INTRODUCTION

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1. Acute myeloid leukemia

Acute myeloid leukemia (AML) refers to a group of marrow-based neoplasms that have clinical similarities and distinct morphologic, immunophenotypic, cytogenetic, and molecular features[1,2].

AML is a clonal, malignant disease of hematopoietic tissue that is characterized by accumulation of abnormal (leukemic) blast cells, principally in the marrow, replacing normal marrow progenitors, with consequent diminished production of red cells, white cells, and platelets[3]. This, in turn, leads to the common clinical manifestations of AML-namely, anemia, infection, and bleeding. As the disease progresses, leukemic blasts pour into the bloodstream, leading to the "weisses blut" described by Virchow in 1845. Eventually, the leukemic cells accumulate in the spleen, lung, brain, and other vital organs[4].

1.1. Epidemiology

1.1.1. Incidence

In adults, AML accounts for 80 to 90% of the cases of acute leukemias[2] and for approximately 25% of all leukemias in the Western world, and therefore is the most frequent form of leukemia[5,6]. Incidence rates are greater in developed countries and in industrialized cities. Worldwide, it is highest in the U.S., Australia, and western Europe[7]. AML has an estimated incidence between 1.6 and 3.7 per 100,000 per year in the U.S. and Western Europe[8]. It is 2-3 per 100,000 persons per year in children, rising to 15/100,000 in older adults with peak incidence in the seventh decade[9], reaching 17 per 100,000 for persons 75 years of age[2]. Age-adjusted incidence is higher in men than in women (4.6 per 100,000 persons versus 3.0)[10] and in whites than in blacks[11].

In Egypt, AML represents 1.9% of all new cancer cases, with a median age of 22 years and a male/female ratio of 1.37 (Figure 1). Among children less than 20 years of age, AML represents 8% of all leukemia cases, with a median age of 8.5 years and a male/female ratio of 1.41[12].

1.1.2. Mortality

If left untreated, AML is rapidly fatal, with most patients dying within a few months of diagnosis[4]. 5-Year survival is only 20%–30%[6] and age-dependent mortality is 2.7 to nearly 18 per 100,000 persons[5]. Although it is possible to support patients for a certain period (median survival, 11–20 weeks)[13], they ultimately die of the leading complications associated with bone marrow failure (i.e., infection and hemorrhage). Some patients are not candidates for cytotoxic therapy, mainly because of older age and/or poor performance status or other active severe medical comorbidities that complicate their care. In such settings, a supportive strategy may be most appropriate[14].

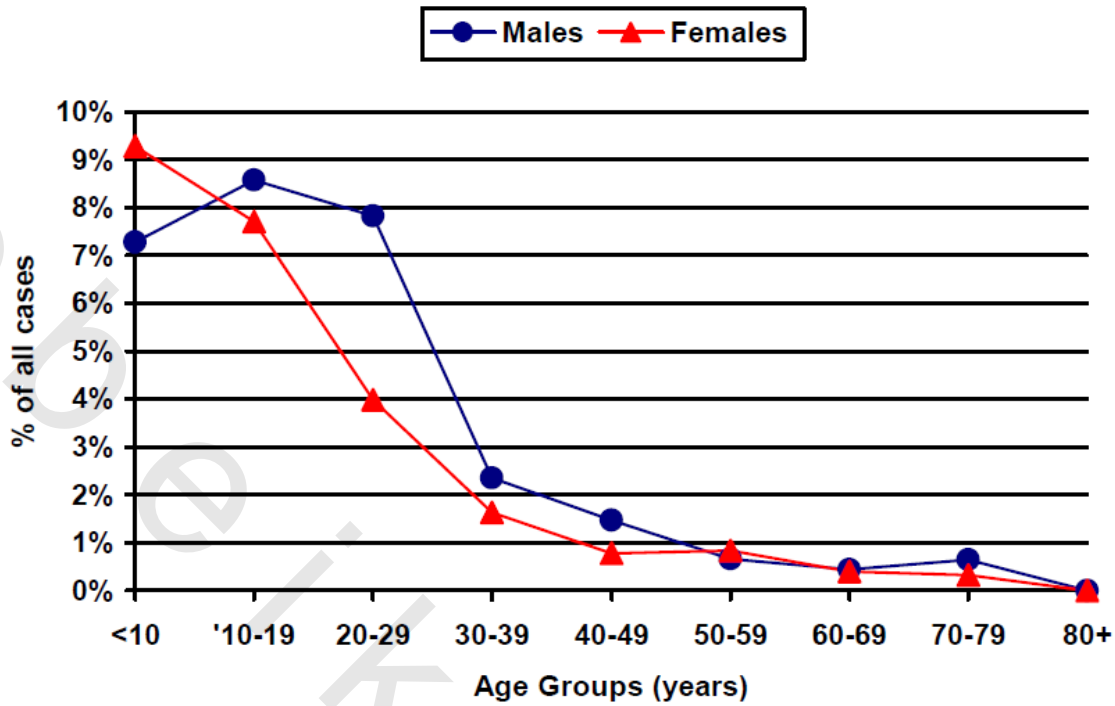

Figure 1: Age specific frequency of acute myeloid leukemia by gender in Egypt (NCI 2002-2003) [12]

As is the case with incidence, the mortality associated with AML varies with age, gender, and race. Age adjusted mortality rates in the U.S increase with age, showing its peak at 17.6 per 100,000 persons in people aged 80 to 84 years[11]. The mortality rate for males is higher than that for females, with the U.S. age-adjusted mortality rate at 3.5 per 100,000 for males and 2.2 per 100,000 for females (2000-2003). AML mortality is greater in whites than in blacks, the U.S. age-adjusted mortality rate was 2.7 per 100,000 for whites and 2.2 per 100,000 for blacks in the year 2000[5,15].

1.1.3. Survival

The overall U.S. survival rate associated with AML from 1996 to 2002 was 21.7%[16]. The 5-year relative survival rate was highest for those who were younger and female. 5-Year survival rate of those age ≤ 55 years was 23%, whereas the corresponding rate for those age >55 years was 11%, while blacks had a slightly better 5-year survival rate than whites (21.5% vs. 19.8%)[5,11].

Survival has improved for young patients[17], but little progress has been made in the elderly. Reasons for worse survival in older patients include a higher percentage of patients with adverse cytogenetics (complex karyotypes and abnormalities of chromosomes 5 and 7) and a higher percentage of cases in which leukemia cells express the multidrug resistance protein. Additionally, older patients tend to have comorbid illnesses and a poor performance status, which contribute to early mortality[2].

1.2. Etiology and pathogenesis

There is considerable heterogeneity between cases of AML as regards morphology, immunological phenotype, associated cytogenetic and molecular abnormalities, and patterns of gene expression[9]. While most cases of AML arise de novo without objectifiable leukemogenic exposure[5], there is an association with irradiation, smoking, some rare

congenital abnormalities, chemical exposure and obesity. Perhaps the most frequently identified cause is progression from other myeloproliferative disorders, e.g. myelodysplasia, or as a consequence of prior chemotherapy for another malignancy[9]. No direct evidence suggests a viral etiology[10]. Myelodysplastic syndromes (MDS) are disorders of bone marrow characterized by hypercellularity, dysplastic maturation, and an excess number of blasts. They may be accompanied by anemia, neutropenia, or thrombocytopenia. A high percentage of these cases progress to AML[1]. The exact risk depends on the type of MDS[18].

1.2.1. Genetic factors

AML results from a series of somatic mutations in either a hematopoietic multipotential cell or, occasionally, a more differentiated, lineage-restricted progenitor cell[19]. Some cases of monocytic leukemia, promyelocytic leukemia, and AML in younger individuals more likely arise in a progenitor cell with lineage restrictions (progenitor cell leukemia)[20,21]. Other morphologic phenotypes and older patients likely have disease that originates in a primitive multipotential cell. In the latter case, all blood cell lineages can be derived from the leukemic stem cell because it retains the ability for some degree of differentiation and maturation[3].

Somatic mutation results from a chromosomal translocation in the majority of patients[22]. The translocation results in rearrangement of a critical region of a proto-oncogene. Fusion of portions of two genes usually does not prevent the processes of transcription and translation; thus, the fusion gene encodes a fusion protein that, because of its abnormal structure, disrupts a normal cell pathway and predisposes to a malignant transformation of the cell. The mutant protein product often is a transcription factor or an element in the transcription pathway that disrupts the regulatory sequences controlling growth rate or survival of blood cell progenitors and their differentiation and maturation[23,24].

A multistep series of mutations are needed to produce acute myeloid leukemia. Leukemogenesis needs, at a minimum, activating mutations in class 1 genes that stimulate signal transduction pathways and induce cellular proliferation, in conjunction with mutations in class 2 genes that affect transcription factors and compromise normal differentiation[25,26]. Mutations leading to activation of the receptor tyrosine kinase *FLT3*, *KIT*, and *RAS* signaling pathway belong to class 1 mutations. *RUNX1/ETO*, *CBFB/MYH11*, and *PML/RARA*, which are fusion transcripts generated by well known recurring chromosomal abnormalities such as t(8;21), inv(16), and t(15;17), respectively, are examples of class 2 mutations[27,28]. Support for this two-hit model comes from demonstration that class 1 and class 2 lesions occur together more commonly than do two class 1 or two class 2 lesions[29,30]. A third class of genes encoding epigenetic modifiers, including, but not limited to, *DNMT3A*, *IDH1*, *IDH2*, *TET2*, *ASXL1*, and *EZH2*, have a major role in pathogenesis, associated with a worse patient outcome and are frequent in older patients[31].

Genetic factors are implicated in the pathogenesis of AML by virtue of its high incidence in patients with syndromes characterized by chromosomal abnormalities or instability, or defective DNA repair[2]. Certain syndromes with somatic cell chromosome aneuploidy, such as trisomy 21 noted in Down's syndrome, are associated with an increased incidence of M7 AML (acute megakaryoblastic leukemia) in early childhood[10]. Childhood monosomy 7 is a unique MDS syndrome of childhood that overlaps with juvenile myelomonocytic leukemia. A rare form of familial monosomy 7 can present in adults[2]. Trisomy 8 mosaicism is a rare constitutional abnormality with features of mental retardation, multiple developmental defects, and an increased incidence of AML[4]. AML also occurs rarely in association with Klinefelter (XXY) and Turner (XO) syndromes[2]. Deletions of all or part of a chromosome

(e.g., chromosome 5, 7, or 9) are common cytogenetic abnormalities. Deletions in chromosomes 5 and 7 and complex cytogenetic abnormalities are increased in frequency in older patients and cases of AML following cytotoxic therapy compared to de novo cases[32]. Because the genes residing on the undeleted homologous segment of chromosome 5 are not mutated, an epigenetic lesion, such as hypermethylation of a gene allelic to one on the deleted segment on chromosome 5, may result in the leukemogenic event[3].

Several DNA-repair syndromes are associated with an increased incidence of AML. Bloom's syndrome is characterized, in 25% of patients, by hematologic malignancies including AML[33]. Almost 50% of patients with Fanconi's anemia develop myelodysplasia or AML by the age of 40 if death from other causes does not occur first[34]. The tumor suppressor gene syndromes Li-Fraumeni syndrome and neurofibromatosis are associated with a higher risk of AML[4].

Several congenital cytopenia syndromes are linked to increased incidence of AML: Blackfan-Diamond syndrome is characterized by a definite increase in AML. Severe congenital neutropenia, also called Kostmann's syndrome, has a 10 to 15% risk of evolving to AML after 8 years of therapy. With long follow-up, more than half of patients with Schwachman-Diamond syndrome developed MDS/AML. Familial leukemia is probably caused by one or both of two factors: a genetic predisposition within a family and/or a common environmental exposure[2,4]. Familial platelet disorder (FPD)/AML is characterized by a 30 to 35% predisposition to develop myeloid malignancies[35]. Another familial leukemia has been associated with 16q22 abnormalities involving the *PEBP2B/CBFB* gene[36]. Germ-line mutations of CCAAT/enhancer-binding protein α (*CEBPA*), runt-related transcription factor 1 (*RUNX1*), and tumor protein p53 (*TP53*) have also been associated with a higher predisposition to AML[10].

1.2.2. Environmental and chemical factors

A vast variety of environmental and chemical exposures are assumed to be associated with a variably elevated risk of developing AML in adults. Exposure to ionizing radiation is linked to AML. Among survivors of the atomic bomb explosions in Japan, an increased incidence of AML was observed, with a peak at 5 to 7 years after exposure[37]. Moreover, therapeutic radiation has been found to increase the risk of secondary AML[5].

Exposure to benzene, a solvent used in the chemical, plastic, rubber, and pharmaceutical industries, is associated with an increased incidence of AML[10]. Toxicity is related to cumulative dosage, and the risk of leukemia was high before safety controls were put into effect in the workplace[2]. In surveys of factories in China, the leukemogenic risk was four to seven times higher in workers exposed to benzene than in the general population, and the average latency was 11.4 years. A dose-response pattern was suggested, with the highest risk in Chinese workers exposed at constant levels of 25 ppm or higher[38].

Smoking has been weakly associated with leukemia[39]. It was found to confer an average 1.2 - 2.3 times increase in the incidence of AML, particularly of FAB subtype M2, with the greatest risk in current smokers[40]. Tobacco smoke contains potentially leukemogenic chemicals, including benzene, urethane, nitrosamines, and radioactive compounds[2].

The number of people exposed to environmental causes of AML probably far exceeds the number who develops the disease. Development of AML after exposure may reflect genetic variation in enzymes that detoxify benzene and other carcinogens, such as NAD(P)H dehydrogenase, quinone 1 (NQO1)[41]. A single 609C→T substitution in *NQO1*, lowers

NQO1 activity; if two alleles are mutant, enzyme activity is absent[42]. Reduced NQO1 activity was 10.47 times more common among patients with de-novo AML[43]. Members of the cytochrome P450 family are also involved in detoxification. Allelic variants of *CYP1A1* are no more common in AML than in the general population, but the *CYP1A1*2B* (Val) allele is 15.9 times more common in patients with -5/-7 and RAS mutations than in other patients with AML[44].

Chemotherapeutic drugs, such as alkylating agents and topoisomerase II inhibitors, have been reported to increase the incidence of AML[5]. Up to 10–15% of patients with AML develop the disorder after treatment with cytotoxic chemotherapy (usually for a solid cancer)[30]. Alkylating agent-associated leukemias occur on average 4-6 years after exposure, and affected individuals have aberrations in chromosomes 5 or 7. Topoisomerase II inhibitor–associated leukemias occur 1–3 years after exposure, and affected individuals often have aberrations involving long arm of chromosome 11 (11q)[10]. Antibiotics, including chloramphenicol, and analgesic agents, including phenylbutazone and derivatives such as sulfinpyrazone, have been associated with marrow suppression, aplastic anemia, agranulocytosis, and, more rarely, AML[2]. Although radiation therapy is less leukemogenic than chemotherapy, leukemia has followed radiation and radioisotope therapy given for a variety of cancers[45]. A 2-fold increase in risk (latency period of 2 to 11 years) has been reported in breast cancer patients receiving adjuvant radiotherapy compared to a 10-fold increase in risk after chemotherapy; combined radiation and chemotherapy resulted in a 17-fold risk[46].

1.3. Pathophysiology

The most common cause of death in AML is bone-marrow failure. The genetic reprogramming of AML blasts renders them ineffective at generating mature red cells, neutrophils, monocytes, and platelets. AML blasts also inhibit normal blasts from differentiating into mature progeny[30]. How growing leukemic clones suppress normal polyclonal residual hematopoiesis is poorly understood[28], but inhibition does not result from "crowding out" of normal blasts because there is no correlation between degree of cytopenia and marrow blast count; rather inhibition may be mediated by various chemokines produced by AML blasts[47].

The principal sign of marrow failure in AML is infection. The range of pathogens is largely limited to common endogenous aerobic gram-positive and gram-negative bacteria, endogenous *Candida* species, and, particularly in warm, humid climates, *Aspergillus* species, which are water-borne or enter via the respiratory tract[48]. Studies of various antibiotics indicate that the main predictor of successful treatment is an increasing neutrophil count. However, cytotoxic chemotherapy, the treatment most often used to effect such a rise, temporarily increases the risk of infection because it damages gastrointestinal mucosa[30].

Potentially fatal organ infiltration, most ominously involving the lung and brain, becomes more likely as the white-blood-cell count rises above 50,000, particularly in the monocytic subtype of AML or if blasts are positive for CD56 surface antigen.[49] In these situations, emergency cytotoxic chemotherapy is needed, although its use is often associated with acute respiratory distress syndrome and tumor lysis syndrome[30].

1.4. Classification systems:

The rapid evolution of classification schemes for AML has resulted from several successive waves of new technology entering the clinical hematology laboratory and being validated in clinical trials. The French-American-British (FAB) classification of 1976 and its later modifications (Table 1) primarily relied upon morphologic examination, cytochemical tests, and limited immunophenotyping to establish lineage-related classification of myeloid blasts[50].

Table 1: French-American-British classification of acute myeloid leukemia[4,10]

Subtype	**Definition**	**Incidence**
M0: AML with minimal differentiation	≥30% blasts <3% myeloperoxidase-positive Myeloid antigen expression	5%
M1: AML without maturation	30% blasts ≥3% myeloperoxidase-positive <10% cells mature beyond blast stage	20%
M2: AML with maturation	>30% blasts ≥3% myeloperoxidase-positive >10% myeloid cells mature beyond blast stage	30%
M3: Acute promyelocytic leukemia (APL)	>30% blasts plus hypergranular promyelocytes Intense myeloperoxidase positivity	10%
M4: Acute myelomonocytic leukemia (AMMoL)	Monocytosis >30% myeloblasts + monoblasts + promonocytes >20% myeloperoxidase-positive >20% nonspecific esterase-positive	20%
M5: Acute monoblastic leukemia (AMoL)	>30% myeloblasts + monoblasts + promonocytes <20% myeloperoxidase-positive >80% nonspecific esterase-positive	10%
M6: Acute erythroid leukemia	≥30% of nonerythroid cells are myeloblasts >50% erythroid elements	4%
M7: Acute megakaryocytic leukemia (AMgL)	>30% blasts (myeloblasts + megakaryoblasts) >30% megakaryocytic elements defined by immunophenotyping or electron microscopy	1%

In the years that followed, additional data including recognition of common cytogenetic abnormalities, clinical history and correlation with clinical outcomes led to the World Health Organization (WHO) classification scheme in 2001, with distinct AML categories defined by: 1) recurrent cytogenetic abnormalities; 2) presence of myelodysplastic changes; 3) history of methylating agent or topoisomerase 2 inhibitor chemotherapy drug exposure, or 4) a ‘not

otherwise specified' category encapsulating the FAB morphologic/cytochemical classification[51]. The defining BM blast percentage for AML was decreased from 30% to 20% at this time, largely because myelodysplastic syndrome (MDS) patients with over 20% blasts had been found to have a poor prognosis very similar to those with AML[32]. A revised WHO classification was published in 2008 (Table 2), and builds on the insights of the 2001 system with an increased number of recurrent cytogenetic abnormalities, and the creation of provisional categories for AML cases with mutations in the *NPM1* or *CEBPA* genes[52].

Table 2: World Health Organization classification of acute myeloid leukemia and related precursor neoplasms[10,53]

AML with recurrent genetic abnormalities
AML with t(8;21)(q22;q22); *(RUNX1-RUNX1T1)**
AML with inv(16)(p13.1q22) or t((16;16)(p13.1;q22); *(CBFB-MYH11)**
APL with t(15;17)(q24;q21); *(PML-RARA)**
AML with t(9;11)(p22;q23); *(MLLT3-MLL)*
AML with t(6;9)(p23;q34); *(DEK-NUP214)*
AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *(RPN1-EVI1)*
AML (megakaryoblastic) with t(1;22)(p13;q13); *(RBM15-MKL1)*
Provisional entity: AML with mutated *NPM1*
Provisional entity: AML with mutated *CEBPA*
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms§
Acute myeloid leukemia, not otherwise specified
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Acute erythroid leukemias
Pure erythroid leukemia
Erythroleukemia, erythroid/myeloid
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis
Acute myeloid leukemia associated with Down syndrome
Blastic plasmacytoid dendritic cell neoplasm

* Diagnosis is AML regardless of blast count

§ Cytotoxic agents implicated in therapy-related hematologic neoplasms: alkylating agents; ionizing radiation therapy; topoisomerase II inhibitors; others

The key role of cytogenetic data is further increased by the stipulation that cases with the t(8;21)(q22;q22), inv(16)(p13.1q22), t(16;16)(p13.1;q22), or t(15;17)(q22;q12) need not have >20% blasts to be diagnosed as AML. Additional weight is given to cytogenetic abnormalities in the AML with myelodysplasia-related changes category, where specific MDS-associated cytogenetic findings can take the place of morphologic evidence of myelodysplasia. Two entities found in patients with Down syndrome are now classified separately, and the therapy-related AML category has been simplified to a single entity for patients with history of exposure to any of a broad range of cytotoxic drugs or ionizing radiation therapy.

Adjustments to the criteria for AML with myelodysplasia-related changes, and the addition of blastic plasmacytoid dendritic cell neoplasm as an AML-related precursor neoplasm are the other significant changes from the WHO 2001 scheme[8].

1.5. Methods for diagnosis and classification

1.5.1. Clinical picture:

The initial clinical manifestations of AML usually are nonspecific and relate to the diminished production of normal blood cell. The onset most often is insidious over the course of several weeks to months, and it is not uncommon for a patient to be seen several times before a blood count is finally taken and the diagnosis of leukemia is suspected[4]. Typically, patients present with signs and symptoms of fatigue, hemorrhage, or infections and fever[54]. Pallor and weakness are caused by anemia. Fever is common and is the presenting feature in 15 to 20% of patients; it is often associated with sweats and results from infection secondary to neutropenia or from leukemia itself. Hemorrhagic signs and symptoms, including petechiae, epistaxis, and easy bruising, may be found in up to one half of patients at diagnosis and correlate with the severity of thrombocytopenia or the presence of disseminated intravascular coagulation (DIC). Up to 50% of patients experience weight loss, but it is usually not severe. Bone pain occurs in <20% of patients. Organomegaly and adenopathy have been reported in up to one half of patients with AML[2].

Leukemic blast cells circulate and enter most tissues in small numbers[3]. Leukemic skin infiltration, or leukemia cutis, occurs in up to 13% of patients with AML during the course of the disease and is associated with involvement of other extramedullary sites, including the central nervous system (CNS)[55]. Gum infiltration is also characteristic of acute monocytic leukemia (AMoL). Benign skin lesions associated with AML include Sweet syndrome and pyoderma gangrenosum; these are generally painful and responsive to steroids[56]. Meningeal disease has been reported to develop in 5 to 20% of children[57] and up to 16% of adults with AML. CNS disease is associated with young age (<2 years), hyperleukocytosis, and the AMoL variants[58]. It is often asymptomatic but may be associated with headache or cranial nerve palsies, particularly V and VII. Ocular involvement may result in blindness and suggests meningeal involvement. Intracerebral masses rarely coexist with leukemic meningitis but have been reported in Acute Myelomonocytic Leukemia with eosinophilia (FAB M4Eo) in association with inv(16)(p13q22) and M5 subtypes[59].

1.5.2. Peripheral blood and bone marrow cell count and morphology

Blood and marrow smears are morphologically examined using a May-Grunwald-Giemsa or a Wright-Giemsa stain. For a diagnosis of AML, a marrow or blood blast count of 20% or more is required, except for AML with t(15;17), t(8;21), inv(16) or t(16;16), and some cases of erythroleukemia. Myeloblasts, monoblasts, and megakaryoblasts are included in the blast count. In AML with monocytic or myelomonocytic differentiation, monoblasts and promonocytes, but not abnormal monocytes, are counted as blast equivalents. Erythroblasts are not counted as blasts except in the rare instance of pure erythroid leukemia[60].

Blood counts vary widely among patients with AML. Cytopenias result from hematopoietic failure and contribute to symptoms and signs. Neutropenia is present in most AML patients [2]. The median presenting leukocyte count is ~ 5,000/µl. Between 25% and 40% of patients have counts < 5000/µl, and 20% have counts > 100,000/µl. Fewer than 5% have no detectable leukemic cells in the blood [10]. Anemia is common in AML and is predominantly normochromic and normocytic. Erythroid precursors in the marrow are often

megaloblastic, particularly in acute erythroleukemia or in AML that has evolved from a myelodysplastic syndrome[2]. Thrombocytopenia, which may be severe at diagnosis, can be associated with DIC[2]. Platelet counts <100,000/μl are found at diagnosis in ~75% of patients, and ~25% have counts <25,000/μl. Both morphologic and functional platelet abnormalities can be observed, including large and bizarre shapes with abnormal granulation and inability of platelets to aggregate or adhere normally to one another[10]. Hyperleukocytosis, arbitrarily defined as a blood blast count >100,000/μl, is rare in AML without a monocytic component; it occurs more commonly in cases of AMoL and AMMoL, and is a medical emergency[61,62].

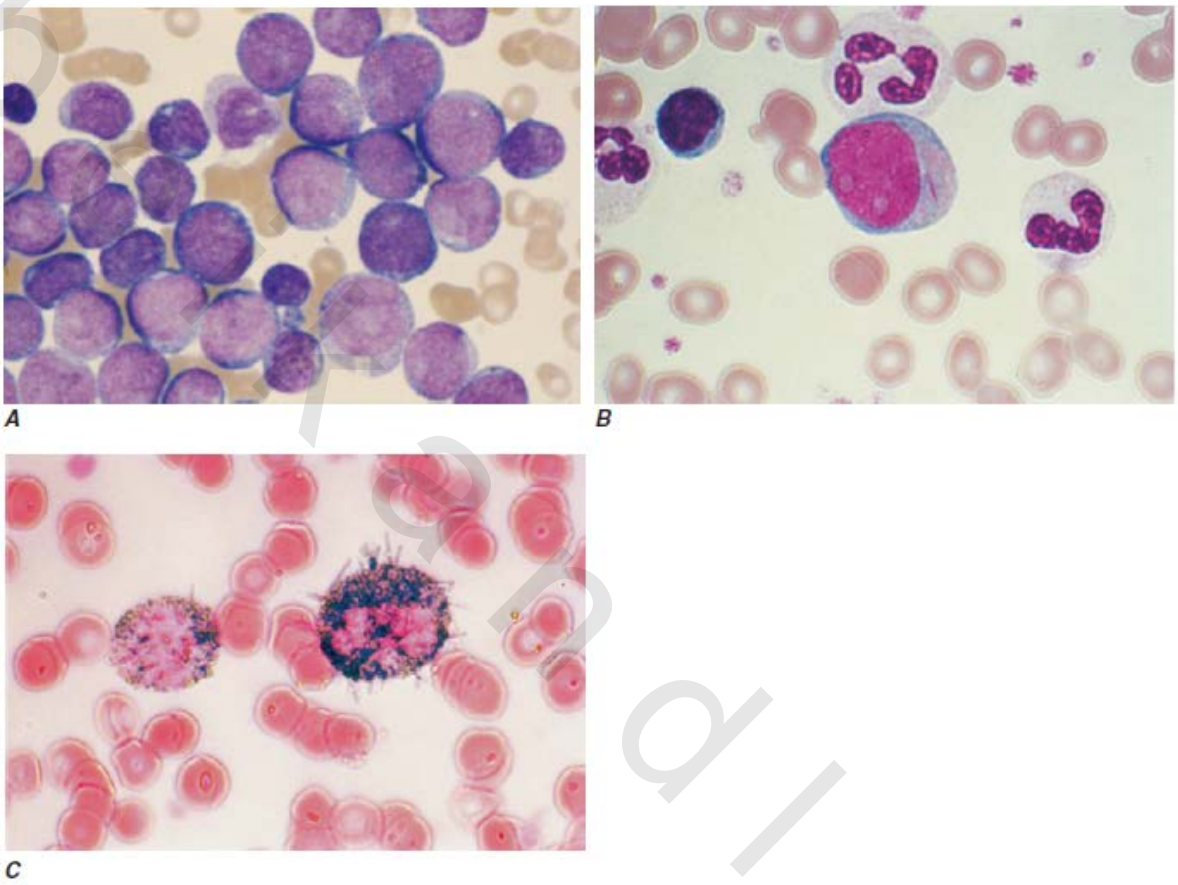

Figure 2: Morphology of AML cells. ***A.*** Uniform population of primitive myeloblasts with immature chromatin, nucleoli in some cells, and primary cytoplasmic granules. ***B.*** Leukemic myeloblast containing an Auer rod. ***C.*** Peroxidase stain shows dark blue color characteristic of peroxidase in granules in AML.[10]

The morphology of the malignant cell varies in difference subsets. In AML the cytoplasm often contains primary (nonspecific) granules, and the nucleus shows fine, lacy chromatin with one or more nucleoli characteristic of immature cells[10]. Auer rods are crystalline cytoplasmic inclusion bodies characteristic of, though not uniformly seen in, all myeloid leukemias (Figure 2)[63].

The appearance of the individual types of AML mirrors the cell type from which they derive (Figure 3). M1 leukemias originate from early myeloid precursors with no apparent maturation toward any terminal myeloid cell type. This is apparent in the lack of granules or other features that mark more mature myeloid cells. M3 leukemias are a neoplasm of promyelocytes, precursors of granulocytes, and M3 cells exhibit abundant azurophilic granules that are typical of normal promyelocytes. M4 leukemias arise from myeloid precursors that can differentiate into granulocytes or monocytes, whereas M5 leukemias

derive from precursors already committed to the monocyte lineage. Therefore, M4 and M5 cells both contain the characteristic folded nucleus and gray cytoplasm of monocytes, whereas M4 cells also contain granules of a granulocytic cytochemical staining pattern. M6 and M7 leukemias cannot be readily identified on morphologic grounds, but immunostaining for erythrocytic proteins is positive in M6 cells, and staining for platelet glycoproteins is apparent in M7 cells[63].

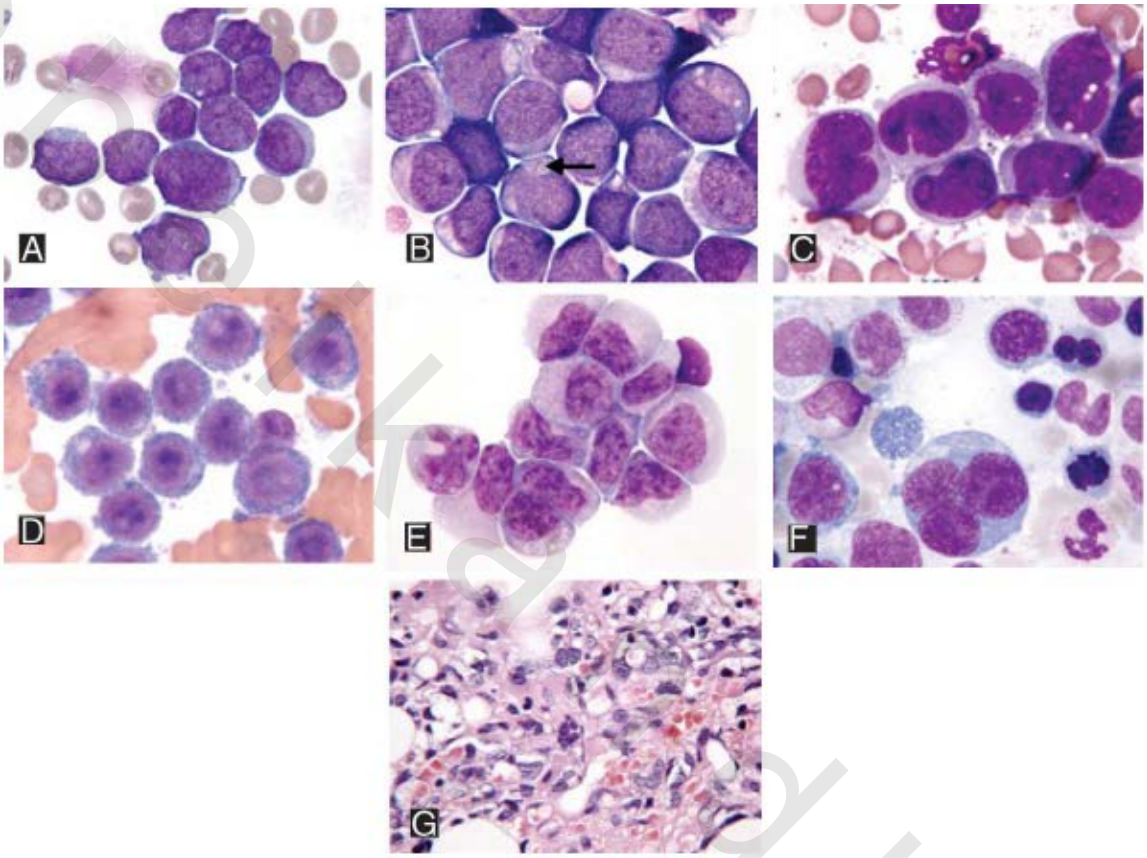

Figure 3: The morphologic spectrum for the acute myeloid leukemias (AMLs) in bone marrow aspirates (**A-F**) and a marrow biopsy specimen (**G**). **A,** Acute myeloblastic leukemia with minimal (FAB AML-M0) or no (FAB AML-M1) maturation. **B,** Acute myeloblastic leukemia with maturation (FAB AML-M2). **C,** Acute myelomonocytic leukemia (FAB AML-M4). **D,** Acute monoblastic leukemia (FAB AML-M5a). **E,** Acute monocytic leukemia (FAB AML-M5b). **F,** Acute erythroid leukemia (FAB AML-M6). **G,** Acute megakaryoblastic leukemia (FAB AML-M7)[4].

1.5.3. Immunophenotyping

AML cases can be categorized according to the combinations of myeloid-associated antigens displayed on the surface of the malignant blast[4]. Flow cytometry (FCM) is the preferred method of characterizing surface and internal antigen expression in leukemia cases[8].

In undifferentiated AML cases, including FAB M0 cases, expression of CD34, CD117, and CD33 is characteristic, but the blasts tend not to express CD65s. In more mature AML types, including most cases of FAB M1 and M2, expression of CD34, CD33, CD13, and CD65s is seen. In leukemias associated with t(8;21), often with M2 morphology, an immunophenotype similar to that with other M2 AML types is present, but expression of the NK marker CD56 and the B lymphoid marker CD19 also is seen. In APLs, blasts uniquely stain strongly with CD15s and weakly with CD15. In addition, they usually do not express CD34 or HLA-DR. In acute myelomonocytic and monocytic leukemias, blasts express CD14, the prototypical monocytic antigen. Early myeloid markers, including CD34 and CD117, generally are absent. Myelomonocytic leukemias associated with inv(16) blasts frequently express the T-cell antigen, CD2. In most acute erythroid leukemias, blasts fail to express early myeloid markers (e.g., CD34) but do express CD36 and CD71 and often express blood group H antigen, the precursor to ABO. In acute megakaryocytic leukemias, blasts react with antibodies to CD41a/CD61 (GPIIb/IIIa). Mature platelets sometimes can adhere to the surface of M5 AML blasts, so that they appear as in M7 leukemias. In true M7 AML, however, expression of CD14 does not occur[4]. As seen here, many AML cases can be demonstrated to express antigens of other hematopoietic lineages: for example, t(8;21)-containing cases often show expression of the B cell antigens CD19, Pax5 or cytoplasmic CD79a; and T cell markers CD2 or CD7 are not uncommon in several subtypes of AML[64].

1.5.4. Cytochemistry

To identify lineage involvement some countries still rely more on cytochemistry, rather than on immunophenotyping, using myeloperoxidase (MPO) or sudan black B (SBB) and nonspecific esterase (NSE) stains. Detection of MPO (if present in ≥ 3% of blasts) indicates myeloid differentiation, but its absence does not exclude a myeloid lineage because early myeloblasts and monoblasts may lack MPO. SBB staining parallels MPO but is less specific[60]. SBB has the advantage of being usable even when the air-dried smears have not been recently prepared. The nonspecific esterase (NSE) activity that characterizes monocytes and monoblasts can be detected by reactivity with alpha naphthyl butyrate esterase, or sodium fluoride-inhibited reactivity with alpha naphthyl acetate esterase. Naphthol-ASD-chloroacetate esterase (CAE) activity is specific for neutrophil and mast cell lineages[8].

Using these stains, AML with minimal differentiation cases are defined by having less than 3% of blasts positive for MPO, SBB or CAE, and lacking NSE activity; the designation of these cases as acute myeloid leukemias relies on immunophenotypic detection of expression of some combination of CD13, CD117 or CD33 and lack of lymphoid differentiation markers. AML without maturation is distinguished from AML with minimal differentiation by having >3% MPO or SBB-positive blasts, or the presence of Auer rods (which should also stain for both MPO and SBB). Monoblasts and promonocytes seen in the acute myelomonocytic or acute monoblastic or monocytic leukemia categories show blast-equivalent cells with NSE reactivity but usually no MPO or SBB reactivity. Acute megakaryoblastic leukemias may show NaF-resistant NSE activity for alpha naphthyl acetate, but are MPO and SBB negative[8]. In acute erythroid leukemia, a periodic acid- Schiff (PAS)

stain may show large globules of PAS positivity. Iron stains may allow for the detection of iron stores, normal sideroblasts, and ring sideroblasts[60].

1.5.5. Cytogenetics and molecular testing

1.5.5.1. Conventional cytogenetics

Conventional cytogenetics analysis is a mandatory component in the diagnostic evaluation of a patient with suspected acute leukemia. Chromosomal abnormalities are detected in approximately 55% of adult AML[65,66]. Seven recurrent balanced translocations and inversions, and their variants, are recognized in the WHO category "AML with recurrent genetic abnormalities." Furthermore, several cytogenetic abnormalities are considered sufficient to establish the WHO diagnosis of "AML with myelodysplasia-related features" when 20% or more blood or marrow blasts are present (Table 2)[52].

Conventional cytogenetics involves the staining of metaphase cells and thus requires dividing cells. Because malignant cells in the marrow are more frequent and have a higher mitotic rate, marrow, rather than peripheral blood, is the preferred source for cytogenetic analysis. Cells usually are cultured for 24 hours, with arrest by short-term incubation with colchicine; then, 20 metaphases typically are analyzed. The abnormalities detected include changes in chromosome number, gains or losses of portions of chromosomes, and reciprocal exchange of genetic material either between two or more chromosomes (translocations) or within a single chromosome (inversions)[4].

1.5.5.2. Molecular cytogenetics

Methanol/acetic acid–fixed cell pellets should be stored so if cytogenetic analysis fails, fluorescence in situ hybridization (FISH) is an option to detect gene rearrangements, such as *RUNX1- RUNX1T1*, *CBFB-MYH11*, *MLL* and *EVI1* gene fusions, or loss of chromosome 5q and 7q material. FISH is frequently necessary to identify *MLL* fusion partners in 11q23 translocations[67,68].

1.5.5.3. Molecular genetics

A marrow (and blood) specimen should routinely be taken for molecular diagnostics. Ideally, DNA and RNA should be extracted and viable cells stored; if cell numbers are limited, RNA extraction should be a priority, because RNA is suitable for molecular screening for fusion genes and leukemia-associated mutations[60]. Molecular diagnosis by reverse transcriptase-polymerase chain reaction (RT-PCR) for the recurring gene fusions, such as *RUNX1-RUNX1T1*, *CBFB-MYH11*, *MLLT3-MLL*, *DEK-NUP214*, can be useful in certain circumstances. RT-PCR, for which standardized protocols were published by the BIOMED-1 group[69], is an option to detect these rearrangements, if chromosome morphology is of poor quality, or if there is typical marrow morphology but the suspected cytogenetic abnormality is not present[70,71].

As mentioned before, somatically acquired mutations have been identified in several genes, for example, the *NPM1* gene, the *FLT3* gene[72], the *CEBPA* gene[73], the myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, *Drosophila*) (*MLL*) gene[74], the neuroblastoma RAS viral (v-ras) oncogene homolog (*NRAS*) gene[75], the Wilms' tumor 1 (*WT1*) gene[76], the v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (*KIT*) gene[77], the runt-related transcription factor 1 (*RUNX1*) gene[78], the tet oncogene

family member 2 (*TET2*) gene[79,80], and the isocitrate dehydrogenase 1 (NADP+), soluble (*IDH1*) gene[81]. The frequencies of these gene mutations vary among cytogenetic groups[82].

AML with mutations in *NPM1* or *CEBPA* have been incorporated in the WHO classification as provisional entities[52]. While testing for *NPM1*, *CEBPA*, and *FLT3* is currently not considered mandatory outside clinical trials, it is recommended that these 3 mutations be analyzed at least in patients with cytogenetically normal AML (CN-AML) who will receive treatment other than low-dose chemotherapy or best supportive care[60].

Cytogenetic and molecular testing additionally impacts therapeutic decision-making for patients with favorable cytogenetics or gene mutation-containing leukemias. Such as patients with low WBC counts and t(8;21), inv(16), t(16;16), or *NPM1* mutation without cytogenetic abnormalities and without *FLT3*-internal tandem duplication, in which allogeneic stem cell transplant does not appear to confer increased survival[83,84].

1.6. Pretreatment evaluation:

The initial evaluation of AML has 2 objectives. The first is to characterize the disease process based on factors such as prior toxic exposure, antecedent myelodysplasia, and karyotypic or molecular abnormalities, which may provide prognostic information that could impact responsiveness to chemotherapy and risk of relapse. The second objective focuses on patient-specific factors, including assessment of comorbid conditions, which may affect an individual's ability to tolerate chemotherapy. Both disease-specific and individual patient factors are taken into consideration when deciding treatment[85].

Most patients are anemic and thrombocytopenic at presentation. Replacement of the appropriate blood components, if necessary, should begin promptly. Because qualitative platelet dysfunction or the presence of an infection may increase the likelihood of bleeding, evidence of hemorrhage justifies the immediate use of platelet transfusion, even if the platelet count is only moderately decreased[10]. About 50% of patients have a mild to moderate elevation of serum uric acid at presentation. Only 10% have marked elevations, but renal precipitation of uric acid and the nephropathy that may result is a serious but uncommon complication. The initiation of chemotherapy may aggravate hyperuricemia, and patients are usually started immediately on allopurinol and hydration at diagnosis. Rasburicase (recombinant uric oxidase) is also useful for treating uric acid nephropathy and often can normalize the serum uric acid level within hours with a single dose of treatment. The presence of high concentrations of lysozyme, a marker for monocytic differentiation, may be etiologic in renal tubular dysfunction, which could worsen other renal problems that arise during the initial phases of therapy[10].

1.7. Prognosis:

A number of clinical and biologic features predict prognosis in AML including clinical state[2]. For the past two decades, the most important prognostic factors in AML have remained: (1) patient age; (2) presenting white blood cell count; (3) whether the AML presents clinically as de novo disease or secondary to antecedent myelodysplasia (MDS) or leukemogenic therapies (therapy-related AML, t-AML); and (4) the presence of specific cytogenetic abnormalities, usually clustered as "favorable," "intermediate," or "unfavorable"[86].

1.7.1. Age and performance status

For many years, it has been appreciated that age is a key prognostic factor in AML, with a steady deterioration in outcome with increasing years. AML occurring in adults older than 55 years of age is associated with an extremely poor prognosis with an overall survival (OS) of less than 20% at 5 years[87]. The significantly poorer outcome seen in older AML patients is, in part, explained by a higher frequency of unfavorable cytogenetic abnormalities and biologic features[86]. Advancing age influences the patient's ability to survive induction therapy. Age also influences outcome because AML in older patients differs biologically. The leukemic cells in elderly patients more commonly express CD34 and the multidrug resistance 1 (MDR1) efflux pump that conveys resistance to natural product-derived agents such as the anthracyclines[10].

Performance status, independent of age, also influences ability to survive induction therapy and thus response to treatment[88]. The main predictor of treatment-related death is the patient's performance status on the Zubrod scale[89]. Age, serum albumin, bilirubin, and creatinine and various indices of morbidity are other predictors of early death, with each probably independent of each other as well as of performance status[90].

1.7.2. Presenting white blood cell count

A high presenting leukocyte count is an independent prognostic factor for attaining a CR. A leukemic cell count greater than 20,000/μl is associated with a lower CR rate and shorter duration of remission[91]. A prolonged symptomatic interval with cytopenias preceding diagnosis or a history of an antecedent hematologic disorder is another pretreatment clinical feature associated with a lower CR rate and shorter survival time. The CR rate is lower in patients who have had anemia, leukopenia, and/or thrombocytopenia for more than 3 months before the diagnosis of AML when compared to those without such a history. Responsiveness to chemotherapy declines as the duration of the antecedent disorder(s) increases[10].

1.7.3. Secondary and therapy-related AML

Secondary AML represents 24%-56% of AML diagnosis in older patients, compared to a prevalence of approximately 8% in younger AML patients[92]. Secondary AML arises from an antecedent MDS or from prior treatment with chemotherapy or radiation therapy for another cancer. Greater than 90% of secondary leukemias are of myeloid origin[5]. Therapy-related AML or AML arising after a myelodysplastic syndrome is usually more resistant to standard treatment than de-novo AML[30].

1.7.4. Cytogenetics and molecular genetics

The karyotype of the leukemic cells is the strongest prognostic factor for response to induction therapy and for survival[66,65]. AML patients are commonly categorized into 3 risk groups, favorable, intermediate, or adverse (Table 3)[93,94].

Pretreatment conventional cytogenetic studies identify an acquired clonal abnormality in approximately 50% - 60% of patients with de novo AML, of which 10% - 12% are complex karyotypes that have very poor outcome. Complex karyotype has been defined as the presence of 3 or more chromosomal abnormalities in the absence of t(8;21), inv(16) or t(16;16), and t(15;17)[95,96]. In approximately 40% - 50% of cases, no karyotypic abnormality is detected using typical banding techniques, yielding an AML with normal karyotype[96].

Table 3: Standardized reporting for correlation of cytogenetic and molecular genetic data in AML with clinical data[60]

Genetic group	**Subsets**
Favorable	t(8;21)(q22;q22); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD (normal karyotype)
	Mutated *CEBPA* (normal karyotype)
Intermediate-I*	Mutated *NPM1* and *FLT3*-ITD (normal karyotype)
	Wild-type *NPM1* and *FLT3*-ITD (normal karyotype)
	Wild-type *NPM1* without *FLT3*-ITD (normal karyotype)
Intermediate-II	t(9;11)(p22;q23); *MLLT3-MLL*
	Cytogenetic abnormalities not classified as favorable or adverse§
Adverse	inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
	t(6;9)(p23;q34); *DEK-NUP214*
	t(v;11)(v;q23); *MLL* rearranged
	-5 or del(5q); -7; abnl(17p); complex karyotype¥

*Includes all AMLs with normal karyotype except for those included in the favorable subgroup; most of these cases are associated with poor prognosis, but they should be reported separately because of the potential different response to treatment.

§For most abnormalities, adequate numbers have not been studied to draw firm conclusions regarding their prognostic significance.

¥Three or more chromosomal abnormalities in the absence of one of the WHO designated recurring translocations or inversions, that is, t(15;17), t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23), t(6;9), inv(3) or t(3;3); indicate how many complex karyotype cases have involvement of chromosome arms 5q, 7q, and 17p.

Patients with t(8;21)(q22;q22), t(15;17)(q22;q21), or inv(16)(p13q22) were found to have a relatively favorable prognosis[87]. Those with t(15;17) have a very good prognosis (~85% cured), and those with t(8;21) and inv(16) have a good prognosis (~50% cured)[10]. In the absence of these changes, the presence of a complex karyotype, monosomies of chromosome 5 or 7, deletions of long arm of chromosome 5 (del[5q]/5q$^-$), 3q abnormalities, t(6;9), inv(3), or 7 predicted an adverse prognosis[87]. The remaining patients, including those with normal karyotype or structural or numerical changes not encompassed by the favorable or adverse risk groups, account for 40% to 49% of all AML cases and had an intermediate prognosis [87,97] with moderately favorable outcome (~40% cured)[10].

Gene mutations and deregulated gene expression allow deciphering the genetic diversity within defined cytogenetic groups, in particular the large and heterogeneous group of patients

with CN-AML. Prognostic significance within CN-AML has consistently been shown for mutations in the *NPM1*, *CEBPA*, and *FLT3* genes alone or in combination in AML patients[84,82,98]. CN-AML patients harboring internal tandem duplication (ITD) of the *FLT3* gene have an inferior outcome compared with cases without *FLT3*-ITD[99,100]. In several, but not all studies, the presence of *NPM1* mutation in CN-AML has been associated with higher CR rates and better relapse-free survival (RFS) and event-free survival (EFS). Approximately 40% of patients with *NPM1* mutations also carry *FLT3*-ITD, and multiple studies have shown that the genotype "mutated *NPM1* without *FLT3*-ITD" represents a favorable prognostic marker, with higher CR rates, and better RFS and overall survival (OS)[84,101,102]. CN-AML with mutations in *CEBPA* is another subset that has been associated with a favorable prognosis. The survival data are very similar to those of AML patients with mutated *NPM1* without *FLT3*-ITD[103,104,105]. In cytogenetically favorable core binding factor (CBF) AML [i.e., AML with t(8;21) or inv(16)/t(16;16)], the presence of a *KIT* mutation has been shown to have an unfavorable influence on outcome in retrospective studies[106,107].

The common focus on obtaining prognostic information before starting treatment should not obscure the prognostic usefulness of information available only after therapy begins. For example, the British MRC Group has shown that the value of cytogenetics in predicting relapse-free survival can be improved by incorporating information on the response to the first course of induction therapy[30]. Patients who achieve CR after one induction cycle have longer CR durations than those requiring multiple cycles[108].

1.8. Treatment:

Conventional treatment for acute myeloid leukemia has two phases, induction and post-remission, which includes stem cell transplantation[28]. Induction therapy attempts to produce complete remission, defined as a marrow with less than 5% blasts with Auer rods absent, a neutrophil count greater than 1000/μl, and a platelet count greater than 100,000/μl[30]. Circulating blasts should be absent. Although rare blasts may be detected in the blood during marrow regeneration, they should disappear on successive studies. Bone marrow cellularity should be >20% with trilineage maturation. Extramedullary leukemia should not be present[10].

Complete remission is the only response that leads to cure and, at the least, to an extension in survival, while consolidation is designed to eliminate residual leukemia cells that persist after induction, prolonging the complete remission. Once a patient has been in remission for 3 years, the likelihood of relapse declines sharply to less than 10%[28, 30]. For patients in morphologic CR, reverse transcriptase polymerase chain reaction (RT-PCR), to detect AML-associated molecular abnormalities, and either metaphase cytogenetics or interphase cytogenetics by fluorescence in situ hybridization (FISH), to detect AML-associated cytogenetic aberrations, are currently used to detect residual disease. Such detection of minimal residual disease may become a reliable discriminator between patients in CR who do or do not require additional and/or alternative therapies[10].

1.8.1. Induction chemotherapy

The backbone of remission induction consists of an anthracycline (daunorubicin) and cytosine arabinoside (Ara-C), a regimen that has not changed in over 30 years[9,92]. Typically, daunorubicin is given at a dose of 45-60 mg/m^2/d × 3 days, in combination with Ara-C, which is administered as a continuous infusion at 100-200 mg/m^2/d × 7 days (frequently referred to as 3+7 or 7+3 chemotherapy)[60,92]. After induction chemotherapy, the bone marrow is examined to determine if the leukemia has been eliminated. If ≥5% blasts exist with ≥20% cellularity, the patient is usually retreated with cytarabine and an anthracycline in doses similar to those given initially, but for 2 and 5 days, respectively[10].

With such regimens, CR is achieved in 60% to 80% of adults under the age of 60 years. No other intervention has been convincingly shown to be better[30,109]. Two-thirds achieve CR after a single course of therapy, and one-third require two courses. About 50% of patients who do not achieve CR have a drug-resistant leukemia, and 50% do not achieve CR because of fatal complications of bone marrow aplasia or impaired recovery of normal stem cells[10]. Induction chemotherapy should be started after the diagnostic work-up has been completed, preferably with minimal delay. Retrospective data suggest that treatment outcome might be adversely impacted when the time from diagnosis to start of treatment increases beyond 5 days[110].

Numerous trials have been done to improve the rate and quality of CR, including the use of anthracyclines other than daunorubicin (eg, idarubicin hydrochloride and mitoxantrone), the addition of a third drug (usually etoposide), use of high-dose instead of conventional dose cytarabine, the use of hematopoietic growth factors such as granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor, and the combination of anthracyclines with fludarabine phosphate or cladribine and intermediate dose cytarabine[28].

High-dose cytarabine-based regimens have very high CR rates after a single cycle of therapy. When given in high doses, more cytarabine may enter the cells, saturate the cytarabine-inactivating enzymes, and increase the intracellular levels of 1-β-D-arabinofuranylcytosinetriphosphate, the active metabolite incorporated into DNA. Thus higher doses of cytarabine may increase the inhibition of DNA synthesis and thereby overcome resistance to standard-dose cytarabine. In two randomized studies, high-dose cytarabine with an anthracycline produced CR rates similar to those achieved with standard 3 and 7 regimens. However, the CR duration was longer after high-dose cytarabine than after standard-dose cytarabine[10].

1.8.2. Supportive/palliative care

It is unusual for induction chemotherapy not to clear most of the leukemic blasts, however, this is at a cost of 3 - 4 weeks of severe pancytopenia. Supporting patients through the period of marrow suppression is crucial to treatment outcome[9]. Adequate and prompt blood bank support is critical to therapy of AML. Blood and platelet transfusions should be administered to alleviate symptoms stemming from anemia and thrombocytopenia, and antibiotics started when appropriate[92]. Platelet transfusions should be given as needed to maintain a platelet count >10,000–20,000/μl. RBC transfusions should be administered to keep the hemoglobin level >80 g/l (8 g/dl) in the absence of active bleeding, DIC, or congestive heart failure[10]. Careful monitoring of biochemical parameters of renal and hepatic function and coagulation is required. A priority is the prevention and management of infection. Most patients will receive prophylactic oral antibiotics and antifungals to minimize the risk of infection during the neutropenic period[9].

Low-dose chemotherapy should only be used in the setting of leukocytosis and/or associated symptoms. Avoiding crowds, refraining from ingestions of raw foods is recommended for the institution of neutropenia[92]. Recombinant hematopoietic growth factors have been incorporated into clinical trials in AML. These trials have been designed to lower the infection rate after chemotherapy. Both G-CSF and granulocyte-macrophage colony-stimulating factor (GM-CSF) have reduced the median time to neutrophil recovery by an average of 5-7 days. This accelerated rate of neutrophil recovery, however, has not generally translated into significant reductions in infection rates or shortened hospitalizations[10].

1.8.3. Post-remission chemotherapy

Various types of post-remission strategies have been evaluated including intensive conventional chemotherapy, prolonged maintenance treatment, and high-dose therapy followed by allogeneic or autologous hematopoietic stem cell transplantation (HSCT)[30,111].

Consolidation therapy and intensification therapy often are interchangeable and refer to an intensive form of post-remission therapy that is intensely myelosuppressive and includes drugs similar to those used in induction, often at higher doses[17]. Patients have fewer treatment-related complications in complete remission than with active AML, making dose intensification an appealing post-remission strategy in patients likely to be sensitive to cytarabine or anthracycline[30]. After achievement of CR, all patients will eventually relapse without further treatment and thus consolidation treatment is essential (with the ultimate goal of cure), provided that patients have adequate organ function[28]. On the other hand, maintenance therapy usually is defined as therapy that is less myelosuppressive than that used to produce remission[17]. In one study there was no benefit in remission duration or OS with 3 years of intensive maintenance compared with autologous HSCT as post-remission therapy, whereas maintenance proved superior for disease-free Survival (DFS) to 1 course of consolidation[112].

If an HLA-matched sibling donor (MSD) is available, allogeneic HSCT is the preferred therapy for most patients up to age 55 to 60 years, if they present with an intermediate-risk karyotype, the most common cytogenetic group, composing approximately 50% of all AML patients. Data have shown that this form of therapy provides the best antileukemic effect as judged by the relapse rate[113,114]. This benefit is attributable to both the high-dose therapy of standard conditioning regimens and a potent graft-versus-leukemia (GVL) effect[115]. However, benefits of allogeneic HSCT have been limited by the high transplant-related mortality (TRM). Meta-analyses of clinical trials that prospectively assigned allogeneic HSCT versus alternative consolidation therapies for AML in first Complete Remission (CR1) on an intent-to-treat donor versus no-donor basis show that allogeneic HSCT offers significant OS benefit for patients with intermediate- and high-risk AML[116,117].

After allogeneic HSCT, autologous HSCT offers the most potent form of post-remission therapy, relying on the antileukemic effect provided by the myeloablative regimen[17]. Autologous HSCT is considered an alternative option for post-remission therapy in patients with favorable- and intermediate-risk cytogenetics, whereas it cannot be recommended in patients with high-risk cytogenetics[118,119]. Outcome after autologous HSCT is at least as good as after the use of post-remission chemotherapy; however, there has been no evidence of an improvement in outcome. Autologous HSCT may offer an advantage in specific subsets of AML[120].

1.8.4. Relapse

Most patients with AML will require salvage therapy because of failure to enter complete remission after initial treatment (primary refractory) or, more typically, relapse after a brief complete remission. The type of therapy should depend on expected outcome with standard salvage regimens, such as high-dose cytarabine or fludarabine plus cytarabine. The factors most predictive of response are the duration of the previous remission and the number of previous salvage attempts[121]. If the first complete-remission lasted less than 6–12 months, standard regimens produce complete-remission rates of 10–20% when used as first salvage and less than 5% in later attempts. By contrast, probability of complete remission after first salvage in patients with first remission lasting more than 1 year was 40–50%[122].

Patients eligible for allogeneic HSCT should receive transplants expeditiously at the first sign of relapse. Long-term disease-free survival is approximately the same (30–50%) with allogeneic HSCT in first relapse or in second remission. Autologous HSCT rescues ~20% of relapsed patients with AML who have chemosensitive disease. The most important factors predicting response at relapse are the length of the previous CR, whether initial CR was achieved with one or two courses of chemotherapy, and the type of post-remission therapy[10].

2. DNA Repair

The human genome, comprising three billion base pairs coding for 30,000-40,000 genes, is constantly attacked by endogenous reactive metabolites, therapeutic drugs and a plethora of environmental mutagens that impact its integrity. Thus it is obvious that the stability of the genome must be under continuous surveillance[123]. The cellular response to DNA damage mainly consists of six biological conserved pathways, known as homologous recombination repair (HRR), non-homologous end-joining (NHEJ), methyltransferase repair, mismatch repair (MMR), base excision repair (BER), and nucleotide excision repair (NER), that operate in a concerted way to minimize genetic information loss due to a DNA lesion[124].

2.1. Homologous recombination repair (HRR)

Double strand breaks (DSB) are detected by MRE11-RAD50-NBS1 complex and converted to 3' ssDNA tails, which are subsequently bound by replication protein A (RPA). Then, RAD52 protein interacts with RPA and promotes RAD51 binding to ssDNA. Subsequently, the RAD51 bound to ssDNA invades a homologous molecule in a reaction stimulated by RAD54. After DNA synthesis and ligation, two Holliday junctions are formed and branch migration can occur. The Holliday junctions are finally resolved by other proteins including Bloom and Werner DNA helicases and ligases[125].

Defects in HRR proteins cause ataxia telangiectasia, Nijmegen breakage, Bloom and Werner human syndromes, as well as hereditary breast and ovarian cancer associated with mutations in *BRCA1* or *BRCA2* genes[124].

2.2. Non-homologous end-joining (NHEJ)

DNA repair by NHEJ initiates when KU70-KU80 dimers (KU complex) bind both DSB ends. In higher eukaryotes the DNA protein kinase catalytic subunit (DNA-PKcs) is subsequently recruited. Once DSB are recognized and the ssDNA filaments of the lesion are processed, a DNA polymerase synthesizes short DNA strands and DNA ends are linked together in the presence of the XRCC4/LIF1-DNA ligase IV/DNL4 complex. DSBs that are not suitable for ligation may be processed by MRE11-RAD50-NBS1 and FEN1/RAD27 nuclease. Given that DNA is repaired by synthesis and ligation without using a homologous sequence, NHEJ is often associated to nucleotide loss[126].

2.3. Methyltransferase repair

Methyltransferases, including MGT1 protein found in eukaryotes, catalyzes the irreversible transfer of methyl groups from DNA to their own cysteine residues[127].

2.4. Mismatch repair (MMR)

The main task of MMR is to remove base mismatches and small insertion/deletion loops (IDL) introduced during replication. In yeast, single base mismatches are recognized by MUTSα (MSH2/ MSH6) and IDL are sensed by MUTSβ (MSH2/MSH3). PCNA protein is also engaged in MMR, maybe supporting the damage detection and strand discrimination steps. Another complex named MUTLα, composed by MSH1 and PMS1 proteins, binds both MUTSα and MUTSβ to promote their efficient binding to mismatches. Finally, EXO1 removes these regions and gaps filling and closing are completed by DNA polymerase and

DNA ligase, respectively[128]. The inactivation of human MMR homologous proteins is cause of hereditary non-polyposis colorectal cancer[124].

2.5. Base excision repair (BER)

BER mainly repairs non-bulky lesions produced by alkylation, oxidation or deamination of bases. During BER, damaged bases are recognized by a specific DNA glycosylase, which cleaves the N-glycosydic bond between the base and the deoxyribose to remove the base [129]. After cleavage, the damaged base is released and an apurinic/apyrimidinic (AP) site is created. An AP site can also occur spontaneously and represents damage by itself. Bifunctional glycosylases have an intrinsic AP lyase activity, which cleaves the sugar phosphate backbone 3' to the AP site. After base was removed from DNA, a non-specific endonuclease (APN1 or APN2) releases the deoxyribose phosphate to produce a gap, which is filled by DNA polymerase β, whereas the FEN1/RAD27 endonuclease eliminates the displaced DNA and the CDC9 ligase closes up the nick. After strand displacement by Pol β, and Pol δ or Pol ε, a flap structure is formed, which is cleaved by FEN1/RAD27. The RAD1-RAD10 and MUS81-MMS4 endonucleases are also believed to play minor roles in BER by processing the DNA 3' end [130]. No human disease is currently known to be associated with defects in BER[124].

2.6. Nucleotide excision repair (NER)

Nucleotide excision repair is a major DNA repair pathway in eukaryotic cells[131]. Bulky DNA adducts, such as UV-light-induced photolesions [(6-4) photoproducts (6-4PPs) and cyclobutane pyrimidine dimers (CPDs)], intrastrand cross-links, large chemical adducts generated from exposure to aflatoxine, benzo[*a*]pyrene and other genotoxic agents are repaired by NER[132,133]. In NER about 30 proteins are involved. Cells defective in NER belong to different complementation groups and UV-hypersensitive disorders such as xeroderma pigmentosum (XP), Cockayne's syndrome (CS), trichothiodystrophy (TTD), UV-sensitive syndrome (UVSS) and a variety of UV-hypersensitive rodent lines, in which the defect can be completed by human genes belonging to the excision repair cross-complementing group (ERCC)[134].

NER consists of two sub-pathways: global genome NER (GGNER), which removes damage in the overall genome, and transcription-coupled NER (TCNER), which specifically repairs the transcribed strand of active genes (Figure 4)[124]. GGNER is thought to be largely transcription-independent and removes lesions from the non-transcribed domains of the genome and the non-transcribed strand of transcribed regions. 6-4PPs, which distort the DNA more than CPDs, are removed rapidly and predominantly by GGNER, such that it may be difficult to detect TCR experimentally. In contrast, CPDs are removed very slowly by GGNER. Their removal occurs more efficiently by TCNER from the transcribed strand of expressed genes[135,136].

During GGNER, recognition of the DNA lesion occurs by XPC–HR23B, RPA–XPA or UV-DDB (consisting of DDB1 and DDB2). DNA unwinding is performed by the XPD and XPB helicase containing transcription/repair complex TFIIH together with XPA and RPA to generate the damaged single stranded DNA ready for incision by the specific endonucleases XPG and ERCC1-XPF. Finally, resynthesis occurs by the replicative DNA polymerases Polδ or Polε and ligation by DNA ligase I[123,137]. On the other hand, during transcription-coupled repair (TCR) the induction of the lesion results in blockage of RNA Polymerase II (RNAPII). This leads to assembly of CSA, CSB and/or TFIIS at the site of the lesion, by which RNAPII

is removed from the DNA or displaced from the lesion, making it accessible to the exonucleases XPF–ERCC1 and XPG cleaving the lesion-containing DNA strand. Resynthesis again occurs by Polδ or Polε and ligation by DNA ligase I[123,138].

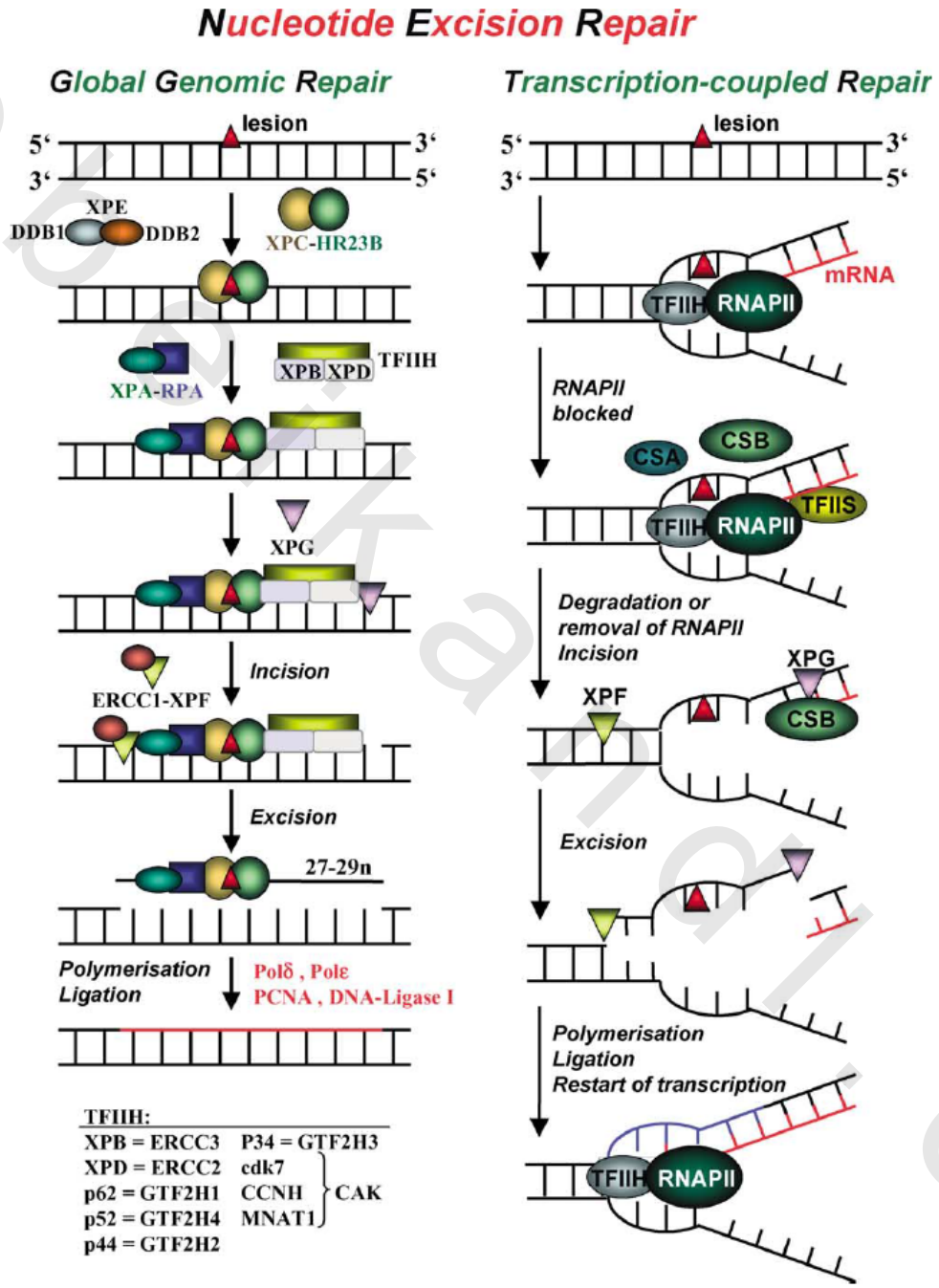

Figure 4: Mechanism of nucleotide excision repair (NER).[123]

2.7. Transcription initiation factor II H (TFIIH)

The transcription initiation factor TFIIH is a remarkable protein complex that has a fundamental role in the transcription of protein-coding genes as well as during the DNA nucleotide excision repair pathway[139]. Mammalian TFIIH is a multiprotein complex with ten subunits that consists of two main functional subcomplexes: the core complex, which is composed of six subunits (xeroderma pigmentosum group B complementing protein (XPB), p62, p52, p44, p34 and p8 also known as TTDA); and the CAK (cyclin-dependent kinase (CDK)-activating kinase) complex, which is composed of CDK7, cyclin H and MAT1. The core and CAK subcomplexes are bridged by the XPD subunit, which interacts with p44 and MAT1 of the core or the CAK subcomplex, respectively (Figure 5)[140,141].

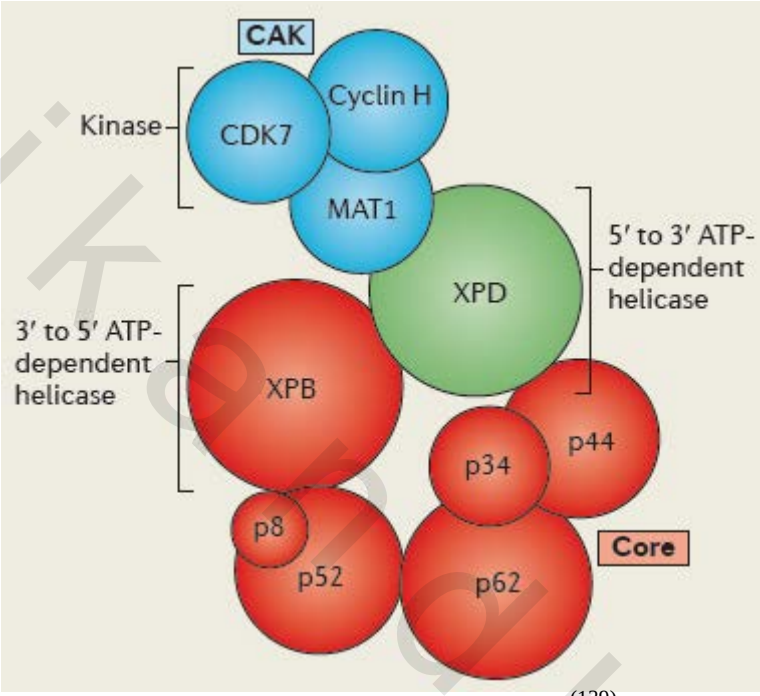

Figure 5: The structure of TFIIH[139]

Electron microscopy and image processing of the TFIIH complex revealed that it forms a ring-like structure with a central cavity that possibly interacts with DNA (Figure 6)[140,142,143]. TFIIH has several intrinsic enzymatic activities: CDK7 is a cyclin-dependent kinase and XPB and XPD are thought to act as ATP-dependent helicases of opposite polarities[139]. The main role of TFIIH in NER is to open the DNA around the lesion and thereby allow the excision of the damaged oligonucleotide and its replacement by a new DNA fragment.[139]

TFIIH was identified as an essential factor for Pol II-mediated transcription, TFIIH also participates in the transcription of ribosomal RNA (rRNA) by Pol I[144] and probably also in the synthesis of 5S rRNA, tRNA and other small RNAs that are transcribed by Pol III.[145]

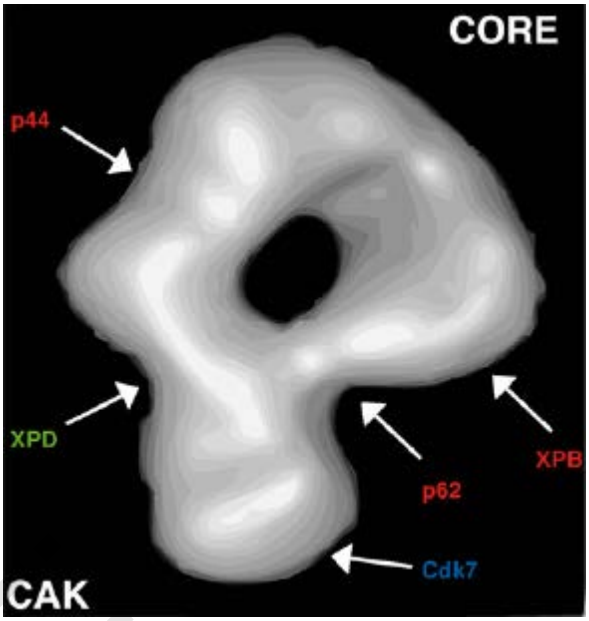

Figure 6: Electron microscopic structure of human TFIIH[143]

2.8. XPD

Xeroderma pigmentosum (XP) was the first DNA-repair disorder to be identified. It is a rare autosomal recessive genetic disorder characterized by numerous skin abnormalities ranging from excessive freckling to multiple skin cancers. The incidence of skin cancer is about 2000-fold greater than in normal individuals. All skin abnormalities result from exposure to sunlight and are caused by inability to repair DNA damage induced in the skin by UV radiation from sunlight. This is caused, in the majority of cases, by defects in NER. XP is genetically heterogeneous. There are eight complementation groups designated XP-A through G and XP-variant (XPV). The *XPD* gene is defective in XP individuals and assigned to the XP-D complementation group [146].

XPD (Rad3 in *S. cerevisiae*) is a superfamily 2 DNA helicase with a 5'-3' polarity. In eukaryotes, XPD is a component of the transcription factor IIH (TFIIH), along with the helicase XPB (Rad25) and eight other protein subunits. TFIIH is essential for RNA polymerase (RNAP) II-mediated transcription initiation and for the NER pathway. In both situations, the role of TFIIH is to open up the DNA duplex, around the promoter or site of DNA damage, allowing access for RNAP or NER factors in the respective pathways[147].

XPD is the structural bridge linking the core TFIIH subunits required for NER to the Cdk-activating kinase (CAK) complex [148]. The helicase activity of XPD is not required for transcription initiation, as point mutations that knock out this function while preserving the protein structure result in transcription competent TFIIH [149]. In contrast, mutations that reduce the helicase activity of XPD cause defects in the NER pathway. Mutations of human *XPD* give rise to three related diseases: xeroderma pigmentosum (XP), trichothiodystrophy (TTD), and combined XP with Cockayne's syndrome (XP/CS) [146]. These have a surprisingly wide spectrum of symptoms, ranging from severe UV sensitivity and a 1000-fold increased propensity to cancer for XP, through to brittle hair, transcriptional and developmental defects for TTD. This reflects the dual role of TFIIH in transcription and repair. Mouse models for XP/CS and TTD syndromes have highlighted the importance of segmental progeria, or premature aging, that is thought to be a consequence of accumulating DNA damage leading to transcriptional defects and apoptosis[150].

XPD is the founding member of a family of DNA helicases in eukaryotes, which includes the FancJ (also known as Bach1, Brip1), Chl1, and Tel helicases. These helicases are involved in a variety of DNA repair and recombination pathways in humans, and all have the conserved cysteine residues diagnostic for the FeS cluster. Some mutations of FancJ that cause Fanconi anemia map close to the FeS domain, suggesting that the FeS cluster is also essential for this enzyme [151].

2.8.1. XPD structure

In all of the NER helicases, the helicase domains that drive helix unwinding are not sufficient for their biological roles in opening DNA bubbles for excision. Rather they require the presence of accessory domains that play roles in duplex splitting or damage recognition. All of the NER helicases have two functional parts: two helicase domains that use ATP binding and hydrolysis to drive conformational change and two accessory domains that help transmit helicase domain changes to the DNA and either interrupt the linear sequence of the helicase domains or are found at the N- or C-termini. Their positions and structures are united only by their diversity [152].

Crystal structures of archaeal XPDs reveal that Rad51/RecA domains, connected by a flexible hinge, share an α-β fold and are called helicase domains 1 and 2 (HD1 and HD2) [153]. HD1 and HD2 pack against each other to form an interface cleft that brings together motifs I, II, V, and VI to form a composite ATP-binding site. This architecture is consistent with an inchworm model where cycles of ATP binding, hydrolysis, and release are coupled to opening and closing of HD1 and HD2, which drives the enzyme along DNA (Figure 7) [154].

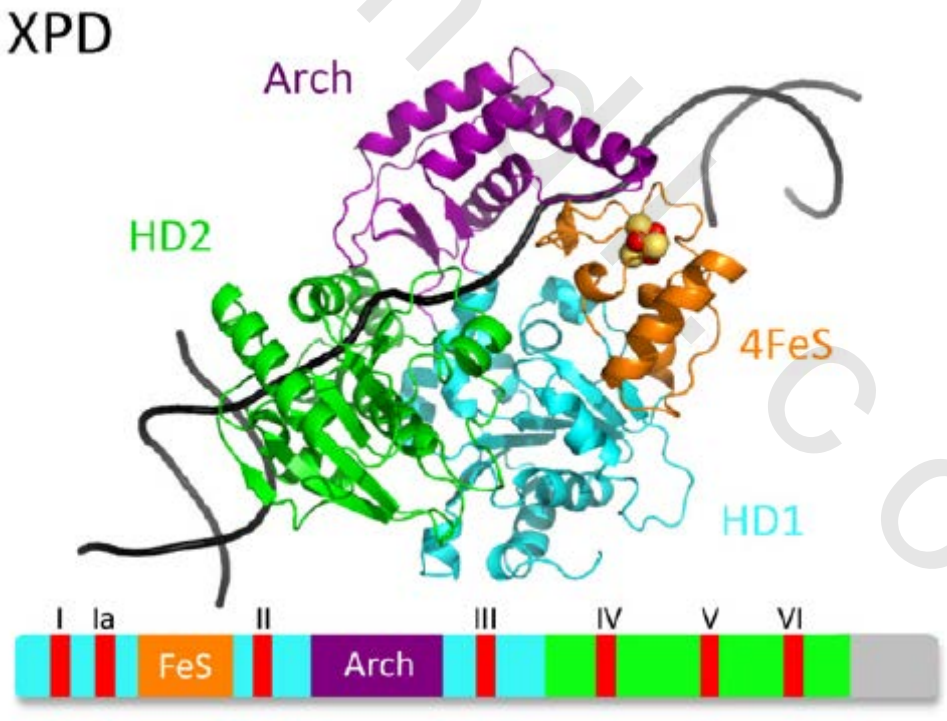

Figure 7: XPD structure[152]

In XPD, HD1 is interrupted by two additional domains, a domain containing a 4FeS cluster and an Arch shaped domain [153]. The 4FeS cluster consists of six alpha helices with four cysteines coordinating the Fe atoms. The Arch domain is also largely α-helical with a

60◦ angle between two sets of helices, which gives rise to the arch-like structure[155]. The importance of the 4FeS cluster is shown by loss of helicase activity when any of the coordinating cysteines is mutated, by the loss of structure of several parts of the molecule when the iron is oxidized and by a common TTD-specific mutation (R112H) being localized in this cluster [151,153]. Moreover, growing evidence supports the role of 4FeS clusters in DNA damage detection and in possible electron transfer along DNA [156].

Although a crystal structure of XPD with DNA has not been published, three independent computational models of XPD from three different organisms agree on the path of ssDNA binding. ssDNA passes through a hole formed by the Arch, FeS, and HD1 domains, travels through a basic channel across the top of HD2, and exits near a helical wedge in HD2 [147,153,157]. These models require conformational flexibility at the hinge between HD1 and HD2 and between the Arch and 4FeS domains to allow for DNA loading (Figure 8) [152]. It is proposed that either the hole itself or a pocket on the side of the hole is able to recognize lesions in the DNA. Arginine-112, which when mutated destroys the FeS cluster and abolishes helicase activity, is part of the wall of this hole [151,157]. The 4FeS domain is inserted between the HD1 Walker A and B motifs, suggesting that its conformation may change when ATP is bound or hydrolyzed and the cluster may help open the dsDNA [155].

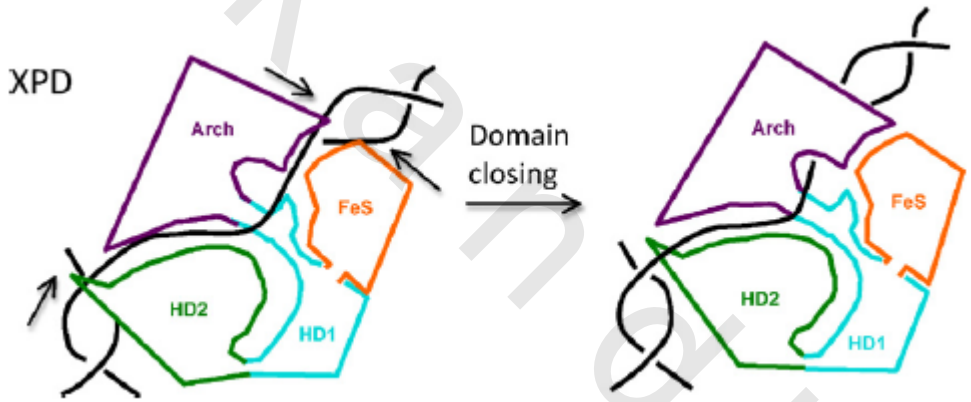

Figure 8: XPD helicase domains and functional conformational flexibility: proposed XPD conformational flexibility. Loading of XPD onto DNA would require an opening of the non-covalent interactions between the Arch and FeS domain, while flexibility between HD1 and HD2 is necessary for translocation along DNA during unwinding[152].

3. Chemotherapeutic agents in AML

AML is treated with 3+7 chemotherapy which comprises daunorubicin that is given at a dose of 45-60 mg/m^2/d × 3 days, in combination with Ara-C, which is administered as a continuous infusion at 100-200 mg/m^2/d × 7 days. These drugs act by damaging DNA of leukemic cells. DNA damage causes activation of DNA-repair mechanisms in the different phases of the cell cycle. Cells resume cell cycle progression once damage has been repaired, whereas cells which fail to repair the DNA damage undergo permanent cell-cycle arrest or apoptosis [158]. The lesions caused by these chemotherapeutic drugs are repaired by enzymes of nucleotide excision repair family (XPA, XPD, XPG, and ERCC1) [159,160].

3.1. Mechanism of action

3.1.1. Cytarabine

Cytarabine (Cytosine arabinoside or Ara-C) is a deoxycytidine analog. It is a cycle S-phase-specific antimetabolite that becomes phosphorylated intracellularly to an active triphosphate form (ara-CTP). Ara-CTP incorporates into DNA resulting in chain termination and inhibition of DNA synthesis and function. Ara-CTP inhibits several DNA polymerases α, β and γ, which, in turn, interferes with DNA chain elongation, DNA synthesis, and DNA repair. Ara-CTP inhibits the enzyme ribonucleotide reductase, resulting in decreased levels of essential deoxyribonucleotides required for DNA synthesis and function [10,161].

Cytarabine undergoes extensive metabolism, with approximately 70%–80% of drug being recovered in the urine as the Ara-U metabolite within 24 hours. Deamination occurs in liver, plasma, and peripheral tissues. The principal enzyme involved in drug catabolism is cytidine deaminase, which converts ara-C into the inactive metabolite ara-U. dCMP-deaminase converts ara-CMP into ara-UMP, and this represents an additional catabolic pathway of the drug. The terminal elimination half-life is 2–6 hours. The half-life of ara-C in CSF is somewhat longer, ranging from 2 to 11 hours, due to the relatively low activity of cytidine deaminase present in CSF [161].

3.1.2. Daunorubicin

Daunorubicin (Daunomycin, Cerubidine or Rubidomycin) is an antitumor anthracycline antibiotic. It is a cell cycle-nonspecific agent. Intercalates into DNA and inhibits topoisomerase II by forming a cleavable complex with DNA and topoisomerase II to create uncompensated DNA helix torsional tension, leading to eventual DNA breaks. Additionally, it inhibits transcription through inhibition of DNA-dependent RNA polymerase. It also forms oxygen free radicals results in single- and double-stranded DNA breaks with inhibition of DNA synthesis and function [10,161].

Daunorubicin is metabolized in the liver with formation of one of its primary metabolites, daunorubicinol, which has antitumor activity. Parent compound and its metabolites are excreted mainly through the hepatobiliary system into feces. Renal clearance accounts for only 10%–20% of drug elimination. The half-life of the parent drug is 20 hours, while the half-life of the daunorubicinol metabolite is 30–40 hours [161].

3.2. Resistance

3.2.1. Resistance for cytarabine can be achieved by a number of mechanisms [161]:

- Decreased activation of drug through decreased expression of the anabolic enzyme deoxycytidine kinase.
- Increased breakdown of drug by the catabolic enzymes, cytidine deaminase and deoxycytidylate (dCMP) deaminase.
- Decreased transport of drug into cells.
- Increased expression of CTP synthetase activity resulting in increased concentrations of competing physiologic nucleotide substrate dCTP.

When given in high doses, more cytarabine may enter the cells, saturate the cytarabine-inactivating enzymes, and increase the intracellular levels of 1-β-D arabinofuranylcytosinetriphosphate (ara-CTP). Thus higher doses of cytarabine may increase the inhibition of DNA synthesis and thereby overcome resistance to standard-dose cytarabine[10].

3.2.2. Resistance for daunorubicin can be achieved by a number of mechanisms [161]:

- Increased expression of the multidrug-resistant gene with elevated P170 levels. This leads to increased drug efflux and decreased intracellular drug accumulation.
- Decreased expression of topoisomerase II.
- Mutation in topoisomerase II with decreased binding affinity to drug.
- Increased expression of sulfhydryl proteins, including glutathione and glutathione-dependent enzymes.

3.3. Toxicities

Concern over potential treatment-related toxicities may result in under-treatment of disease. Paradoxically, administration of full-dose daunorubicin, for example, may result in a reduction in early deaths by affecting a more rapid CR [92].

The hematologic toxicity of high-dose cytarabine based induction regimens has typically been greater than that associated with 3 and 7 regimens. This toxicity occurs more commonly in patients with renal impairment and in those >60 years of age. The increased toxicity observed with high-dose cytarabine has limited the use of this therapy in elderly AML patients[10].

3.3.1. Toxicities of cytarabine therapy [161,162]

- Myelosuppression is dose-limiting. Leukopenia and thrombocytopenia are common. Nadir usually occurs by days 7–10, with recovery by days 14–21. Megaloblastic anemia is also observed.
- Nausea and vomiting. Mild to moderate emetogenic agent with increased severity observed with high-dose therapy. Anorexia, diarrhea, and mucositis usually occur 7–10 days after therapy.
- Cerebellar ataxia, lethargy, and confusion. Neurotoxicity develops in up to 10% of patients. Onset usually five days after drug treatment and lasts up to one week. In most cases, CNS toxicities are mild and reversible. Risk factors for neurotoxicity include high-dose therapy, age older than 40, abnormal renal function with serum creatinine 1.2 mg/dl, and abnormal liver function.

- Transient hepatic dysfunction with elevation of serum transaminases and bilirubin. Most often associated with high-dose therapy.
- Acute pancreatitis.
- Ara-C syndrome. Described in pediatric patients and represents an allergic reaction to cytarabine. Characterized by fever, myalgia, malaise, bone pain, maculopapular skin rash, conjunctivitis, and occasional chest pain. Usually occurs within 12 hours of drug infusion. Steroids appear to be effective in treating and/or preventing the onset of this syndrome.
- Pulmonary complications include noncardiogenic pulmonary edema, acute respiratory distress, and *Streptococcus viridans* pneumonia. Observed with high-dose therapy.
- Erythema of skin, alopecia, and hidradenitis are usually mild and self-limited. Hand-foot syndrome observed rarely with high-dose therapy.
- Conjunctivitis and keratitis. Associated with high-dose regimens.
- Seizures, alterations in mental status, and fever may be observed within the first 24 hours after Intrathecal (IT) administration.

3.3.2. Toxicities of daunorubicin therapy [161,162]

- Myelosuppression. Dose-limiting toxicity with leukopenia being more common than thrombocytopenia. Nadir occurs at 10–14 days with recovery by day 21.
- Nausea and vomiting. Usually mild, occurring in 50% of patients within 1–2 hours of treatment.
- Mucositis and diarrhea are common within the first week of treatment but not dose-limiting.
- Cardiotoxicity. Acute form presents within the first 2–3 days as arrhythmias and/or conduction abnormalities, EKG changes, pericarditis, and/or myocarditis. Usually transient and mostly asymptomatic.
- Cardiotoxicity. Chronic form associated with a dose-dependent, dilated cardiomyopathy and congestive heart failure. Incidence increases with cumulative doses greater than 550 mg/m^2 .
- Strong vesicant. Extravasation can lead to tissue necrosis and chemical thrombophlebitis at the injection site.
- Hyperpigmentation of nails, rarely skin rash, and urticaria. Radiation recall skin reaction can occur at prior sites of irradiation. Increased hypersensitivity to sunlight.
- Alopecia is universal. Usually reversible within 5–7 weeks after termination of treatment.
- Red-orange discoloration of urine. Lasts 1–2 days after drug administration.