significant comorbid conditions as well as poorer performance status [161]. This

form of HSCT has improved the accessibility to transplant patients with AML or other hematological malignancies who are not eligible for standard conditioning therapy for allogeneic HSCT [162]. However, RIC-allogeneic HSCT appears to be safe and permits durable donor engraftment [162]. The antineoplastic potency of RIC-allogeneic HSCT relies primarily on the GVL effect of the allograft rather than ablating all residual leukemia disease [161]. The anti-leukemic GVL effect is derived from the donor cells infused [138]. TRM appears to be less with RIC-allogeneic HSCT than that observed with conventional myeloablative allogeneic HSCT [138]. RIC-allogeneic HSCT for AML has the following limitations: (1) insufficient anti-leukemic effect in the preparative conditioning therapy, (2) high relapse rates, (3) possible higher incidence of engraftment failure, (4) unclear benefit of using conventional pre-transplant cytoreductive treatment, (5) incidence and severity rates of chronic GVHD similar to myeloablative transplants, and (6) most of the available data have been obtained from small single-center reports or larger but retrospective multicenter studies rather than prospective trials [138].

In patients with high-risk AML ineligible for conventional allogeneic HSCT, RIC regimen can result in long-term remissions and chronic GVHD that reduces relapse rate and improves OS as well as DFS [163]. In these patients, novel approaches are also required to reduce post-HSCT relapses [163]. Allogeneic HSCT from related or unrelated donors after conditioning therapy with low-dose TBI and fludarabine relies almost exclusively on GVL effects and can result in long-term remissions in older patients and in patients who are medically unfit for the standard-myeloablative conditioning therapy [164]. Compared to fludarabine, cytarabine and idarubicin nonablative conditioning regimen, the more myelosuppressive conditioning therapy with fludarabine and melphalan provided better disease control, but higher TRM and morbidity in patients with high-risk MDS and AML subjected to allogeneic HSCT [165]. RIC-allogeneic HSCT using fludarabine, busulfan and alemtuzumab conditioning treatment is safe and has minimal toxicity even in high risk patients with advanced disease in whom conventional myeloablative conditioning therapy is contraindicated [162].

In a four arm clinical study that included 51 patients who were followed up for at least 100 days, RIC-allogeneic HSCT in these patients with AML using clofarabine and/or fludarabine in addition to IV busulfan daily conditioning therapy, the following results were obtained: (1) clofarabine had sufficient immunosuppressive capacity to consistently support the engraftment of allogeneic progenitor cells, (2) clofarabine and IV busulfan \pm ATG appeared to be a highly active conditioning regimen in patients with advanced largely chemotherapy-refractory myeloid leukemia, (3) the use of fludarabine and clofarabine in addition to busulfan was safe, and (4) in pre-transplant conditioning therapy, a combination of 2 nucleoside analogs (fludarabine and clofarabine) may synergistically increase anti-leukemic efficacy without a concomitant increase in clinical toxicity [166].

Treosulfan in combination with fludarabine as RIC therapy for allogeneic HSCT in patients with secondary AML or MDS is a feasible and an effective regimen that enables engraftment in almost all patients subjected to transplantation [167]. Achieving a stringent CR in AML patients is critical for the success of RIC-allogeneic HSCT [161]. However, the definition of CR was updated in 2003 and the new definition requires no evidence of leukemia by flow

cytometry in addition to having morphological remission [161]. Despite being associated with GVHD, TRM and relapse, RIC-allogeneic HSCT produces outcomes similar to allogeneic HSCT using myeloablative regimens [138].

8.3. Autologous HSCT

Autologous HSCT is an effective therapeutic modality in AML with the possibility of long-term survival, particularly in patients with standard-risk AML [168,169]. Several historical randomized trials have reported significantly lower relapse rates after autologous HSCT than after conventional chemotherapy [170,171]. Autologous HSCT has the following advantages: (1) faster hematological engraftment reflected by the recovery of blood indices, (2) the possibility of applying this form of transplant in older patients, (3) lower incidences of transplant-related complications, (4) faster immune reconstitution, and (5) absence of GVHD [169]. However, autologous HSCT has few disadvantages including: lack of GVL effect, insufficient number of peripheral stem cells at mobilization, and higher incidence of relapse [169]. Faster recovery of blood counts following autologous HSCT has the following advantages: shorter duration of hospitalization, decreased need for blood product transfusion, and reduction in the days of IV antimicrobials [172].

Oral busulfan is the historical backbone of the busulfan-cyclophosphamide conditioning regimen for autologous HSCT [170]. The use of IV busulfan, instead of the oral form, simplifies the autograft procedure and confirms the usefulness of autologous HSCT in AML. As in allogeneic HSCT, IV busulfan is associated with the uncommon complication of VOD in the autologous transplant setting [170].

BM is the traditional source of stem cells for HSCT [172]. Since 1994, the use of peripheral blood stem cells has resulted in more rapid engraftment kinetics and lower rates of NRM [170]. The use of G-CSF-mobilized peripheral blood stem cells use has been associated with more rapid engraftment presumably due to the higher number of infused CD34+ cells and with reduced morbidity and TRM compared to autologous BM transplantation [172,173]. Therefore, autologous peripheral blood HSCT has recently replaced autologous BM transplantation based on reduction in morbidity, resource utilization and duration of hospitalization [174]. Nevertheless, the outcomes of peripherally collected autologous stem cells and autologous stem cells collected by BM harvesting are similar [172].

Phase I and II studies have shown that patients with favorable- risk cytogenetics benefit from autologous HSCT with a reduction in relapse rate and improvement in leukemia-free survival [174]. However, in patients with high-risk AML, autologous HSCT is not a valuable option, while allogeneic HSCT is a valid therapeutic potential provided CR or control of the disease is achieved [175]. In elderly individuals with AML, there are limitations to the utilization of autologous HSCT [176]. Fortunately several new agents that can be used alone or in combinations may become more useful than autologous HSCT in this age group [176].

In elderly patients with AML, autologous HSCT has been performed, but the published studies lack randomization and have included highly selected patients [177]. However acceptable toxicity and relatively low TRM have been reported [177]. Relapses following autologous

HSCT were also the main cause of treatment failure [177]. In patients with de novo AML belonging to the age group of 60 - 70 years: more than 25% of patients benefit from standard intensive chemotherapy and autologous HSCT has a tolerable toxicity and may have a positive impact on leukemia-free survival [178].

The outcome of autologous HSCT widely varies among patients with AML [179]. Delayed hematological recovery in patients with AML in CR1, subjected to autologous HSCT, is associated with favorable outcome, that is, longer OS and longer time to progression [179]. A two-step approach to autologous HSCT is as follows: (1) HiDAC consolidation, and (2) conditioning therapy with busulfan and etoposide followed by infusion of peripherally collected autologous stem cells has produced excellent stem cell yields and has allowed a high proportion of patients to receive this intended therapy [180].

9. Maintenance therapy following HSCT

Disease recurrence is a major cause of treatment failure after HSCT for AML [117]. Disease relapse post-HSCT is a devastating event for patients with hematologic malignancies [116]. Maintenance therapy following HSCT offers the possibility of avoiding or delaying relapse, but its role remains unclear in most diseases treated by HSCT [116]. GVHD is a major cause for NRM and morbidity after HSCT. Decitabine maintenance after HSCT may eradicate MRD and facilitate a GVL effect. Lower doses of decitabine (15 mg/m² for 5 days every 6 weeks) are well tolerated after HSCT [117]. Decitabine maintenance may have a favorable effect on the incidence of GVHD by enhancing the effect of T-regulatory lymphocytes [117]. One study has shown that approximately 43% of patients are able to tolerate 8 cycles of low dose decitabine maintenance following HSCT. The lower incidence of GVHD and the lack of decitabine toxicity indicate that a longer period of administration may be required [117]. Methods that are used to monitor MRD following HSCT include: (1) MFC, (2) PCR for gene overexpression (PCR-GE) in disease compared to healthy tissue, (3) PCR for sequence, somatic mutation or splice variant specific to tumor (PCR-NUT), and (4) next-generation sequencing (NGS) [95,102-104,116].

9.1. Immunotherapy in AML

Myeloid forms of leukemia are particularly suited for immunotherapeutic interventions because of the following reasons: (1) myeloid leukemias express both HLA class I and class II molecules and their down-regulation is infrequently observed in leukemic blasts, (2) leukemic blasts typically exist in physical niches within the BM microenvironment and/or peripheral blood components that are relatively accessible to antigen-specific T-cells and other non-antigen-specific immunocytes, (3) myeloid disorders are often characterized by chromosomal translocations that result in chimeric proteins which are unique leukemia antigens that may offer target specificity, and (4) the window of immune recovery or reconstitution following cytotoxic-based induction therapy or HSCT offers a unique opportunity to prime an immune response or circumvent potential leukemia-induced peripheral tolerance [181]. The following are the types of immunotherapies that are available for AML patients: (1) allogeneic HSCT

including RIC-allogeneic HSCT, (2) DLIs as well as GVL effects of HSCT and DLI, (3) autologous anti-leukemic T-cell infusions, (4) adoptive transfer of allogeneic or autologous T-cells and natural killer (NK) cells, (5) vaccination with leukemic cells, (6) use of peptides and DNA vaccines such as: peptide vaccines, granulocyte monocyte-colony stimulating factor (GM-CSF) secreting tumor vaccines and whole tumor cell vaccines, (7) use of immunomodulatory drugs, (8) DC immunotherapy including: monocyte or BM-derived-DCs and leukemia-derived-DCs, (9) treatment with cytokines such as IL-2, (10) administration of monoclonal antibodies such as GO, and (11) WT₁ antigen targeting [182-187].

Cytotoxic chemotherapy can successfully induce remissions in adult patients with AML, but such remissions are not usually sustained as the disease has high probability of relapse [182]. In AML patients durable remissions can be achieved in less than 30% of patients as most of these patients respond initially to combination chemotherapy, but relapse later on [182,183]. At relapse, blast cells are usually resistant to the drugs to which the patient has been exposed and also frequently to other cytotoxic agents. In such cases, immunological mechanisms for blast killing appear crucial [183]. There is evidence; obtained from tissue culture, animal and clinical studies, that stimulated donor T-cells can recognize and kill leukemic blasts through the recognition of alloantigens, differentiation antigens or leukemia-specific antigens as targets [183].

Strategies to prevent relapse of AML include: consolidation chemotherapy, HSCT and immunotherapy [182]. The principle of anti-leukemic adoptive immunotherapy, as coined by Georges Mathe in 1965, is an activity of allogeneic immunologically competent cells against the host leukemic cells [188]. The best known model and the most commonly used method of anti-leukemic adoptive immunotherapy is allogeneic HSCT [188]. The following provide evidence supporting the GVL effects of allogeneic HSCT: (1) lower relapse rate amongst recipients of HLA-identical sibling transplants than recipients of syngeneic transplants, (2) lower relapse rate among patients who develop GVHD following allogeneic HSCT, (3) decreased GVHD but increased relapse rate after T-cell depleted allografts, (4) anti-leukemic effect and curative potential of DLI in patients with leukemia who relapse after HSCT, and (5) the effectiveness of non-myeloablative allogeneic HSCT [188]. Wider application of immunotherapies such as allogeneic HSCT and RIC therapy has altered the landscape and could offer a potential for cure to an increasing number of older patients with AML [186]. RIC-allogeneic HSCT in older AML patients, after achievement of CR, is feasible and has improved DFS over conventional chemotherapy [186].

Immunotherapeutic approaches such as improvement in pre-HSCT conditioning regimens and the use of DLI to induce GVL effect will ultimately improve the outcome of HSCT and decrease the burden of AML [182]. The GVL effect of HSCT has shown that the immune system is capable of eradicating AML [189]. Allogeneic HSCT and DLI have the potential to eradicate leukemic cells by means of allogeneic T-cells [185]. Adoptive immunotherapy has the potential to provide long-term survival and even cure in patients with leukemia [188]. Novel approaches using NK cells and T-cells have been developed to provide more direct anti-leukemic therapy while sparing the risk of toxicity to normal tissues [188].

Emerging clinical data indicate that GO is efficacious not only in acute promyelocytic leukemia but also, once combined with conventional chemotherapy, in favorable and intermediate risk AMLs [190]. Data from several cooperative groups indicate that older patients with AML benefit from the incorporation of GO into the induction therapy [186]. Also, the clinical results of GO strongly support the utility of CD33 targeted therapeutics [190]. The early recognition that some AMLs may predominantly or entirely involve committed myeloid progenitor cells led to efforts underlying LSCs with antibodies recognizing the CD33 differentiation antigen [190]. Targeted therapy with GO has produced remissions in relapsed AML and the drug appears promising when used in combination with the standard chemotherapy for the treatment of newly diagnosed AML [191].

Targeted alpha-particle immunotherapy offers the potential of more efficient tumor cell killing, while sparing the surrounding normal cells, than beta-particles [192]. Clinical studies on alpha particle immunotherapy for AML have focused on the myeloid cell surface antigen CD33 as a target using the humanized monoclonal antibody lintuzumab. Studies have demonstrated the safety and efficacy of bismuth-213 labeled lintuzumab in the treatment of AML as it has produced remissions in some patients with AML after partial cytoreduction with cytarabine [192]. The second generation construct that contains actinium-225 (225 Ac-lintuzumab) has also been shown to have significant anti-leukemic activity even in elder patients with low-dose cytarabine [192]. Preclinical studies have shown that anti-CD45 monoclonal antibodies, as part of conditioning therapy prior to HSCT, are useful [192]. MRD reflected by the persistence of LSCs below the detection limit by conventional methods, causes a high rate of disease relapses [187]. Assessment of MRD is critical in monitoring the effectiveness of immunotherapy in AML patients as the ultimate goal of AML treatment is the eradication of MRD [182,187].

Four decades after the initial attempts at vaccination for AML, using bacilli Calmette-Guerin and irradiated autologous leukemic cells, interest in immunotherapy in AML has been revived [182,193,194]. Phase I and II studies using different DC vaccination protocols have been designed [187]. DC-based vaccination has resulted in the induction of potent anti-leukemic cytotoxic T cells, but with limited clinical efficacy [187]. To improve the effectiveness of DC-based vaccination, the optimal timing of vaccination during the course of AML has to be determined, although current literature and cumulative experience indicate that immunotherapy may be most effective in the state of MRD following induction and post-remission chemotherapy [187].

The development of antigen-targeted immunotherapy of AML has been accelerated by: (1) the GVL effect of HSCT on residual leukemic cells, and (2) the identification of leukemia associated antigens (LAAs) that can serve as potential targets for immunotherapy [189]. There are several LAAs and these together with other molecules that may serve as potential targets in AML immunotherapy are included in Table 10 [185,187,195,196]. Criteria that should be met for selection of ideal LAAs which can be used in AML targeted therapy include that they should: (1) be leukemia specific as they are expressed in leukemia cells with minimal or no expression in normal tissues, (2) display high and homogeneous expression in most leukemic blasts including LSCs, (3) play a defined oncogenic role and are important for the leukemia phenotype, (4) be immunogenic as they possess strong immunogenic properties, (5) demonstrate clinical utility and effectiveness, (6) show expression in hematopoietic stem cells and (7) display expression in T-cells particularly activated T cells [189,195].

Antigen or molecules	(percentage)	Antigen or molecule	(percentage)
AURKA (Aurora kinase A)	(37%)	CD 25 ; IL-2 receptor α	-
PRAME (preferentially exposed antigen in melanoma)	(32%)	CD 33 ; siglec - 3	> 80%
PHAMM (CD 168, receptor of hyaluronic mediated motility)	(60-70%)	CD 44 ; H-CAM	100%
WT ₁ (Wilms tumor 1)	(73-100%)	CD 45	-
CLL-1 (c-type lectin-like molecule)	(92%)	CD 47 (integrin-associated protein immunoglobulin superfamily)	100%
MUC (mucin-1)	(67-70%)	CD 96 (tactile Ig superfamily)	30%
mHAgs (minor histocompatibility antigens)	-	CD 123 (IL-3 receptor α)	100%
HBG-2	(42%)	h Tert	
CCNA-1	(82%)	HSJ 2	
BAALC	(30%)	MPP 11	
PRTN-3	(48%)	NE (neutrophil elastase)	
RBPJk	-	PR ₁	
Proteinase 3	-	DRAP and cyclin A ₁	

Table 10. Leukemia-associated antigens and other molecules that the potential targets in AML immunotherapy

Improved understanding of NK cell biology has provided insights on selection of donors for HSCT and on the immunotherapeutic options in non-HSCT settings [186]. Novel immunotherapeutic approaches and identification of novel target antigens and improved donor selection for allogeneic HSCT hold promise for a variety of treatment options for older patients having AML in the near future [186]. The identification of LAAs and the observation that administration of allogeneic T- cells can mediate a GVL effect paved the way to develop active and passive immunotherapeutic strategies respectively [185]. Promising targeted passive immunotherapy approaches include: the administration of anti-CD33 antibodies and the adoptive transfer of LAA-specific T cell or KIR ligand-mismatched NK cells [185]. Whole tumor cell vaccines or DC-based vaccines also hold promise in boosting the immune system for the induction of anti-leukemia immune responses. The aim of these immunotherapeutic strategies is the eradication of AML blasts by the immune system [185]. Treatment of older patients with AML is slowly but steadily changing with emphasis on supportive care and potentially curative therapeutic approaches such as certain forms of allogeneic HSCT [186].

Recent advances in immunology and identification of promising LAAs open the possibilities of eradicating MRD by antigen-specific immunotherapy following the administration of cytotoxic chemotherapy [184]. Progress in inducing antitumor immune responses together

with strategies to attenuate immunosuppressive factors will establish immunotherapy as an important armament to combat AML [184]. Although immunotherapeutic trials have shown improvement in immunogenicity and clinical outcomes, severe adverse events have been encountered in highly avid engineered T-cell therapies indicating the importance of having a balance between effectiveness and adverse effects related to the use of advanced immunotherapy. Such a balance between clinical efficacy and safety will become a main issue in the era of advanced future immunotherapy [184].

9.2. Novel and targeted therapies in AML

Development of effective targeted cancer therapeutic depends upon distinguishing disease-associated or driver mutations, which have causative roles in the pathogenesis of malignancy, from passenger mutations that are dispensable for cancer initiation maintenance [197]. Therapeutic strategies in AML are shown in Table 11 [15]. Molecular targeted therapies and novel agents that are being evaluated in clinical trials are included in Table 12 [17,58,198-213].

Number	Therapeutic approach	Examples		
(1)	Epigenetic regulation	(a) histone deacetylase inhibitors: vorinostat, panobinostat and belinostat (b) DNA methyltransferase inhibitors: azacytidine and decitabine		
(2)	Induction of differentiation	(a) Arsenic trioxide (b) Retinoid X receptor agonists: ATRA		
(3)	Inhibition of angiogenesis	a- Thalidomide	b- Lenalidomide	c- Bortezomab
(4)	Modulation of drug resistance	(a) Valspodar	(b) Zosuquidar	
(5)	Modification of traditional chemotherapeutics	(a) Nucleoside analogs: clofarabine, sapacitabine and elacytarabine (b) Alkylating agents: irofulven, temodar and onrigin (c) Topoisomerase II inhibitor: hycamtin		
(6)	Immunotherapy	a- Monoclonal antibodies: myelotarg, lintuzumab and avastin b- T-cell targeted therapies		
(7)	Inhibition of signaling pathways	(a) Tyrosine kinase inhibitors: sorafenib, modostaurin and lestaurtinib. (b) Cell cycle inhibitors: ONO 1910. Na (c) Farnesyl transferase inhibitors: sarasar and zarnestra (d) mTOR inhibitors: afinitor, temsirolimus, PI-103 and GSK 21110183 (e) PARP inhibitors: ABT-888 (f) MEK 1/2 inhibitors: AZD 6244, GSK1120212, AS703026 and PD98059 (g) Bcl2 inhibitors: Obatoclax, Oblimersen and ABT-263 (h) XIAP inhibitors: AEG -35156 (i) Aminopeptidase inhibitors: tosedostat		

Table 11. Therapeutic strategies in patients with acute myeloid leukemia

10. Small molecule inhibitors in AML

Many patients with AML who initially respond to induction chemotherapy will eventually relapse or develop refractory disease [115,213]. Targeted therapy with small molecule inhibitors (SMIs) represents a new therapeutic intervention that has been successful in treating several cancers [213]. Examples of SMIs that include inhibitors of the targets: FLT3, HDAC, heat shock protein, CXCR4, proteasomes and Aurora kinases are shown in Table 12 [213-233]. There has been great interest in generating selective SMIs that target critical pathways of proliferation and survival of blasts in AML. SMIs have been developed to modulate the activity of proteins encoded by mutated or over-expressed genes in patients with AML [213]. Clonal evolution and pharmacodynamics are potential obstacles to the clinical development of SMIs for the treatment of AML. Multi-targeted agents and the combination of SMIs with cytotoxic chemotherapy may improve the efficacy of treatment [213]. The analysis of patient samples is an important tool to investigate resistance mechanisms, and discover and validate biological markers that could be used for the prediction and assessment of treatment response [213].

Molecular target	Phase of study	Small molecule inhibitors or new drugs in clinical trials	
Aurora kinase	I	* Alisertib * AMG 900	
Farnesyl transferase	1/2	- Tipifarnib - Lestaurtinib	
Histone deacetylase	1/2/3	- Valproic acid - Panobinostat - Vorinostat - Entinostat	
Proteasome inhibitor	2	- Bortezomib - Ixazomib	
CXCR ₄	1/2	* Plerixafor * BL- 8040 * BMS - 936564	
HSP 90	1/2	- Ganetespil	
FLT 3	1/2/3	- Crenolanib - Sorafenib - Midostaurin	- Quizartinib - Sunitinib - Listaurtinib
C-Kit	1/2	- Nilotinib - Dasatinib	
Hedgehog pathway	1/2	PF - 04449913	
WTN pathway	1	* PRI-728	

Molecular target	Phase of study	Small molecule inhibitors or new drugs in clinical trials
		* CWP - 232291
IDH 1/2	1	* AG - 120 * AG - 221
Molecular target		Small molecule inhibitors or new drugs in clinical trials
mTOR pathway		- Rapamycin - Everolimus - Temsirolimus
Nucleoside analogs		- Clofarabine - Sapacitabine
DNA hypomethylating agents		- Azacytidine - Decitabine
Immunomodulatory agents		- Lenalidomide
Monoclonal antibodies		- Gemtuzumab
Bcl-2		* ABT - 199 * ABT - 737
Retinoids		- ATRA
MEK1		- E 6201
Alkylating agents		- Bendamustine
Statins		- Pravastatin
Mitochondrial translation inhibitor		- Tigecycline
EGFR inhibitor		- Erlotinib
Oncogene eIF4 E inhibitor		- Ribavirin
VEGFR inhibitor		- Pazopanib
CDK inhibitor		- Flavoperidol
RAS mutation		- GSK1120212 - MSC 19363698
JAK 2 mutations		INCB 018424

Table 12. Molecular targeted therapies and new drugs in AML clinical trials

10.1. FLT3 inhibitors

FLT3-ITD activating mutations are present in one fifth to one third of adult patients with AML and they are associated with poor prognosis [197,203,204]. FLT3-ITD mutations represent a

driver lesion and can be a valid therapeutic target in AML [197]. Agents that target FLT3 mutations are under development for the treatment of patients with AML and they may offer a potential paradigm change in the current standard treatment of AML [17]. The introduction of FLT3 inhibitors for the treatment of AML may be the start of a new era in the treatment of AML after many years of exclusive dependency on cytotoxic chemotherapy [17]. Examples of FLT3 inhibitors include: sorafenib, midostaurin, sunitinib and lestaurtinib [17]. Early FLT3 inhibitors including midostaurin, sunitinib and lestaurtinib have demonstrated significant promise in preclinical models of FLT3 mutant AML. Unfortunately, in early clinical trials, many of these agents had failed to achieve robust and sustained FLT3 inhibition as they caused only transient decreases in peripheral blast counts [204]. The second-generation FLT3 inhibitors, such as quizartinib, have demonstrated enhanced FLT3 specificity and have been well tolerated in early clinical trials. Several FLT3 inhibitors have reached phase III clinical trials and a variety of phase I and II trials in order to explore the role of these novel agents in conjunction with conventional chemotherapy and HSCT [204]. Molecular insights provided by FLT3 inhibitors have shed light on the various mechanisms of drug resistance and have provided a rationale supporting the use of combinations of conventional chemotherapeutic regimens and novel targeted treatments [204].

Most patients with FLT3-ITD-positive AML show initial favorable response to FLT3 inhibitors followed by the development of drug resistance [203]. After the use of FLT3 inhibitors in patients with single FLT3-ITD-mutated AML, a new tyrosine kinase mutation may arise, a phenomenon that is associated with the evolution of drug resistance [205]. Acquired resistance to selective FLT3-ITD-positive AML is an emerging clinical problem in the treatment of FLT3-ITD and this resistance is associated with poor prognosis [203,205,206]. There are several mechanisms of resistance to FLT3 inhibitors and they include: (1) FLT3 receptor and ligand expression, (2) up-regulation of the compensatory signaling pathways, (3) acquired mutations in the tyrosine kinase domain (TKD) of FLT3, (4) mutations in other kinase genes, and (5) up-regulation of anti-apoptotic proteins [203]. One of the most common mechanisms of resistance to inhibitors is the acquisition of secondary FLT3-TKD mutations which primarily consist of point mutations in the activation loop TKD₂ and in the ATP-binding pocket of TKD [203].

Midostaurin and lestaurtinib are multi-targeted kinase inhibitors that include FLT3 as one of their targets [207]. They are more appropriate for the treatment of newly diagnosed AML as they are broad-spectrum agents [207]. Quizartinib is a highly selective FLT3 inhibitor and is less toxic to FLT3-ITD AML primary samples *in vitro*. It may be useful in relapsed patients in whom high FLT3 ligand levels may necessitate a highly potent agent that is able to prevent ligand binding to FLT3 [207]. In patients with relapsed/refractory AML, particularly those harboring FLT3-ITD mutations, quizartinib has shown clinical activity with acceptable toxicity profile [208]. Quizartinib is a potent and selective FLT3-TKI with activity against both FLT3-mutant and wild-type AML [209]. However, the quality and duration of responses achieved are suboptimal [209]. The combination of quizartinib and chemotherapy may improve the outcome, while the combination of quizartinib and agents that tackle BM microenvironment may enhance response rates [209]. Sunitinib has anti-leukemic activity in patients who become resistant to sorafenib indicating that sequential therapy with different FLT3 inhibitors may

provide clinical benefit [203]. Clinical activity of sorafenib monotherapy in FLT3-ITD- positive adult AML patients including induction of CRs has been described [203]. However, clinical responses have not been durable in most patients [203].

Ponatinib is a multikinase inhibitor that has demonstrated clinical efficacy in patients with chemotherapy resistant AML having with FLT3-ITD mutations [210]. It exhibits activity against AC-220-resistant FLT3-ITD/F691 gatekeeper mutation, but it is highly ineffective against FLT3-ITD activation loop mutations, particularly at the D835 residue [210]. Crenolanib is a novel TKI that has demonstrated an inhibitory activity against drug-resistant AML primary blasts with FLT3-ITD and D835H/Y mutations [211]. It is effective against FLT3-ITD containing secondary kinase domain mutations, suggesting that crenolanib may be a useful therapeutic agent against TKI-naïve and drug-resistant FLT3-ITD-positive AML [211].

The dual Aurora-B/FLT3 inhibitors represent a significant development in the treatment of AML [212]. These dual inhibitors may overcome FLT3 inhibitor resistance, partly due to inhibition of Aurora kinase, and thus may benefit patients with FLT3-mutated AML [206]. Orally bioavailable dual FLT3-Aurora kinase inhibitors with improved properties are currently under development [206]. More potent novel therapies that are useful in the management of relapsed or heavily pretreated AML having high circulating levels of FLT3 ligand include: FLT3-ITD-specific small molecules and monoclonal antibodies that target FLT3. Also, the use of sensitive methods to monitor FLT3 mutations during treatment may allow individualized treatment with the currently available kinase inhibitors [207].

10.2. HDAC inhibitors

In t(8,21) AML, the AML₁/ETO fusion protein promotes leukemogenesis by recruiting class I HDAC-containing repressor complex to the promoter AML1 target genes [214]. Recruitment of HDACs is an important epigenetic mechanism of transcriptional dysregulation and gene silencing in AML [215]. Modulation of protein lysine acetylation through the inhibition of HDACs is one of the therapeutic strategies to treat AML patients who are unfit for intensive chemotherapy [216]. HDAC-inhibitor-mediated differentiation therapy is a potent and molecularly rational therapeutic strategy in t(8,21) AML. Epigenetic modifying enzymes such as: HDACs, P300 and PRMT1 are recruited by AML-1/ETO thus providing a molecular rationale for targeting these enzymes to treat AML with t(8,21) [217]. Although early phase clinical assessment indicated that treatment with HDAC inhibitors may be effective in t(8,21) AML, rigorous preclinical studies to identify the molecular and biological events which may determine therapeutic responses have not been established [217]. The only HDAC inhibitors that have been investigated in clinical trials of AML are butyrate derivatives, valproic acid (VPA) and desipeptide [218]. HDAC inhibitors can mediate anti-leukemic effects in AML, but their clinical benefits are limited, thus further studies including combination therapies are warranted [218]. VPA is a HDAC inhibitor that is being utilized as a disease-stabilizing therapy as it improves normal blood values and has a minimal risk of clinically relevant toxicity [216]. However, VPA has not been investigated in randomized clinical trials [216]. VPA has been shown to cause growth arrest and induce differentiation of malignant cells via HDAC inhibition [214]. VPA may effectively target AML1/ETO-driven leukemogenesis through disruption of aberrant HDAC inhibitor function. Therefore, VPA should be integrated in the novel therapeutic approaches for AML₁/ETO-positive AML [214].

Autophagy is a catabolic pathway that is upregulated during times of nutrition limitations or stress to maintain cellular metabolism and organelle integrity [219]. In AML1/ETO- positive AML cells, HDAC inhibitors induce autophagy which acts as a pro-survival signal to limit HDAC-induced cell death. In contrast to the fusion oncoproteins, promyelocytic leukemia-RARA (PML-RARA) and breakpoint cluster region-abelson, AML1/ETO is not degraded by either basal or drug-induced autophagy [219]. Combined treatment with HDAC inhibitors and autophagy inhibitors such as chloroquine has resulted in a massive accumulation of ubiquitinated proteins that correlated with increased cell death [219]. The combination of VPA induced autophagy in the cells of patients with t (8,21) AML and chloroquine therapy has enhanced cell death. Because VPA and chloroquine are well-tolerated drugs, their combination could represent an attractive treatment option for AML1-ETO- positive leukemia [219].

10.3. Heat shock proteins

Heat shock proteins (HSPs) such as HSP-90 are often over expressed in AML and are involved in the regulation of apoptosis, proliferation, autophagy and cell cycle progression. Hence, they are considered as possible therapeutic targets in the management of AML [220]. Phase I and II clinical trials have revealed that HSP-90 inhibition can mediate anti-leukemic effects in vivo. Further studies including their combination with conventional chemotherapy are needed to clarify their efficacy and toxicity in the future treatment of AML [220].

11. Targeted therapies for CBF-AML

Core binding factor (CBF)-AML is a favorable AML subset defined cytogenetically by t (8,21) or inv (16) / t (16,16) rearrangement disrupting RUNX1 or CBFB transcription factor functions [221]. The receptor tyrosine kinase (KIT) is expressed in the vast majority of AML subsets and frequent activations of KIT gene mutations have been associated with a higher risk of relapse [221]. Romidepsin has differential anti-leukemic and molecular activity in CBF-AML [215]. The development of romidepsin in the treatment of CBF-AML should focus on drug combinations that target related mechanisms of gene silencing such as DNA methylation [215].

A phase II study evaluated dasatinib as maintenance therapy in 26 patients with high-risk CBF-AML with KIT mutations in first CR showing that dasatinib can be safely administered as single-agent maintenance in AML patients in CR, but does not seem to prevent relapse, because the activity of dasatinib may be impaired by spontaneous and/or dasatinib-driven clonal devolution [221].

11.1. Clofarabine

Clofarabine is a second generation purine nucleoside analog that incorporates the characteristics of 2 other purine analogs: fludarabine and cladribine [222,223]. It inhibits DNA polymerases and ribonucleotide reductase, thus inducing apoptosis in cycling and non-cycling cells [222]. Clinical trials have shown the activity of clofarabine in adult AML both as a single agent and in combination with other cytotoxic drugs [223]. Clofarabine has also been used as front-

line therapy combined with standard induction treatment, idarubicin and cytarabine, in newly diagnosed AML and its use has been shown to be effective (longer OS and EFS compared to chemotherapy alone) and relatively safe [224,225]. Clofarabine is cytotoxic to leukemic cells that are resistant to cytarabine [226].

11.2. Lenalidomide

The highly encouraging results of lenalidomide in the treatment of del (5q) in low-risk MDS have not been reproduced in del (5q) AML. However, in a study that included 33 patients, ≥ 60 years without del (5q) and having low circulating blasts at diagnosis, lenalidomide had produced CR rates of 50% [227]. The clear activity of lenalidomide in a subset of AML patients should promote efforts to identify patients who are likely to respond to the drug, thus allowing the rational use of this agent either alone or in combination with other drugs [227].

11.3. Obatoclox

Over-expression of Bcl-1, Bcl-xL and/or Mcl-1 has been associated with resistance to chemotherapy in AML cell lines and ultimately poor clinical outcome [228]. Obatoclox (a novel inhibitor of anti-apoptotic Bcl-2 family proteins and a pan-Bcl-2 inhibitor) enhances cytarabine-induced apoptosis by enhancing DNA damage or double strand breaks and improves outcome in AML patients harboring Bcl-2 proteins [228]. In a small cohort of elderly AML patients with primary chemorefractory disease, a combination of low-dose azacytidine, ATRA and pioglitazone (peroxisome proliferator-activated receptor γ ligand) has induced CRs, thus bimodulatory therapy may bypass genetically based chemotherapy resistance in AML [229].

11.4. CXCR4 antagonists

The chemokine receptor (CXCR4) and its ligand stromal derived factor-1 (SDF-1) are important key players in the cross-talk between leukemia cells and BM microenvironment or stroma niche [230-232]. SDF-1 regulates the process of homing and engraftment of LSCs into the BM and inhibition of its receptor CXCR4 induces mobilization of leukemic cells into the circulation [232]. CXCR4 expression is associated with poor prognosis in AML patients with or without FLT3 genetic mutations [231]. SDF-1 α / - CXCR4 interactions contribute to the resistance of leukemic cells to signal transduction inhibitor- and chemotherapy-induced apoptosis in systems that mimic the physiologic microenvironment [233]. Disruption of these interactions with CXCR4 inhibitors represents a novel strategy of sensitizing leukemia cells by targeting their protective BM microenvironment [233]. Preclinical and clinical studies using CXCR4 antagonists in combination with chemotherapy have demonstrated that blocking CXCR4 is a novel promising approach in the treatment of AML [230-232].

11.5. Monoclonal antibodies

Approximately 87.8% of AML cells express CD33 and 9.7% of AML cells express CD123, without concomitant CD33 expression. Therefore, nearly all AMLs can be treated with monoclonal antibodies directed against CD33 and CD123 [234]. Identification of targets or

antigens on the cell surface of leukemic cells, particularly LSCs, has recently attracted particular attention and new targeted therapies are under development. Tailored immunotherapy targeting CD33 and CD123 is likely to enhance treatment efficacy in the majority of AML patients [234].

CD33 is present on the leukemic blasts from the majority of patients with AML and MDS [235]. GO is a humanized anti-CD33 monoclonal antibody that was approved by the food and drug authority (FDA) in the USA in 2000 for the treatment of AML in first relapse in patients older than 60 years who are unfit for more intensive chemotherapy [235,236]. In recipients of HSCT, the main adverse effects of GO treatment are anaphylactic reactions, adult respiratory distress syndrome, hepatotoxicity and VOD [235-238]. GO has been shown to be effective in CD33 positive de novo AML even in younger adults and children either a single agent or in combination with conventional induction chemotherapy. However, its use has not improved OS and some studies have shown significant early TRM [237,239-241].

The IL-3 receptor α chain (CD123) has been identified as a potential immunotherapeutic target because it is overexpressed in AML compared to normal HSCs [242]. CD123 chimeric antigen receptor (CAR) T-cells specifically target CD123 positive AML cells [242]. AML patient-driven T cells can be genetically modified to lyse autologous tumor cells. CD123-CAR-T cells are a promising immunotherapy for treating high-risk AML [242]. CD123-specific CARs strongly enhance anti-AML-CIK (cytokine-induced killer) functions, while sparing normal HSCs/progenitor cells thus paving the way to develop novel immunotherapeutic approaches for the treatment of AML [243]. The strategy of redirecting CIK cells with CD123 CAR should soon find a place in the plethora of novel alternative approaches used to treat AML, because the advantages in immune efficacy and in vivo persistence of CAR-redirected T-cells would represent a relevant beneficial effect [244]. CD123 based myeloablation may also be used as a novel conditioning therapy for HSCT [245]. Eventually anti-CD123 CAR-based strategy, coupled with a suicide gene system, could truly represent a major advancement in the field of AML therapeutics, providing a novel magic bullet for AML therapies, particularly for high-risk transplanted patients with MRD or as an alternative biological therapy for older patients in whom standard chemotherapeutic approaches are not applicable [246].

CD123 (IL-3-receptor α chain) has been identified as a potential immunotherapeutic target as it is overexpressed on AML LSCs and AML blasts rather than HSCs [247]. Two fusion proteins (anti-CD3 Fv- Δ IL3 and disulfide-stabilized anti-CD3 Fv- Δ IL3) display anti-leukemic activity against CD123-expressing cell lines and leukemic progenitors both in vitro and in vivo. Therefore, these 2 fusion proteins could be the promising candidates for future immunotherapy in AML [247].

11.6. Leukemia stem cells

Tumors possess a minor fraction of cancer stem cells (CSCs) which maintains the propagation of the malignancy [248]. In many cancers, it is difficult to completely eliminate the CSCs by chemotherapy or radiotherapy, thus recurrence or relapse of cancer usually occurs [248]. Perhaps the Holy Grail in cancer therapy today is CSCs or cancer initiating cells [248]. Despite the recent increase in understanding the pathogenesis of AML, the disease remains with poor

outcome due to the overwhelming relapse rate [249]. Given the general lack of significant success in treating AML with conventional therapies, new approaches to treat the disease are warranted. The coming years will witness the performance of active clinical trials on targeted therapies against LSCs [249].

In 1994, CSCs were first described in AML cells by Dick and co-workers who dissociated LSCs from the bulk of AML cells [248,250]. The immature LSCs resided within the CD34 positive/CD38 negative subpopulation and they represented a small fraction of the total leukemic blasts [248]. The discovery of LSCs has important clinical implications [250]. LSCs are relatively insensitive to current therapies and they are considered amongst the leading causes of treatment failure and relapse in patients with AML [247,248,250]. Many of the critical biological properties of LSCs have been elucidated and these include the following: (1) distinct replicative properties, (2) cell surface phenotypes, (3) increased resistance to chemotherapy, and (4) involvement of growth-promoting chromosomal translocations [248]. In recent years, research has focused on the characterization of LSCs population which is the disease compartment most difficult to eliminate with conventional chemotherapy and the disease compartment most responsible for relapse [249].

LSCs represent a rare self-renewing cellular subpopulation in AML and their property of resistance to chemotherapy is associated with poor outcome. The characterization of genes which express surface markers of LSCs is likely to reveal novel targets that may improve therapeutic outcomes [251]. However, studies have shown that gene expression profiling of LSCs lacks reproducibility. Also, diverse signatures obtained from the analysis of LSC gene expression profiles confirm the heterogeneity of AML [251]. Analysis of gene sets that are essential for regulating LSC functions and improving the reproducibility and clinical characteristics of the relevant LSC signatures at gene levels are vital for biologic, therapeutic and prognostic levels in AML [251].

Although the field of specific therapeutic targeting of LSCs is still in its infancy as it is relatively new, it is a highly promising battleground that may reveal the Holy Grail of cancer therapy and may undoubtedly result in novel strategies to treat not only AML, but also leukemias in general [248]. Due to the unique features of LSCs, drugs are designed to target these cells and to eliminate them, but such drugs and therapies must be tested in the setting of clinical trials [249]. To be successful, novel therapeutic options in AML should aim at eradicating LSCs. Recently, the identification of targets on the cell surface of LSCs has been receiving particular attention [247]. Also, the tumor cell microenvironment or niche is an important therapeutic target, thus Rac inhibitors and various anti-integrin antibodies may be appropriate therapeutic modalities [248]. However, targeting LSCs can be achieved by: (1) targeting the key signal transduction pathways, namely: PI3K, Wnt and Rac, (2) targeting specific cell surface molecules such as CD33, CD44 and CD123 with effective cytotoxic monoclonal antibodies, (3) statins that have shown promising potential in targeting LSCs, and (4) inhibitors of ATP-binding cassette transporter proteins that are being extensively studied in combating drug resistance which is a frequent characteristic feature of LSCs [247,248].

11.7. Dendritic cells

The ability of DCs to activate T-cells is dependent upon their activation state [252]. Myeloid progenitors are prominent source of DCs under homeostatic conditions. LSCs and leukemic blasts can give rise to malignant DCs [253]. Immune tolerance to AML may be initiated at the level of the innate immune system. A specific subset of DCs, called CD8 α ⁺DCs, may be responsible for mediating tolerance in AML, thus targeting the innate immune system may be beneficial in AML [252].

Leukemia-derived DCs can express leukemia antigens and may either induce anti-leukemic T cell responses or favor tolerance to the leukemia, depending on the co-stimulatory or inhibitory molecules or cytokines [253]. Active immunotherapy aimed at the generation of specific CTLs may represent a powerful approach to target LSCs in the setting of MRD [253]. To fully activate CTLs, leukemia antigens have to be successfully captured, processed and presented by mature DCs [253,254]. DC-based immunotherapy is a promising therapeutic strategy for the elimination of MRD in patients with AML [254]. Immunotherapy may be most effective in the setting of MRD after successful induction and PRT [253,254]. AML patients who are at high risk of relapse and who are not eligible for HSCT are particularly suited for such therapeutic approach [253,254].

Studies have shown that DC-vaccination has resulted in potent anti-leukemia CTL-responses and that DC-vaccination protocols remain a promising supplementary strategy in the treatment of leukemia [253]. The vaccination procedure includes: (1) the choice of LAAs, (2) the source of DCs, (3) the DC maturation protocol, and (4) the way the application has to be defined and standardized [253]. The timing and application of potential co-treatment including chemotherapy/HSCT or immunomodulatory therapies have to be considered carefully [253].

12. Personalized therapy for AML

Over the past 7 years, the application of advanced technology in genetic sequencing has revolutionized our understanding of AML biology [255]. Knowledge of somatic mutations in AML and their clinical relevance has increased our ability to determine prognosis depending on the pre-treatment risk stratification [125,255].

In many targeted agents, the initial response may be impressive, but ultimately the duration of response is often modest due to the evolution of drug-resistant malignant cell populations [125,255]. Additionally, the predominant clones at AML relapse may differ from the clones encountered at presentation prior to the administration of chemotherapy [255]. Therefore, repeat molecular profiling and drug susceptibility testing at relapse following targeted therapy allows the determination of the mechanisms of resistance and the potential use of combined targeted therapies to overcome drug resistance [125,255]. The ability to determine drug sensitivity in the laboratory prior to administration of specific therapy (an approach similar to antimicrobial susceptibility testing in microbiological disease) has long been an aspiration in AML management [255]. However, personalized therapy may be more appropriate for certain groups of patients such as older AML patients and those having relapsed disease [97,125,255].

13. Conclusions and future directions

AML is a heterogeneous disease, with multifactorial etiology, characterized by specific chromosomal abnormalities and genetic mutations. AML has numerous complications including: anemia, bleeding diathesis, venous thrombosis, extramedullary involvement, leukostasis, electrolytic disturbances and various infectious complications. Once the diagnosis of AML is established, the disease should be classified into the appropriate subtype according to the WHO and FAB classification systems, then patients should be thoroughly evaluated to determine their fitness for therapy.

The management of AML in older patients is a real therapeutic challenge. However, treatment of AML in older subjects should be tailored according to the circumstances of each patient taking into consideration the age, comorbidities, performance state and the risk category according to the cytogenetic and molecular profiles. Recently, several therapeutic options have been made available for older patients with AML. Interestingly, targeted therapies and monoclonal antibodies are more tolerable and more efficacious than the standard chemotherapies. Philadelphia chromosome positive de novo AML is extremely rare and has several distinguishing features. The available therapeutic options include: cytotoxic chemotherapy, TKIs and allogeneic HSCT. The prognosis of this type of AML is generally poor and the median survival is relatively short. Recently, the incidence of t-AML has been found to be increasing due to the longer survival of cancer patients and the wider utilization of chemotherapy, radiotherapy and immunosuppressive agents. This form of AML can be treated with chemotherapy, allogeneic HSC, investigational agents or the best supportive care.

The aims of induction therapy in patients with AML are induction of CR and restoration of normal BM function. Cytarabine and anthracyclines are still the backbone of the frontline therapy of AML. Response to the induction phase of chemotherapy is evaluated by BM examination, cytogenetic analysis, mutational studies and MRD by flow cytometry and PCR.

The evolution of new genetic mutations and MDR genes contribute to drug resistance that may be encountered during AML treatment. Drug resistance in AML can be treated with salvage chemotherapy and investigational therapies. For relapsed/refractory AML, the following treatment options are available: salvage chemotherapeutic regimens, allogeneic HSCT, immunotherapy with DLI, several novel and targeted therapies, appropriate clinical trials and the best supportive care. PRTs in patients with AML include: (1) consolidation cycles of chemotherapy, (2) autologous or allogeneic HSCT, and (3) maintenance therapy, in patients at high risk of relapse, with the recently introduced novel therapies that can be administered even after successful HSCT.

The indications of allogeneic HSCT in patients with AML are: (1) AML refractory to induction therapy, (2) refractory AML in CR2, (3) intermediate- or high-risk cytogenetics, (4) AML with extramedullary disease, (5) AML secondary to MDS or chronic MPNs, and (6) t-AML not having favorable cytogenetics. Myeloablative allogeneic HSCT is usually performed in younger patients having good performance state and no major systemic dysfunction, while RIC-allogeneic HSCT is generally offered to older patients with AML and patients who are unfit to receive myeloablative-conditioning therapies. The antineoplastic potency of RIC-

allogeneic HSCT relies mainly on the GVL effect of the allograft rather than the ablation of all residual leukemic cells. Currently, several myeloablative and non-myeloablative conditioning therapies are available for AML patients who are eligible for HSCT. Also, several donor or stem cell sources are being utilized such as MRD, MUD, and UCB as well as the haploidentical form of HSCT. In AML patients subjected to allogeneic HSCT, the following strategies to enhance immune reconstitution can be employed: DLI, in vitro T-cell depletion, rapamycin to promote expansion of T-regulatory cells and cyclophosphamide administered on day 3 post-allogeneic HSCT to reduce alloreactive lymphocytes. Autologous HSCT is indicated for older patients with comorbid medical conditions who are not candidates for allogeneic HSCT. Several lines of immunotherapy can be used for AML patients and these include: (1) allogeneic HSCT including RIC-allografts, (2) DLIs and GVL effect of RIC-allogeneic HSCT, (3) autologous anti-leukemic T-cell infusions, (4) adoptive transfer of T-cells and NK cells, (5) vaccination with tumor or leukemic cells and peptides, (6) immunomodulatory agents, (7) DCs and ILs, and (8) monoclonal antibodies and WT1 antigen targeting. Recently, several lines of novel and targeted therapies have been evolving in the management of AML and these include: FLT3 inhibitors, HSPs, HDAC inhibitors, purine analogs, CBF targeting agents, Bcl-2 inhibitors, CXCR4 antagonists and monoclonal antibodies.

The prognosis of AML is very variable and depends on the: age of the patient at diagnosis, comorbidity index, performance status and the specific karyotype. The incorporation of gene mutational analysis as well as gene expression and micro-RNA profiling in the diagnostics of AML is likely to enrich the risk stratification and consequently more targeted and novel therapeutics may be utilized in the frontline management of AML in the future. The recent advancements in the diagnostics and therapeutics have facilitated the introduction of personalized therapy in patients with AML. Regular evaluation of molecular profiling is essential in the modern management of AML. Drug susceptibility testing particularly at the time of relapse, resembling antimicrobial susceptibility testing in microbiology laboratories, may become a reality in the future.

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