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Insights into Acute Myeloid Leukemia: Critical Analysis on its Wide Aspects

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Abstract

Acute myeloid leukaemia (AML) is a heterogeneous, most common type of acute leukaemia that involves mutation in haematopoietic and progenitors stem cells (HPSCs) leading to uncontrolled division, self-renewal and differentiation. Though it was untreatable about a half century ago, AML is now considered to be treatable in up to 40% of adults and those who are at or under 60 years of age. For the post-induction treatment, the mutation testing and cytogenetics are still an important prognostic tool. The AML treatment remains unchanged for almost three decades, although the field is advanced with the discovery of new drugs and deep understanding of the disease biology. Still, many people are relapsing and are dying eventually from the disease. This review discusses the broader aspects of AML, reflecting some of the most important and productive areas of research on the subject and was conducted using thoroughly searching databases, including Health Research and Development Information network Plus (HERDIN Plus), google scholar and PubMed to critically analyses the recent advances, available treatments and future prospectives of AML.

Keywords: AML; Pathogenesis; Treatment; Relapse

1. INTRODUCTION

Numerous molecular and cytogenetic alterations characterize a group of heterogeneous hematologic malignancies like AML. Since last decade, basic and translational research drastically expanded our understanding of the pathology and AML genetic diversity. It has molecular abnormality in FMS like tyrosine kinase 3 (*FLT3*) due to certain mutations[1]. *FLT3* has mutations like internal tandem duplications (ITD), accounting for 30% of the mutations in AML patients, which are mostly associated with relapse[2,3]. In tyrosine kinase domain (TKD) activation loop, the *FLT3* mutations predominantly at residue D835, are found in 7% of the patients with uncategorized prognosis[4].

As prognostic and diagnostic markers recurrent chromosomal structural are well-established, which suggest that somatic mutations are having preferred role in pathogenesis[5]. Approximately 45% of the AML patients

have normal karyotypes, and structural abnormalities are lacking in many of these genomes evaluated with single nucleotide polymorphism array and high density comparative genomic hybridization[6,7]. Repeated mutations in *TET2, FLT3, CEBPA, KIT* and *NPM1* are identified by target sequencing[8] similarly in IDH1 and DNMT3A it is discovered by massively parallel sequencing[9,10].

It has been reported in 2011 that mutations are mostly not carried by AML patients, in any of currently recognized driver genes associated with pathogenesis of AML[11]. Patients associated with favourable risks of their cytogenetic profile i.e. those with *MYH11-CBFB, RUNX1-RUNX1T1* or *PMLRARA* fusions have good outcomes with chemotherapy-based consolidation regimens, while allogenic transplantation is required for the patients having unfavourable-risk profile, such as complex alterations or monosomy karyotype, in order to improve their prognosis in the first remission[12]. Though intermediate cytogenetic risks are mostly found in majority of AML patients, chemotherapeutic consolidation is enough for some of the patients, while others have poor outcomes. A better classification of intermediate risk is of prime concern these days to establish new biomarkers in this regard[13]. *KIT, FLT3, CEBPA* and NPMI are incorporated into standard of care testing by new algorithm classification. For the patients with intermediate risk profile, the prognostic information may be provided in mutations of novel AML genes i.e. *IDH1/2, TET2* and *DNMT3A*[8]. All existing classification schemes are not completely accurate, hence thorough study of epigenetics and genetic changes are relevant in understanding AML pathogenesis, and are required in better therapy approaches and classification, French American British (FAB) and World Health Organization (WHO) classification of AML is given in **Fig. 1** and **Fig. 2** respectively.
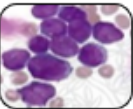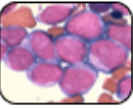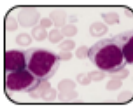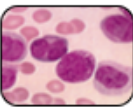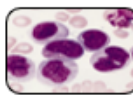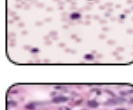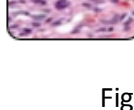

FAB CLASSIFICATION

M0: Undifferentiated acute myeloblastic leukemia (5%)

M1: Greater number of myeloblasts with <10% granulocytic differentiation.

M2: Myeloblasts in great number with granulocytic differentiation >10% , NSE <20%.

M3: Promyelocytes that are hyper granular with many Auer rods on CAE or Wright-stain and variant form cells with reniform nuclei, multilobed or bibbed, primeval cells with multiple Auer rods or relative scarcity of Hypergranular promyelocytes.

M4: >20% but <80% NSE-butyrate positivity in Monocytic cells

M5: Monocytic cells with >80% NSE positivity. (a) Monocytic differentiated (b) Monocytic, differentiated.

M6: >30% myeloblasts with more than 50% erythroblasts eliminating the erythroid cells.

M7: Acute megakaryoblastic leukemia <5%

Fig. 1. FAB classification of Acute Myeloid Leukemia

Fig. 2 WHO classification of AML

2. DIAGNOSIS

Aspiration from bone marrow is a widely prescribed method for diagnosing AML, although it can also be performed by analysing peripheral blood. Bone marrow trephine biopsy is a must if the aspirate is inspirable, dilute or hypocellular. If biopsy and aspiration samples are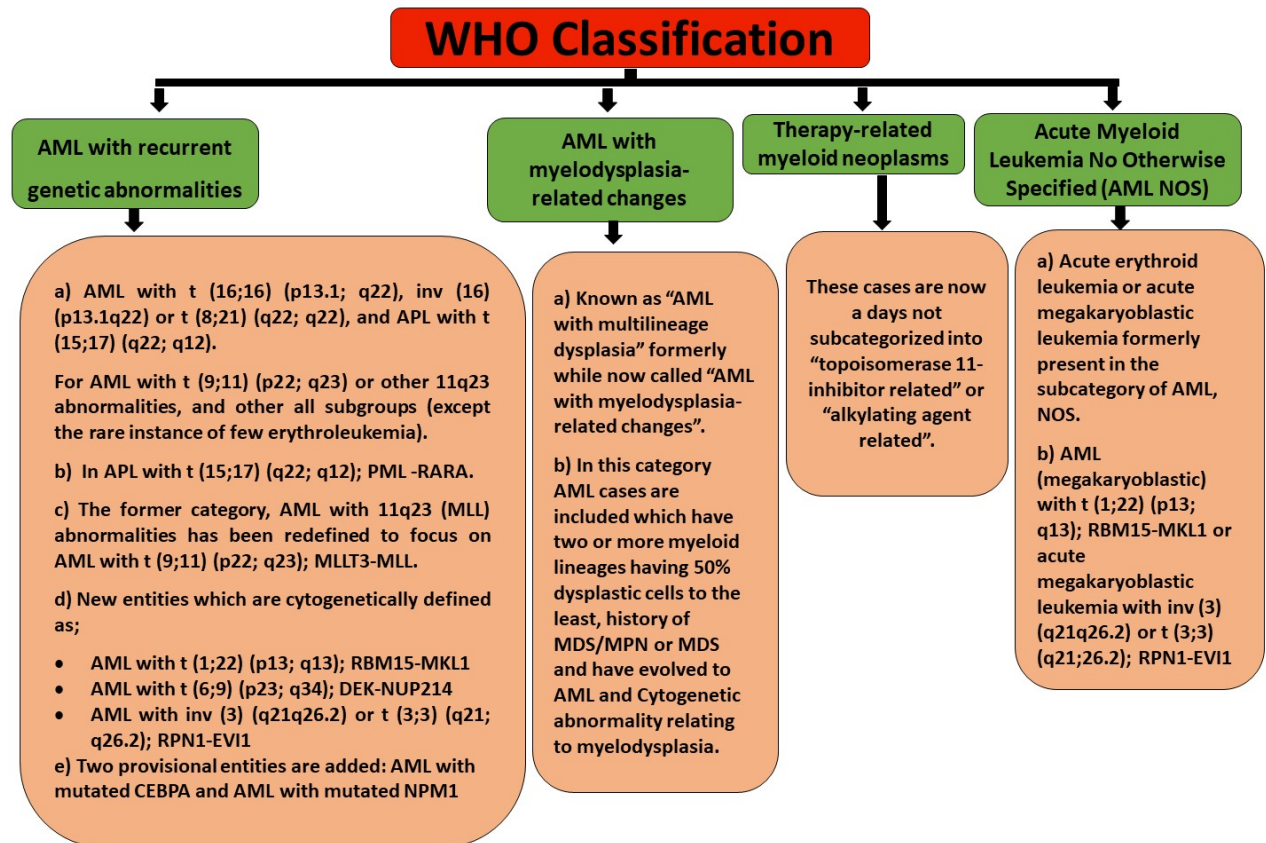 inadequate, then it might be useful to locate clot sections. Studying morphology, cytogenetics, immunophenotyping, molecular genetics and molecular cytogenetics and genome-wide studies are among the main diagnostic procedures.

2.1. Morphology

The bone marrow aspirate is used for the diagnosis of suspected AML patient. Biopsy of marrow trephine is done by dry tapping (punctio sicca). Contrary to this, for morphological examination, bone marrow and blood smears are made following wright-Giemsa staining. It is suggested that the normal nucleated cells count is 500 on marrow smear and 200 leukocytes cells on blood smear. 20% of marrow or blood blast is least count for diagnosis of AML, except few cases of erythroleukemia and AML with t (16;16), inv(16), t (8;21) t(15;17). Megakaryoblasts, myoblasts and monoblasts are included in blast count. In AML characterization, myelomonocytic or monocytic differentiation, promonocytes and monoblasts are usually equivalent to blast with exception of abnormal monocytes. Erythroblasts in erythroid leukemia are counted as blasts in very rare cases. Identification of lineage is based on cytochemistry in most countries rather than using non-specific esterase (NSE) strain, myeloperoxidase (MPO), sudden black B (SBB) and immunophenotyping. In the myeloid lineage, the hallmark enzyme is MPO, and immunohistochemistry, flow cytometry and cytochemical staining can detect it. If more than 3% of blast cells are reported to be cytochemically positive for MPO, the diagnosis of AML is clear, but its absence does not rule out a myeloid lineage because MPO may not be present in

primary monoblasts and myeloblast. MPO is more specific and equivalent to SBB staining. In monocytes and monoblasts, the activity of diffuse cytoplasm is shown in stains of NSE[6].

2.2. Immunophenotyping

In newly diagnosed acute leukemia, lineage is determined by immunophenotyping using flow cytometry[14]. There is no consensus on the cut-off point for a positive marker of acute leukemia, expression of markers by 20% or more of leukemic cells is a criterion commonly used for markers mostly[15], while lower cut-off (10%) is applied for highlighted markers which are CD34, CD3, MPO, TdT and CD117. Blast count cannot be used for morphologic evaluation as substitute of flow cytometry[16]. Immunotyping is used in the diagnostics of AML with minimal differentiation. It is important for the diagnosis of minimally differentiated AML, acute ambiguous lineage leukemia, and acute megakaryoblastic leukemia. Early hematopoiesis-associated antigens which are HLA-DR, CD38 and CD34 are expressed in most cases and has no monocytic or myeloid maturation markers, while cytochemistry shows no MPO. MPO antigens can be positive in at least a fraction of blasts using flow cytometry. In acute megakaryoblastic leukemia, twenty percent or more blasts are present, of which 50 percent or more are of megakaryocytic lineage; megakaryoblasts typically express one or more CD61 or/and CD41 platelet glycoproteins and less generally, CD42. Rare leukemia is an ambiguous lineage of acute leukemia, comprised of cases that show no signs of lineage differentiation i.e, acute undifferentiated leukemia (AULs) or those cases contain blasts representing markers of two or more lineages mixed phenotype acute leukemia (MPAL). AULs lack lineage-associated markers, but they frequently express HLA-DR, CD34, and/or CD38. MPAL can contain either one blast population with different lineage markers on the same cell, or separate blast populations with different lineages, or a combination. With or without an underlying genetic abnormality, many subsets are covered by MPAL. BCR-ABL1 positive acute leukemia may present as MPAL, immunophenotypically. This leukemia should be treated as an ALL with chemotherapy containing an ABL1 tyrosine kinase inhibitor, but not as an AML[17,18].

2.3. Cytogenetics

In suspected patients of AML, conventional cytogenetic analysis is done for diagnoses. In about 55% cases of AML in adults, there are chromosomal abnormalities[19]. In Seven recurrent inversions, balanced translocations and their variants, are reported in the WHO group of "AML with recurrent genetic abnormalities". In addition, multiple cytogenetic abnormalities are considered necessary to assess the WHO diagnosis of "AML with myelodysplasia-related characteristics" when 20% or more marrow or blood blasts are present. The chromosomes condense and become distinguishable during the metaphase of mitosis or meiosis when they align at the centre of the dividing cell, this phase chromosomes can be used for karyotyping.

2.4. Molecular cytogenetics

Fluorescence in-situ hybridization (FISH) can be used to identify the loss of 7q and 5q chromosome material, gene fusions or gene reshuffling, such as *RUNX1, RUNX1T1, CBFB-MYH11, MLL* and *EVI1*, acetic acid/methanol cell pellets fixed by acid should be kept earlier if cytogenetic analysis fails[20].

2.5. Molecular genetics

Fusion transcript (chimeric RNA) can be used for molecular screening of associated mutation and genes related to Leukemia. RNA can be extracted preferentially if the number of cells is less however, RNA and DNA are used in ideal situation with the storage of viable cells. In certain conditions, reverse transcriptase-Polymerase Chain Reaction (RT-PCR) for the reoccurring fusion genes, such as *MLLT3-MLL, CBFB-MYH11, RUNX1RUNX1T1,* and *DEK-NUP214* are useful for molecular diagnosis. The frequency of these gene mutations among cytogenetic groups varies[13]. AML with CEBPA or NPM1 mutations have been added as provisional entities in the WHO classification. Screening for these two markers and for FLT3 mutations should be performed in clinical trials. Although testing for *NPM1, FLT3* and *CEBPA* outside trials is presently not considered compulsory, these three mutations should be checked in cytogenetically normal AML (CN-AML) patients who will get treatment other than best supportive care as or low-dose chemotherapy as per panel recommendations

2.6. Genome-wide studies

The current genomic studies have investigated novel genetic abnormalities, making it easy to systemically characterize cancer genomes[21]. The discovery of novel leukemic sub-categories and prognostic signatures included micro-RNA and gene expression profiling[22]. In AML pathogenesis novel mechanism involved, i.e., Uniparental Disomy (UPD), genome-wide single nucleotide polymorphism (SNP)-based mapping arrays were established, providing both copy number and allele-specific details[23]. A mitotic recombination event results in acquired UPD, in the affected genomic region of pre-existing mutation, cause a homozygous cell. Numerous current studies present the SNP genotyping impact, as a method for gene discovery[24]. While genomic copy number analyses may continue to be helpful in selecting candidate leukemia genes, high-throughput DNA sequence analysis is now expected to be possible at an affordable cost, which may eventually lead to the development of comprehensive oncogene mutation profiling strategies specific to the disease and allele[10]. Finally, functional genetic methods, such as large-scale RNA interference screens, have infinite potential for detecting novel cancer genes. For example, as in a recent study RNA interference was used for identification of RPS14 by grading down-regulation of multiple candidate genes; for the MDS 5q syndrome fundamental gene[25].

3. BIOBANKING

In clinical trials, it is strongly recommended to store patients' leukemic blood and marrow during pre-treatment. The informed consent of the patient is the requirement for biobanking, this consent enables a wide range of correlative laboratory studies that often involve DNA analysis. Viable cells (stored at -196°C liquid nitrogen) and nucleic acid (RNA and DNA should be stored at -80°C) are included in pre-treatment samples. Optional storage can be carried out for germline DNA (e.g., skin biopsy, sputum or from a buccal swab), sample of plasma, methanol acetic acid-fixed cell pellet (cytogenetic), and after treatment of complete remission (CR) the frozen cells (at MRD monitoring and relapse at defined time of before and during follow-up and treatment), proper conditions are needed for storage. Complete count of blood and Cytogenetics, Bone marrow aspirate, Bone marrow trephine biopsy, differential count, immunophenotyping, *CBFB-MYH11*, *RUNX1-RUNX1*T1, *PML-RARA* are included in further tests in the start of investigation and work-up AML patients, other procedures are medical history and demographics performance status (ECOG/WHO score). Biochemical and coagulation tests, analysis of comorbidities, eligibility assessment for allogeneic HSCT, lumber puncture and assessment through prognostic marker includes *CEBPA*, *NPM1, FLT3* gene mutation, *RUNX1*, MLL, WT1, RAS, *KIT,* TP53, *TET2* , ERG, IDH1 gene mutation, EVI1, MN1, BAALC gene expression and the detection of minimal residue diseases[13].

4. AML1-*CBFb* (CORE-BINDING FACTOR SUBUNIT Beta)

AML-associated t(8;21) translocation cloning resulted in the identification of AML1/ETO, which codes the AML1-CBFb DNA-binding subunit, a transcription factor that is essential for normal hematopoietic system development and that regulates a number of haematopoiesis-specific genes. In 40 percent of FAB subtype M2 AML patients, t(8;21) translocation is present but this is just not common only in this subtype. The resultant fusion gene joins; AML1 N- terminal and DNA-binding and *CBFB* -interaction domains, with C-terminal of 8;21 translocation gene (ETO) on 8 no chromosome[26]. Contrary, the resultant protein binds easily. Target sequences regulated by AML1 suppress AML1 mediated activation but not activate transcription[27]. The ETO interaction with the nuclear co-repressor complex represses transcription. In FAB subtype M4Eo AML patients, the t (16;16) (p13; q22) and inv(16) (p13; q22) mutations are mainly (but not only) seen[28,29]. In these the myosin smooth-muscle heavy-chain gene *MYH11* on 16p13 chromosome is fused with the core binding-factor complex on 16q22 chromosome of *CBFb* subunit[30].

CBFb-MYH11 chimeric product form when *CBFb* N-terminal portion, with AML1-interaction domain, is fused to C-terminal domain of *MYH11*. It suppresses AML1-mediated transcriptional activation, converts AML1 into functionally inactive complexes within cytoplasm[31].

5. TREATMENT

AML treatment aims to prevent relapse and induce remission, where remission is less than 5% bone marrow blasts along with peripheral blood count recovery. More sensitive immunological and molecular genetic

techniques are now present, that should be able to accurately characterise remission status; while clinically, they have not yet been tremendously validated. Induction and post-induction are the two phases of treatment. In the past few years, alternative agents prospective randomized trials have proved that idarubicin or mitoxantrone show more efficacy in younger patients than daunorubicin, but both have resulted in more extended cytopenia, in addition to all these therapies, metallic nanoparticles have potential anticancer acitivity[31,32]. In induction therapy, personalized treatment produced relevant advantages, therefore, prospective stratification might include properties of AML. Nuclear receptor family 4A (NR4A) are tumour suppressors silenced in AML. Dihydroergotamine displays AML selective NR4Ad-dependent antileukemic affect in cytogenetically distinct human AML cells and delays progression in mice[33]. Sub-family of NR4A consists of three transcription factors that regulate apoptosis, inflammation, mitogenesis, genotoxicity and hematopoiesis[34]. Antibody P67.6 showed that a hydrolytic release site was needed for selectivity in tissue culture and for efficacy in athymic xenograft models[35]. A P67.6-NAc-gamma calicheamicin 1,2 Dimethyl hydrazine (DMH) conjugate[36] showed specific and strong antileukemic affect in AML models. To minimize its potential for immune response in human trials, antibody P67.6 has undergone complementary determining regions-based humanization. More powerful redesign of this conjugate is gemtuzumab ozogamicin, which has been approved by FDA to treat relapsed AML. Randomized trials have indicated that among young patients mitoxantrone or idarubicin have better activity than daunorubicin[36]. To determine whether equal doses were used[37], studies comparing mitoxantrone and idarubicin are ongoing. Allogenic bone marrow transplant from an HLA-matched sibling is the best treatment[38] because risk of relapse is less than 20%, 45-55% survival rate has been reported for myeloablative treatment[39].

6. RELAPSE

As part of a clonal evolution of one patient, genetically distinct AML clones exist, evolve and are directly responsible for diagnosis or possible relapse due to a presumed choice of chemotherapy. According to the National Cancer Institute guidelines, the concept of relapse is more than 5% of blasts in aspirates of bone marrow or the occurrence of extramedullary leukemia in patients with reported CR in past.[41]. Mostly relapse occur within 1-3 years of treatment[42]. The period from CR or relapse or death, irrespective of the cause, was defined as disease free survival.

Children who experience bone marrow allogenic transplantation had a decreased risk of relapse, However, this was neutralized by a high risk of mortality, so there was no change in disease-free survival and overall survival[43]. Similarly designed U.S collaborative trial found no better results for disease free survival[44]. In all these trials, only a few patients in CR have undergone transplant because of the relapse, which occur either due to residual disease or if autograph is contaminated with leukemic cells. Gene-marking studies have shown that autograph enhances relapse chances and to get rid of the contaminated cells ex vivo, no efficient technique is available[45].

Age, remission period and cytogenetics determine course of action after treatment failure[46]. Patients with t (15;17), t (8;21), or inv (16), if undergo relapse after a year in remission, have 20% survival chance with succeeding therapy. In all other groups, first relapse must be prevented because of the bad treatment response. For children and young adults with first relapse or no response to first-line induction therapy, HSC transplant after marrow ablation is suggested. Bone marrow transplant immediately after relapse or chemotherapy is comparable[40] but arranging a transplant is a challenge. A few patients cannot attain second remission, so marrow transplant is not considered for them. In second remission, autologous transplant or HLA matched allogenic transplant leads to 30% survival rate, however, the knowledge about HLA partially matched related donors or matched unrelated donors is still not that much present[47].

7. CONCLUSIONS

Although acute myeloid leukemia is an occasional disease, it is the malignant neoplasia for many cancer-related deaths. Molecular pathogenesis is studied using cytogenetic analysis of AML for approximately more than three decades. AML is the most common type of leukemia in adults, but all leukemias have a low survival rate. Although treatment outcomes in adults have consistently improved over the past 20 years, safety changes remain limited in the elderly. AML needs special attention in its treatment, novel compounds should be synthesized, decrease in relapse and increase of disease-free survival time needs prior surveillance.
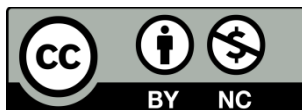
CONFLICT OF INTEREST

No conflict of interest

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