Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet

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Abstract
In 2003, an international working group last reported on recommendations for diagnosis, response assessment, and treatment outcomes in acute myeloid leukemia (AML). Since that time, considerable progress has been made in elucidating the molecular pathogenesis of the disease that has resulted in the identification of new diagnostic and prognostic markers. Furthermore, therapies are now being developed that target disease-associated molecular defects. Recent developments prompted an international expert panel to provide updated evidence- and expert opinion-based recommendations for the diagnosis and management of AML, that contain both minimal requirements for general practice as well as standards for clinical trials. A new standardized reporting system for correlation of cytogenetic and molecular genetic data with clinical data is proposed.
1. Introduction

In 1990 and 2003, expert working groups published recommendations for diagnosis, standardization of response criteria and treatment outcomes, and reporting standards for clinical trials in acute myeloid leukemia (AML). These have been widely adopted in general practice, within clinical trials, and by regulatory agencies. During recent years, considerable progress has been made in deciphering the molecular genetic and epigenetic basis of AML and in defining new diagnostic and prognostic markers. A growing number of recurring genetic changes have been recognized in the new World Health Organization (WHO) classification of AML. Furthermore, novel therapies are now being developed that target some of the genetic lesions. All these developments prompted an international expert panel to provide updated recommendations for the diagnosis and management of adult patients with AML, excluding acute promyelocytic leukemia (APL) for which recommendations were published separately. These recommendations provide standard requirements for general practice and for clinical trials. However, to accelerate progress in research the panel strongly recommends entry of AML patients on clinical trials, and storage of biosamples to enable correlative laboratory studies.

The following statements and recommendations are based on studies that predominantly were performed in the United States and Europe. Specific dosages and interventions may vary among different countries and populations.

2. Methods

2.1 Composition of the panel

The panel included 19 members with recognized clinical and research expertise in AML, of whom 13 came from European Union countries, 5 from the United States, and 1 from Japan. The panel met 4 times.

2.2 Scope of the review

Computerized literature searches of the PubMed, Cochrane, and Medline databases in the English language were conducted using key words relevant to the various sections of this article. Relevant abstracts presented at the 2006, 2007, and 2008 meetings of the American Society of Hematology, the European Hematology Association, and the American Society of Clinical Oncology were also reviewed. The categories of evidence and consensus were those used by the National Comprehensive Cancer Network (NCCN; www.nccn.org/professionals/physician_gls/PDF/aml.pdf). The vast majority of recommendations were category 2A recommendations, ie, they are based on low-level evidence and there is uniform panel consensus. The benefits are closely balanced with the risks and burdens, and the best action may differ depending upon circumstances.
3. WHO classification

The recent WHO classification reflects the fact that an increasing number of acute leukemias can be categorized based upon their underlying cytogenetic or molecular genetic abnormalities, and that these genetic changes form clinico-pathologic-genetic entities (Table 1).\(^3,5\) The subgroup “AML with recurrent genetic abnormalities” comprises several primary AML entities. “AML with t(8;21)(q22;q22); RUNX1-RUNX1T1” and “AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11” are considered as AML regardless of bone marrow blast counts. In “APL with t(15;17)(q22;q12); PML-RARA”, RARA translocations with other partner genes are recognized separately. The former category “AML with 11q23 (MLL) abnormalities” was re-defined in that “AML with t(9;11)(p22;q23); MLLT3-MLL” is now a unique entity; balanced translocations other than that involving MLLT3 should be specified in the diagnosis. Three new cytogenetically defined entities were incorporated: “AML with t(6;9)(p23;q34); DEK-NUP214”; “AML with inv(3)(q21;q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1”; and “AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1”, a rare leukemia most commonly occurring in infants. Two new provisional entities defined by the presence of gene mutations were added, “AML with mutated NPM1 [nucleophosmin (nucleolar phosphoprotein B23, numatrin)]”, and “AML with mutated CEBPA [CCAAT/enhancer binding protein (C/EBP), alpha]”. There is growing evidence that these two gene mutations represent primary genetic lesions (so-called class II mutations)\(^6\) that impair hematopoietic differentiation. Mutations in the fms-related tyrosine kinase 3 (FLT3) gene are found in many AML subtypes and are considered class I mutations conferring a proliferation and/or survival advantage. AML with FLT3 mutations are not considered a distinct entity, although determining the presence of such mutations is recommended by WHO since they have prognostic significance.

The former subgroup termed “AML with multilineage dysplasia” is now designated “AML with myelodysplasia-related changes”. Dysplasia in ≥50% of cells in ≥2 hematopoietic cell lineages was the diagnostic criterion for the former subset. However, the clinical significance of this morphological feature has been questioned.\(^7,8\) AMLs are now categorized as “AML with myelodysplasia-related changes” if a) they have a previous history of myelodysplastic syndrome (MDS) or myelodysplastic/myeloproliferative neoplasm (MDS/MPN) and evolve to AML with a marrow or blood blast count ≥20%; b) they have a myelodysplasia-related cytogenetic abnormality (listed in a footnote to Table 1); or c) if ≥50% of cells in ≥2 myeloid lineages are dysplastic.

“Therapy-related myeloid neoplasms” has remained a distinct entity; however, since most patients have received treatment using both alkylating agents and drugs that target topoisomerase II for prior malignancy, a division according to the type of previous therapy is often not feasible. Therefore, therapy-related myeloid neoplasms are no longer sub-
categorized. Myeloid proliferations related to Down syndrome are now listed as distinct entities.

4. Diagnostic procedures

4.1 Morphology

A bone marrow aspirate is part of the routine diagnostic work-up of a patient with suspected AML. The panel considers a marrow trephine biopsy optional, but it should be performed in patients with a dry tap (punctio sicca).

Blood and marrow smears are morphologically examined using May-Grünwald-Giemsa or Wright-Giemsa stain. It is recommended that at least 200 leukocytes on blood smears and 500 nucleated cells on marrow smears be counted, with the latter containing spicules. For a diagnosis of AML, a marrow or blood blast count of $\geq 20\%$ 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.

To identify lineage involvement some countries still rely more on cytochemistry, rather than on immunophenotyping (usually by flow cytometry), using myeloperoxidase (MPO) or Sudan black B (SBB) and non-specific esterase (NSE) stains. Detection of MPO (if present in $\geq 3\%$ 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. NSE stains show diffuse cytoplasmic activity in monoblasts (usually $>80\%$ positive) and monocytes (usually $>20\%$ positive). 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.

4.2 Immunophenotyping

Immunophenotyping using multiparameter (commonly at least 3- to 4-color) flow cytometry is used to determine lineage involvement of a newly diagnosed acute leukemia (Table 2). There is no general consensus on the cut-off point for considering an acute leukemia to be positive for a marker. For most markers, a commonly used criterion is $\geq 20\%$ of leukemic cells expressing the marker, whereas for selected markers (eg, cytoplasmic CD3, MPO, TdT, CD34, CD117) a lower cut-off has been applied ($10\%$). Quantification of expression patterns of several surface and cytoplasmic antigens is necessary for lineage assignment, to diagnose mixed phenotype acute leukemia (MPAL), and to detect aberrant immunophenotypes allowing for measurement of minimal residual disease (MRD). Flow cytometry determination of blast count should not be used as a substitute for morphological evaluation.
Immunophenotyping is required to establish the diagnosis of AML with minimal differentiation, acute megakaryoblastic leukemia, and acute leukemias of ambiguous lineage. AML with minimal differentiation is an AML without morphologic and cytochemical evidence of myeloid differentiation. Most cases express early hematopoiesis-associated antigens (eg, CD34, CD38, and HLA-DR) and lack most markers of myeloid and monocytic maturation; while MPO is negative by cytochemistry, detection of intracytoplasmic MPO antigens may be positive by flow cytometry in at least a fraction of blasts. Acute megakaryoblastic leukemia is a leukemia with ≥20% blasts of which ≥50% are of megakaryocytic lineage; megakaryoblasts typically express one or more of the platelet glycoproteins CD41 and/or CD61, and less commonly CD42. Acute leukemias of ambiguous lineage are rare leukemias and comprise those cases that show no evidence of lineage differentiation (ie, acute undifferentiated leukemia, AUL) or those with blasts that express markers of more than one lineage (ie, MPAL). AULs often express HLA-DR, CD34, and/or CD38, but by definition lack lineage-associated markers. MPAL can either contain distinct blast populations of different lineages, or one blast population with markers of different lineages on the same cell, or a combination of both. MPAL as defined by WHO encompasses several subsets, with or without an underlying genetic abnormality (Table 1).

**BCR-ABL1** positive acute leukemia immunophenotypically may present as MPAL (Table 1). Such leukemias should not be treated as AML, but rather as ALL with incorporation of an ABL1 tyrosine kinase inhibitor with chemotherapy. Also, in **BCR-ABL1** positive leukemias, the differential diagnosis of chronic myelogenous leukemia (CML) blast crisis should be considered.

Some AMLs with recurrent genetic abnormalities are associated with characteristic immunophenotypic features. For example, AMLs with t(8;21) frequently express the lymphoid markers CD19 or, to a lesser extent, CD7; they may also express CD56; AMLs with inv(16) frequently express the T-lineage associated marker CD2; and AMLs with **NPM1** mutation typically have high CD33 but absent or low CD34 expression.

### 4.3 Cytogenetics

Conventional cytogenetics analysis is a mandatory component in the diagnostic evaluation of a patient with suspected acute leukemia. Chromosome abnormalities are detected in approximately 55% of adult AML. Seven recurrent balanced translocations and inversions, and their variants, are recognized in the WHO category “AML with recurrent genetic abnormalities”. Furthermore, a number of cytogenetic abnormalities are considered sufficient to establish the WHO diagnosis of AML with myelodysplasia-related features when ≥20% blood or marrow blasts are present (Table 1).
A minimum of 20 metaphase cells analyzed from bone marrow is considered mandatory to establish the diagnosis of a normal karyotype, and recommended to define an abnormal karyotype. Abnormal karyotypes may be diagnosed from blood specimens.

4.4 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.

4.5 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, since RNA is suitable for molecular screening for fusion genes and leukemia-associated mutations.

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, 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.

Somatically acquired mutations have been identified in several genes, eg, the NPM1 gene, the FLT3 gene, the CEBPA gene, the myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila) (MLL) gene, the neuroblastoma RAS viral (v-ras) oncogene homolog (NRAS) gene, the Wilms tumor 1 (WT1) gene, the v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (KIT) gene, the runt-related transcription factor 1 (RUNX1) gene, the tet oncogene family member 2 (TET2) gene, and the isocitrate dehydrogenase 1 (NADP+), soluble (IDH1) gene. The frequencies of these gene mutations vary among cytogenetic groups.

AML with mutations in NPM1 or CEBPA have been incorporated in the WHO classification as provisional entities. Screening for these two markers as well as for FLT3 mutations should be done in clinical trials. While testing for NPM1, CEBPA, and FLT3 is currently not considered mandatory outside clinical trials, the panel recommends that these three 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.

4.6 Genome-wide studies

Recent progress in genomics technology has resulted in the identification of novel genetic abnormalities and holds the promise of making the systematic characterization of
cancer genomes feasible.\textsuperscript{35,36} For example, gene- and microRNA-expression profiling have proven valuable for the discovery of novel leukemia subgroups and of prognostic signatures.\textsuperscript{37-39} The introduction of genome-wide single nucleotide polymorphism (SNP)-based mapping arrays, providing both copy number and allele-specific information, led to the identification of a novel mechanism involved in the pathogenesis of AML, ie, uniparental disomy (UPD).\textsuperscript{40} Acquired UPD is due to a mitotic recombination event and may render a cell homozygous for a pre-existing mutation located in the affected genomic region. The power of SNP genotyping as a tool for gene discovery is shown by several recent studies.\textsuperscript{31,32,41,42} While analyses of genomic copy number will continue to be informative with regard to selection of candidate leukemia genes, it is also hoped that high-throughput DNA sequence analysis will become possible at an affordable cost, which may ultimately result in the development of comprehensive, disease- and allele-specific oncogene mutation profiling strategies.\textsuperscript{43} Finally, functional genetic approaches, such as large-scale RNA interference screens, have great potential for the identification of novel cancer genes. An example is a recent study in which graded down-regulation of multiple candidate genes by RNA interference was used to identify \textit{RPS14} as a causal gene for the MDS 5q- syndrome.\textsuperscript{44}

4.7 Biobanking

Within clinical trials, we strongly recommend storing patients' pretreatment leukemic marrow and blood within a biobank. A prerequisite for biobanking is the patient's informed consent that ideally should allow a broad spectrum of correlative laboratory studies that also include analysis of germline DNA. Pretreatment samples should include nucleic acid (DNA and RNA, stored at -80°C) and viable cells (stored at -196°C). For further optional storage we advise saving germline DNA (eg, from a buccal swab, skin biopsy, or sputum), a plasma sample, a methanol/acetic acid-fixed cell pellet (from cytogenetic analysis), and frozen cell pellets from various time points during and after treatment [ie, at the time of complete remission (CR), at relapse; and for MRD monitoring at defined time points during treatment and follow-up], stored under appropriate conditions.

4.8 Other diagnostic tests

Additional diagnostic tests and procedures in the initial work-up of a patient with AML are given in Table 3.

5. Prognostic factors

Prognostic factors may be subdivided into those related to patient characteristics and general health condition and those related to characteristics particular to the AML clone. The former subset usually predicts treatment-related mortality (TRM) and becomes more important as patient age increases while the latter predicts resistance to, at least, conventional therapy.

5.1 Patient-related factors
Increasing age is an adverse prognostic factor.\textsuperscript{45,46} Even after accounting for risk factors, such as cytogenetics, molecular genetics, type of AML (ie, \textit{de novo} AML; AML with previous history of MDS or MDS/MPN; therapy-related AML), and performance status, older patients have worse outcomes than younger patients, suggesting the effect of unknown age-related factors. Nonetheless, calendar age alone should not be a reason for not offering potentially curative therapy to an older patient,\textsuperscript{46} since age is not the most important prognostic factor for either TRM or resistance to therapy. Attention should be given to a careful evaluation and documentation of comorbidities. In a recent study of patients \textgreater 60 years of age receiving induction therapy (idarubicin 12 mg/m\textsuperscript{2} for 3 days, cytarabine 1.5 g/m\textsuperscript{2} for 3 days),\textsuperscript{47} scoring of baseline comorbidities using the hematopoietic cell transplantation comorbidity index (HCTCI)\textsuperscript{48} was predictive of early death rates and overall survival (OS). Comorbidity scoring is a current field of investigation and should contribute to a better definition of the patient considered “unfit” for intensive chemotherapy.

5.2 AML-related factors

AML-related prognostic factors includes white blood count (WBC), existence of prior MDS, previous cytotoxic therapy for another disorder (see section 9), and cytogenetic and molecular genetic changes in the leukemic cells at diagnosis. Various other factors, such as splenomegaly and elevated serum lactate dehydrogenase (LDH) levels, have been reported to confer some prognostic effect but with variable consistency among studies. The significance of a prognostic factor is always dependent on the therapy given to a patient.

5.2.1 Cytogenetics

The karyotype of the leukemic cells is the strongest prognostic factor for response to induction therapy and for survival.\textsuperscript{16,17} Younger adult patients are commonly categorized into three risk groups, favorable, intermediate or adverse.\textsuperscript{49-52} The most appropriate risk group assignment for a number of the rarer cytogenetic abnormalities, eg, del(7q), isolated trisomy 8, del(9q), t(v;11)(v;q23) other than t(9;11), and del(20q), remains uncertain due to limitations of sample size and differences in treatment schedule among studies. The impact of secondary genetic lesions in cases with balanced translocations or inversions requires further investigation. With the possible exception of trisomy 22 in AML with inv(16) or t(16;16) that has been associated with an improved relapse-free survival (RFS),\textsuperscript{53,54} no such impact has been shown for other secondary cytogenetic changes.

Complex karyotype, which occurs in 10-12\% of patients, has consistently been associated with a very poor outcome.\textsuperscript{55} Complex karyotype has been defined as the presence of \textgeq 3 (in some studies \textgeq 5) chromosome abnormalities in the absence of t(8;21), inv(16) or t(16;16), and t(15;17), since in most studies increased karyotype complexity in these subgroups did not adversely affect outcome. As indicated in the new WHO classification, cases with other recurring genetic abnormalities, such as AML with t(9;11) or
t(v;11), AML with inv(3) or t(3;3), and AML with t(6;9) should also be excluded, since these groups constitute separate entities. The nonrandom pattern of abnormalities within complex karyotypes includes a paucity of balanced rearrangements, and a predominance of chromosomal imbalances; losses most frequently affect 5q, 17p, and 7q, and gains 8q, 11q, and 21q. Prominent features of complex karyotype cases are the frequent loss of 17p and/or TP53 gene mutation, occurring in about two-thirds of the cases, and a high prevalence of high-level DNA amplifications. Recently, a new cytogenetic category has been proposed that distinguishes AML of particularly unfavorable risk, ie, the monosomal karyotype. In this study, the monosomal karyotype was defined by the presence of one single monosomy (excluding isolated loss of X or Y) in association with at least one additional monosomy or structural chromosome abnormality (excluding core binding factor [CBF] AML).

One striking observation is the increasing incidence of adverse versus favorable cytogenetic abnormalities with increasing age. This, at least in part, contributes to the poorer outcome of AML in older adults. A number of cytogenetic risk classifications have been proposed for elderly patients.

5.2.2 Molecular genetics

Gene mutations and deregulated gene expression have been identified that allow us to decipher the genetic diversity within defined cytogenetic groups, in particular the large and heterogeneous group of patients with CN-AML (Figure 1). Prognostic significance within CN-AML has consistently been shown for mutations in the NPM1, CEBPA, and FLT3 genes alone or in combination in younger adult patients.

CN-AML patients harboring internal tandem duplication (ITD) of the FLT3 gene have an inferior outcome compared with cases without FLT3-ITD. There is also evidence that outcome may be more related to the level of the mutated allele, rather than its mere presence, or to the insertion site of the ITD. The prognostic relevance of FLT3 tyrosine kinase domain (TKD) mutations (at codons D835 and I836) remains controversial.

In several, but not all studies, the presence of NPM1 mutation in CN-AML has been associated with higher CR rates and better RFS and event-free survival (EFS). Of note, approximately 40% of patients with NPM1 mutations also carry FLT3-ITD, and multiple studies have showed that the genotype “mutated NPM1 without FLT3-ITD” represents a favorable prognostic marker, with higher CR rates, and better RFS and OS that is reminiscent of that seen in patients with inv(16) or t(8;21). This favorable impact of mutated NPM1 (with or without FLT3-ITD) on survival endpoints also seems to hold up among patients of older age. 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. Two recent studies suggest
that there is heterogeneity among mutated CEBPA cases, in that only cases with double mutations, usually biallelic, have a favorable outcome.\textsuperscript{90,91} It remains an open question whether the presence of a FLT3-ITD impacts on prognosis in patients with mutant CEBPA.\textsuperscript{65,92} In cytogenetically favorable CBF AML [ie, 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.\textsuperscript{93-97}

There is a growing list of genetic abnormalities that are being investigated (Table 3). These include mutation analyses of the WT1,\textsuperscript{28,98-101} RUNX1,\textsuperscript{30,102,103} TET2,\textsuperscript{31,32} and IDH1 genes,\textsuperscript{33} and the analyses of gene expression signatures,\textsuperscript{37,38} or of deregulated expression of single genes, such as EVI1,\textsuperscript{19} ERG,\textsuperscript{104} MN1,\textsuperscript{105,106} and BAALC\textsuperscript{107} genes.

5.2.3 Monitoring of minimal residual disease

The monitoring of MRD as determined by RT-PCR detecting leukemia-specific targets (eg, gene fusions, gene mutations, overexpressed genes), or by multiparameter flow cytometry identifying leukemia-associated aberrant phenotypes remains an active field of investigation.\textsuperscript{108} Despite technical developments, there is still, except for APL,\textsuperscript{4} a paucity of large prospective trials demonstrating its clinical utility.\textsuperscript{108} Potentially useful applications of MRD monitoring include early assessment of response to therapy to improve risk stratification and guide post-remission therapy; and post-treatment monitoring to detect impending relapse and guide preemptive therapy. The kinetics of RUNX1-RUNX1T1 and CBFB-MYH11 decline has been found to correlate with risk of relapse, or to represent a prognostic factor independent of other pre-treatment variables.\textsuperscript{108-110} Following consolidation therapy, low-level PCR-positivity can be detected in patients even in long-term remission of CBF AML. Normalizing to $10^4$ copies of ABL1 in accordance with standardized criteria, transcript levels below 12 to 10 copies appear to predict long-term remission.\textsuperscript{109,110} Real-time quantitative (RQ)-PCR assays have been developed for other fusion gene targets such as MLLT3-MLL and DEK-NUP214, but data are very scarce due to the low frequencies of these leukemias.\textsuperscript{108} NPM1 mutations likely provide one of the most promising new targets and studies are ongoing to evaluate the clinical utility of MRD monitoring in AML with NPM1 mutation.\textsuperscript{111}

The major advantage of using flow cytometric detection of MRD lies in its applicability to virtually all patients. Although its sensitivity, as reported in previous studies, is at least one log below that of RQ-PCR assays, the sensitivity will likely be improved by the use of 6- to 8-color laser technology. Among 8 studies,\textsuperscript{108} 7 demonstrated that immunophenotypic detection of MRD in AML following induction and consolidation provides independent prognostic information. Validation, using larger patient cohorts and modern technology, is ongoing.
5.3 **Standardized reporting system for genetic abnormalities**

The panel proposes a standardized reporting system for genetic abnormalities when presenting data correlating genetic findings with clinical outcome allowing for a better comparison of data among studies (Table 4). This standardized report includes data from cytogenetic analysis and from mutation analyses of the *NPM1, CEBPA*, and *FLT3* genes.

6. **Response criteria and survival outcomes**

Most response criteria and survival measures as described in the previous recommendations have been widely used by clinicians and cooperative groups (for definitions see Tables 5 and 6).\(^2\) Response criteria should meet the specific objective of a study, eg, in phase II and III clinical trials CR or CR with incomplete blood recovery (CRi) should be the appropriate endpoint, whereas in phase I clinical trials, the criteria of partial remission (PR) and morphologic leukemia-free state may also be useful. Other response criteria are cytogenetic CR (CRc)\(^{112-115}\) and molecular CR (CRm) (Table 5).

*Response assessment.* Following conventional induction therapy with 3 days of an anthracycline and 7 days of cytarabine (“3+7”) or therapies of comparable intensity, response assessment is commonly performed between day 21 and 28 after start of therapy. The exact timing may vary among protocols and should meet the specific objectives of the study.

*Early response assessment.* Early response assessment may be required in investigational studies to evaluate the antileukemic efficacy of a novel agent, or to guide subsequent treatment, eg, with protocols applying intensified induction regimens. It is made at 7-10 days following chemotherapy. Bone marrow at that time is usually hypoplastic or aplastic, documenting the anti-leukemia effect.

*Response assessment during follow-up period.* Within clinical trials, it is usually recommended to perform repeat marrow aspirates every 3 months for the first two years; in some cases, surveillance continues every 6 months for the following 2-3 years. Most relapses occur within 1-3 years after the end of therapy. Standardized time points are necessary if MRD monitoring is performed. Outside clinical trials, repeat marrow aspirates may not be needed, and should be done only if blood counts become abnormal.\(^{116}\) Blood counts should be done every 1-3 months for the first two years, then every 3-6 months up to five years.

7. **Management of younger adults (18-60 years)**

7.1 **Induction therapy**

Three days of an anthracycline (eg, daunorubicin, at least 60 mg/m\(^2\) [higher doses are being explored], idarubicin, 10-12 mg/m\(^2\), or the anthracenedione mitoxantrone, 10-12 mg/m\(^2\)) and 7 days of cytarabine (100-200 mg/m\(^2\) cont. iv) (“3+7”) currently remains the standard for induction therapy. With such regimens, CR is achieved in 60-80% of younger adults. No other intervention has been convincingly shown to be better.\(^{117,118}\) 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.\textsuperscript{119}

\textit{Alternative anthracyclines, high-dose cytarabine, additional agents given with conventional induction chemotherapy.} Randomized studies have compared daunorubicin at a dose of 45-60 mg/m\textsuperscript{2} with other anthracyclines, such as idarubicin\textsuperscript{120-123} or aclacinomycin,\textsuperscript{124} with amsacrine,\textsuperscript{125} or with mitoxantrone.\textsuperscript{126} With respect to OS, it is not clear whether any agent is superior to daunorubicin at equivalent doses. High-dose cytarabine (HiDAC) combined with daunorubicin in induction has been studied by the Southwest Oncology Group (SWOG) (2 g/m\textsuperscript{2}/q12h on days 1-6),\textsuperscript{127} and the Australian Leukemia Study Group (ALSG) (3 g/m\textsuperscript{2}/q12h on days 1, 3, 5, and 7)\textsuperscript{128} in prospective randomized trials, and by the Eastern Cooperative Oncology Group (ECOG) (3 g/m\textsuperscript{2}/q12h on days 1, 3, and 5)\textsuperscript{129} and SWOG (cytarabine 100 mg/m\textsuperscript{2} cont. iv on days 1-7, followed by HiDAC 2g/m\textsuperscript{2}/q12h on days 8-10; “3+7+3”)\textsuperscript{130} in phase II trials. Neither randomized trial showed a higher CR rate with HiDAC, and both demonstrated increased toxicity. In a trial by the AML Cooperative Group (AMLCG), 1 versus 2 courses with HiDAC (3 g/m\textsuperscript{2}/q12h on days 1-3) in induction produced equal CR rates, disease-free survival (DFS) and moderate toxicity.\textsuperscript{131} Therefore, it is not generally recommended that HiDAC be included in induction regimens outside clinical trials.

Attempts to increase response rates by the use of additional cytotoxic agents (thioguanine, etoposide, fludarabine, topotecan), or modulators of multidrug resistance (MDR) in general have failed.\textsuperscript{132-137} Sensitization of leukemic cells with hematopoietic growth factors, such as granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF), has been studied in order to increase cytotoxicity of chemotherapy.\textsuperscript{138} The Dutch-Belgian Hemato-Oncology Cooperative Group (HOVON) and Swiss Group for Clinical Cancer Research (SAKK) showed priming with G-CSF resulted in a significantly better DFS, and in the intermediate-risk group also a significantly better OS.\textsuperscript{139} Similarly, in a study by the Acute Leukemia French Association (ALFA) group, priming with GM-CSF resulted in a higher CR rate and a better EFS, in particular in the intermediate-risk group, albeit without influencing OS.\textsuperscript{140} In contrast, in a study by the AMLCG, G-CSF priming did not impact OS or RFS.\textsuperscript{141} Priming with growth factors remains an active field of clinical investigation; it cannot be recommended in routine practice. Another area of induction therapy research is the evaluation of gemtuzumab ozogamicin (GO) administered with conventional chemotherapy (see section 11.1).

\textbf{7.2 Post-remission therapy}

\textbf{7.2.1 Post-remission strategies}
Various types of post-remission strategies have been evaluated including intensive conventional chemotherapy, prolonged maintenance treatment, and high-dose therapy followed by autologous or allogeneic hematopoietic stem cell transplantation (HSCT).117,118

**High-dose cytarabine.** A landmark study performed by Cancer and Leukemia Group B (CALGB) showed that 4 cycles of HiDAC (3 g/m²/q12h on days 1, 3, and 5) is superior to 4 courses of intermediate- (400 mg/m² continuous iv on days 1-5) or standard-dose (100 mg/m² continuous iv on days 1-5) cytarabine; patients were scheduled to also receive 4 courses of monthly maintenance treatment.142 This beneficial effect of cytarabine dose intensification, however, was restricted to patients with CBF AML and, to a lesser extent, to patients with CN-AML, whereas outcome of patients with other cytogenetic abnormalities was not affected by cytarabine dose.143 There remain open questions regarding the number of cycles, the most appropriate dose and schedule, and the role of combining HiDAC with other agents. Outcome results similar to those after HiDAC consolidation may be obtained using other intense chemotherapy regimens. However, use of prolonged intensive consolidation,144 or of multiagent chemotherapy does not appear to be superior to HiDAC alone.145,146

**Maintenance therapy.** 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 DFS to 1 course of consolidation according to the S-HAM protocol (HiDAC 1g/m²/q12h on days 1, 2, 8, and 9; mitoxantrone 10 mg/m² on days 3, 4, 10, and 11).131 Maintenance chemotherapy is generally not routinely administered outside of clinical trials for patients with non-APL AML.

**Autologous hematopoietic stem cell transplantation.** 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.50,148,149 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.150

**Allogeneic hematopoietic stem cell transplantation.** Allogeneic HSCT as a post-remission strategy is associated with the lowest rates of relapse. This benefit is attributable to both the high dose therapy of standard conditioning regimens and a potent graft-versus-leukemia (GVL) effect.151 However, benefits of allogeneic HSCT have been limited by the high TRM. Single prospective trials have neither shown a definitive advantage nor disadvantage in OS of allogeneic HSCT for patients with AML in first CR (CR1).152-155 Meta-analyses of clinical trials that prospectively assigned allogeneic HSCT versus alternative consolidation therapies for AML in 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.156-158

The value of allogeneic HSCT needs to be reassessed based on the identification of AML-related genetic changes that profoundly impact on prognosis, on the availability of different transplant sources (bone marrow, blood) and donor types (matched related, unrelated and haploidentical donors, umbilical cord stem cell grafts), and in the light of the use of reduced-intensity conditioning (RIC) regimens.65,159 Finally, it is important to consider TRM that may vary between <15% up to 50%. It is essential to assess whether the benefit of the reduced relapse rate outweighs TRM or will be offset by a high TRM. Comorbidity scores, such as the HCTCI,48,160 provide useful guidance in these decisions. Furthermore, a composite risk score, previously established for CML,161 that includes patient age, disease stage, time interval from diagnosis to transplant, donor type, and donor-recipient gender combination, has been shown to be highly predictive of TRM, leukemia-free survival, and OS also in patients with AML.162 Further risk factors include cytomegalovirus serum status of recipient and donor,163 and non-HLA genetics, ie, SNPs or microsatellites of cytokines, cytokine receptor genes, or genes associated with innate immunity.164

Thus, for individual clinical decision making, it is recommended to take into account both the disease risk as best assessed by the cytogenetic and molecular genetic profile of the leukemia and the risk associated with the transplant itself as assessed by comorbidity and other transplant-related risk indices.

7.2.2 Post-remission therapy according to cytogenetic and molecular genetic risk

Favorable-risk AML. Post-remission therapy with repetitive cycles of HiDAC (3 g/m²/q12h on days 1, 3, and 5) is considered a reasonable choice for younger adult patients with CBF AML,53,54,165,166 and also for AML with mutated NPM1 without FLT3-ITD and with mutated CEBPA.65

For CBF AML, retrospective studies by CALGB suggest that ≥3 cycles of HiDAC (cumulative dose: 54-72 g/m²) are superior to only one cycle (18 g/m²).164,165 No advantage has been shown for autologous or allogeneic HSCT in front-line treatment.53,156-158,167-169 Nonetheless, there are subsets of CBF AML that do rather poorly (eg, t(8;21) with high WBCs, CBF AML with KIT mutations or molecular disease persistence); allogeneic HSCT may be considered in these patients, especially for those with a low transplant risk (eg, European Bone Marrow Transplant [EBMT] risk 0-1, CMV negative serostatus, no comorbidity), although such a strategy should be investigated within a clinical trial.

A study by the German-Austrian AML Study Group (AMLSG) provided evidence that those AML patients whose molecular genetic profile predicts a favorable prognosis, such as CN-AML with mutated NPM1 without FLT3-ITD, may also not benefit from allogeneic HSCT.65 Thus, in general, patients with such favorable-risk AML are not considered
candidates for allogeneic HSCT, unless they have a very low transplant risk or new transplant strategies (eg, RIC HSCT) are evaluated within a clinical trial.

Intermediate-risk AML. For the remaining patients with CN-AML (intermediate-I) and those with intermediate-2 karyotypes (Table 4), repetitive cycles of HiDAC (3-4 cycles; 3 g/m²/q12h on days 1, 3, and 5) are currently widely used by many cooperative groups; however, outcome for most of the subsets remains unsatisfactory. There is accumulating evidence that allogeneic HSCT is an attractive option for those patients who are at high risk of relapse. The benefit might be highest for patients with a low or intermediate transplant risk. A beneficial effect has been shown for patients with intermediate-risk cytogenetics in general,156-159 and for patients with CN-AML and unfavorable molecular markers, ie, those who lack the favorable genotypes of mutated \textit{NPM1} without \textit{FLT3}-ITD or mutated \textit{CEBPA}.65 In particular, although evidence from prospective trials is not available, allogeneic HSCT should be considered in patients whose leukemic cells have \textit{FLT3}-ITD.65,170

Adverse-risk AML. For most patients with high-risk cytogenetics, outcome remains dismal with conventional consolidation therapy.49-52,143 An allogeneic HSCT from a matched related donor is currently considered the treatment of choice for patients with unfavorable cytogenetics in CR1; this recommendation is based on results from single studies50,155,156,171 as well as from meta-analyses.156-158 The US Intergroup Study demonstrated an advantage for allogeneic HSCT for patients with unfavorable cytogenetics with a survival of 44% versus 15% for patients receiving only a single cycle of HiDAC consolidation chemotherapy, although the number of patients was small and the consolidation limited.50 Data from the European Organization for Research and Treatment of Cancer (EORTC) / Gruppo Italiano Malattie Ematologiche d’Adulto (GIMEMA) AML-10 trial and from three consecutive trials of the HOVON-SAKK group with a larger number of patients demonstrate a benefit for allogeneic HSCT, among younger patients with adverse cytogenetics.155,156

The outcome after allogeneic HSCT from fully matched unrelated donors (defined by molecular high resolution HLA typing) appears to be similar compared with allogeneic HSCT from matched related donors. The Center for International Blood and Marrow Transplant Research (CIBMTR) recently reported a long-term survival probability of 30% for AML patients with adverse cytogenetics transplanted in CR1 from matched unrelated donors.172 Given the dismal results after conventional chemotherapy, allogeneic HSCT from either matched related or unrelated donors in CR1 is therefore considered a reasonable treatment option for patients with unfavorable cytogenetics.

7.3 Primary refractory disease

Several studies have shown that lack of early blast clearance or non-response to the first induction cycle are major predictors for poor outcome, and conventional chemotherapy offers almost no chance of cure for these patients.148,173,174 Consequently, allogeneic HSCT
has been widely used for these patients. Retrospective studies show outcome is limited by a high relapse rate and a high non-relapse mortality leading to OS rates of 20-30%. To improve on these results alternative conditioning regimens are being investigated. One approach evaluated in a multicenter trial is a sequential strategy of intensive chemotherapy followed, after three days of rest, by RIC for allogeneic HSCT, and prophylactic administration of donor lymphocyte infusions. A prerequisite for success for such a transplant strategy is rapid identification of a suitable matched donor. HiDAC, if not used for first induction, with or without an anthracycline may be considered for salvage therapy prior to allogeneic HSCT. Patients with induction failure who are not eligible for allogeneic HSCT should be considered for clinical trials evaluating novel agents.

8. Management of older patients (\(\geq 60\) years)

Although the prognosis of AML probably worsens with each year of increasing age, “older” patients are generally considered those \(\geq 60\). Older patients are more likely to suffer treatment-related early death and to exhibit therapeutic resistance. Increasing age is associated with factors predictive of early death, eg, poor performance status or various comorbidities, and of treatment resistance, eg, adverse cytogenetics, secondary AML, or the MDR phenotype. Even after accounting for these associations, older age remains an important predictor of poor outcome. Evidence is accumulating to indicate that in older individuals age-dependent leukemia-specific differences also account for reduced treatment response. Older age \textit{per se}, however, should not be a reason to withhold intensive therapy. Studies suggest that remission induction chemotherapy provides better quality of life and longer survival than supportive care only. Thus, these patients often deserve being offered the option of standard chemotherapy. Older patients from a clinical practice perspective may be divided according to whether they are 60-74 or \(\geq 75\) years of age.

8.1 Patients age 60-74

\textit{Induction therapy.} For patients with performance status <2 and no comorbidities, standard induction therapy is often a plausible option resulting in CR rates averaging 50% and rates of death in aplasia or from indeterminate cause below 15%. Similar to younger adults, induction therapy generally consists of 3 days of an anthracycline (eg, daunorubicin 45-60 mg/m\(^2\) or an alternative anthracycline at equivalent dose), and 7 days of cytarabine (100-200 mg/m\(^2\) continuous iv). Dose reduction may be considered for individual patients. Both American and European cooperative group studies have found that the choice of anthracycline (daunorubicin or idarubicin, or the anthracenedione mitoxantrone) is of little consequence, assuming equitoxic doses are administered. The AMLCG over 15 years has used 60 mg/m\(^2\) daunorubicin with acceptable toxicity. The HOVON/SAKK/AMLSG recently
showed that daunorubicin can be dose-intensified to 3 x 90 mg/m² in older patients up to 65 years with more CRs and better survival, without marked additional toxicity.188

The degree of acceptability of administering standard induction therapy depends greatly on cytogenetics. Adverse cytogenetics is a strong independent prognostic factor for failure to achieve CR and OS.61-63 For this subset of older patients, CR rates are ≤30%, and OS is <5%. Thus, although the karyotype may be unknown at diagnosis in most centers, patients known to have adverse cytogenetics, even those with a good performance status and lacking comorbidities, may be considered for investigational therapies, or, if such therapies are unavailable, for mild cytoreductive therapy only. Recent data suggest delays in initiating therapy may not be harmful in older patients, thus allowing individualized approaches.119

Post-remission therapy. Randomized studies in elderly patients achieving CR are biased since only a low proportion of the initial study cohort is randomized and the majority of these patients have intermediate- or favorable risk cytogenetics and lack significant comorbidities. These studies have generally compared “more” versus “less” post-remission therapy. In the British Medical Research Council (MRC) AML11 study, 4 post-remission courses of moderate intensity were compared with 1 course, with equivalent survival in the 2 arms.189 The CALGB compared 2 relatively intense cycles (cytarabine 500 mg/m²/q12h; mitoxantrone 5 mg/m²/q12h, each for 6 doses) with 4 less intensive cycles (cytarabine 100 mg/m² continuous iv on days 1-5) and found no differences.190 In the AMLCG92 trial, older patients benefited with longer remission duration from monthly myelosuppressive maintenance (cytarabine 100 mg/m²/q12h x 10 with an anthracycline or thioguanine) when compared with a single course of the S-HAM regimen (cytarabine dosage: 500 mg/m²/q12h on days 1, 2, 8, and 9).147 The French ALFA 9803 trial found that 6 cycles of outpatient consolidation (daunorubicin 45 mg/m² or idarubicin 9 mg/m² on day 1 and cytarabine 60 mg/m²/q12h s.c. on days 1-5) gave superior DFS and OS than 1 cycle of “4+7” consolidation.187 Despite its longer duration, the outpatient arm required less time in hospital and fewer red cell and platelet transfusions. In the AMLSG AML HD98-B trial, intensive consolidation (idarubicin 12 mg/m² on days 1 and 3; etoposide 100 mg/m² on days 1-5) was superior to a mild one-year oral maintenance therapy (idarubicin 5 mg p.o. on days 1, 4, 7, 10, and 13; etoposide 100 mg p.o. on days 1 and 13; q4 weeks).191 From these data no clear recommendation can be given. For patients without adverse cytogenetics, good performance status and no significant comorbidity, standard “3+7” induction followed by repetitive cycles of modest dose consolidation may be an acceptable norm, with recent results from the Swedish National Registry suggesting that this approach is associated with longer survival than lower doses of similar therapy.46
There is growing evidence that AML with a favorable genetic profile, ie, CBF AML and AML with mutated NPM1 (with or without FLT3-ITD), may benefit from dose escalation during consolidation.\textsuperscript{61-63,81-84,188} A recent AMLSG study suggests that AML with mutated NPM1 without FLT3-ITD may benefit from the addition of all-trans retinoic acid to intensive induction and consolidation therapy, although this finding awaits confirmation by further studies.\textsuperscript{81,192}

\textit{Allogeneic HSCT using reduced-intensity conditioning.} Allogeneic HSCT in older patients has become an active promising field of investigation.\textsuperscript{193-201} Non-myeloablative or RIC regimens have been developed to reduce TRM in older or medically less fit patients. A retrospective study from the Cooperative German Transplant Study Group of 368 patients (median age, 57 years; range, 50-73) suggests that matched unrelated and matched sibling donor allogeneic HSCT (72% had received RIC regimens) result in comparable survival in older AML patients.\textsuperscript{202}

Nevertheless, current data are difficult to interpret due to small patient cohorts, heterogeneity of conditioning regimens applied, and, most importantly, the considerable inherent patient selection bias in the higher age segment.\textsuperscript{200} Therefore, allogeneic HSCT should be performed within clinical trials. A prospective comparison of allogeneic HSCT from matched related and unrelated donors using RIC with conventional consolidation therapy has been launched by the EBMT group together with several cooperative groups (ClinicalTrials.gov Identifier: NCT00766779).

8.2 Patients Age $\geq$ 75

An alternative to standard dose induction should be sought for patients $\geq$ 75 (and probably $\geq$ 65) with a performance status of 2 or 3, comorbidities, or organ dysfunction. In a randomized trial,\textsuperscript{203} low-dose cytarabine (LDAC; 20 mg twice daily s.c. for 10 days) was associated with longer survival than hydroxyurea (sufficient dose to keep WBC $<10\times10^9$/L) and thus might be considered for such patients, but the magnitude of benefit is not so great as to make hydroxyurea or supportive care an unreasonable option. Even with this low-intensity approach, there was a 30-day mortality of 26%. Furthermore, there is no benefit of LDAC in patients with adverse cytogenetics.\textsuperscript{203,204} The choice of therapy very much depends on a patient’s wishes. Any discussion of choice of therapy must refer to observations that 74\% of older patients estimated that their chances of cure with “3+7” were $\geq$ 50\%; in contrast, 85\% of their physicians estimated this chance to be $<10\%$.\textsuperscript{205}

For patients age $\geq$ 75 but with a good performance status and no comorbidities, selection of treatment may again be contingent on cytogenetics and to a lesser extent on type of AML (\textit{de novo} vs. secondary following MDS or MDS/MPN). Patients age $\geq$ 66 with CBF AML have a 75\% CR rate and only a 16\% death rate with cytarabine-containing therapy, making standard therapy a very plausible option.\textsuperscript{206} Furthermore, some such
patients if particularly healthy might be candidates for more aggressive consolidation including moderate-dose cytarabine.\textsuperscript{61-63,83} Recent data suggest that patients age \( \geq 60 \) with CN-AML and mutated \textit{NPM1} (with or without \textit{FLT3}-ITD) benefit from standard 3+7 regimens.\textsuperscript{81-84}

### 8.3 Cautions and future directions

Patients entered onto clinical trials of either standard or investigational therapy represent a very small, and likely biased, subset\textsuperscript{207} and reported results overestimate the effectiveness of therapy in the general population of older AML.\textsuperscript{46,208} Physicians decide to give treatment to some patients but not others based on generally accurate perceptions of how patients will fare after such treatment,\textsuperscript{203} or on strict protocol exclusion criteria.\textsuperscript{207} Examining 2,657 Medicare beneficiaries age >65 in American Surveillance, Epidemiology, and End Results (SEER) registries, Menzin et al.\textsuperscript{208} found that 70% did not receive “chemotherapy”, ranging from 56% of patients age 65-74 to 94% of patients age \( \geq 85 \). In the Swedish Adult Acute Leukemia Registry, a true population-based registry, 198 of 727 (27%) patients age 65-74 and 800 of 1,115 (72%) patients age >74 did not receive intensive chemotherapy but palliation only.\textsuperscript{46}

A second problem is the heterogeneity present in any sizable defined group, but hidden by the terms “older patient”, “medically unfit patient”, or “patient ineligible for standard intensive chemotherapy”. Thus, there is a need to consider multiple variables. Besides age, the most important covariates are cytogenetics and secondary AML (following MDS or MDS/MPN), WBC, performance status, and comorbidities.\textsuperscript{207} No specific comorbidity index has yet been developed for older patients with AML; thus, only organ dysfunctions are currently taken into account.

As is apparent from the many new induction therapies being studied in older patients, it is easier to recognize that a patient needs investigational therapy than it is to specify what that therapy should be. Part of this difficulty reflects the tendency to report results of single-arm trials without reference to even a historical control group.\textsuperscript{210} Current examples of investigational therapies include clofarabine, cloretazine, azacitidine (or decitabine) +/- histone deacetylase inhibitors or GO, “3+7” or LDAC combined with GO, and FLT3 inhibitors in patients with activating \textit{FLT3} mutations (see section 11). Tipifarnib has been compared with best supportive care in older patients and was found not beneficial.\textsuperscript{211}

### 9. Therapy-related AML

Therapy-related AML (t-AML) is a recognized clinical syndrome occurring as a complication following cytotoxic and/or radiation therapy. With emerging new therapies, eg, treatment with monoclonal antibodies, small molecules, anti-hormone agents and growth factors, the term “t-AML” has blurred and its definition needs to be revisited. Therefore, it is important that previous therapies be meticulously documented and reported.
The etiology and specific factors that predispose to therapy-related myeloid neoplasms largely remain elusive. Various genetic pathways and cooperating mutations are involved in its pathogenesis. Two main groups have been described, one comprising leukemias arising 5-7 years following therapy with alkylating agents or irradiation and associated with abnormalities of chromosome arms 5q and/or 7q, and a second occurring with a shorter latency, within 2-3 years, after therapy with agents targeting topoisomerase II that are often associated with a translocation involving bands 11q23 (MLL) or 21q22 (RUNX1). However, there appear to be more pathways comprising chromosomal rearrangements and mutations in multiple genes (eg, TP53, RUNX1, RAS).

Survival of t-AML patients has been poor compared with that of patients with de novo AML. A number of factors may explain the poor outcome, such as persistence of the primary malignant disease, injury to organs from prior therapy, depletion of normal hematopoietic stem cells, damage to marrow stroma (in particular by radiation therapy), chronic immunosuppression or dysfunctional phagocytes leading to colonization with pathogenic or antibiotic-resistant bacteria and fungi, and refractoriness to transfusion support. Finally, t-AML is associated with a higher frequency of unfavorable cytogenetics. In multivariable analyses, however, t-AML appears to remain an independent adverse prognostic factor. Scarce data are available regarding whether t-AML changes outcome in general, or only in specific subsets. A recent study suggests that survival is also poorer in patients with therapy-related CBF AML compared with de novo CBF AML.

There is a paucity of prospective treatment data since patients with t-AML have often been excluded from front-line clinical trials. There are no randomized studies comparing standard AML therapy to other forms of treatment. In a review of 644 t-AML patients treated with various standard AML chemotherapy regimens, only 182 (28%) achieved a CR. Individual small series report CR rates of 40-50%. The treatment most likely to cure t-AML is allogeneic HSCT. In case series, OS appears to be about 20-30%. Nonmyeloablative, RIC allogeneic HSCT is under investigation for those who are not eligible for standard myeloablative HSCT. The EBMT Registry reported on 65 t-AML patients who underwent autologous HSCT; the 3-year OS was 35%.

Thus, primary considerations for treatment of t-AML patients should include the status of the primary cancer, the patient’s performance status, the presence of complications from primary therapy, and the leukemic karyotype. Patients with t-AML should be encouraged to participate in prospective trials that are designed for other AML patients with similar genetic changes. Patients who have an HLA-matched donor should be considered for allogeneic HSCT.

10. Relapsed AML
In the majority of patients with AML who achieve a CR, the leukemia will recur within 3 years after diagnosis. In general, the prognosis of patients following relapse is poor and treatment options unsatisfactory.²³²-²³⁵

10.1 Prognostic factors in relapsed AML

Because of the poor prognosis of patients in relapse, it would be useful to be able to assess whether treatment with curative intent is a realistic possibility for a particular patient. Long-term survival will depend on the ability to successfully induce a remission and the probability to consolidate with HSCT. Patients with an early relapse (ie, duration of CR1 <6 months), adverse cytogenetics, or older age have a poor outcome. An estimate of long-term survival can be obtained by assessing a defined set of prognostic factors as shown in Table 7.²³⁴ The prognostic score furnishes a basis for recommendations regarding treatment with curative objectives, or palliative therapy, or therapy in the context of a phase I/II study.

10.2 Reinduction of remission

There is a lack of prospective controlled studies evaluating different treatments in relapsed AML, and therefore no generally established standard. A commonly accepted approach is to define a treatment that is directed at achieving a new remission and that leads to HSCT. One mainstay of second-line treatment is cytarabine which can be employed at intermediate (1 g/m²) and high dose (2-3 g/m²) levels. An example of a salvage regimen is cytarabine (3 g/m²/q12 h on days 1, 3, 5, and 7) combined with daunorubicin (50 mg/m²) or idarubicin (10 mg/m²) on days 2, 4, and 6.²³⁶ Another schedule is single agent cytarabine for six days (2-3 g/m²/q12 h).²³⁷ Regimens that include cytarabine at high doses cannot be safely applied in patients aged ≥60 years because of an unacceptably high risk of toxicity. Another schedule is mitoxantrone (10 mg/m² per day) and etoposide (100 mg/m² per day) given for five days.²³⁸,²³⁹ GO (see section 11.1) can produce remissions in older patients, especially those relapsing after a prolonged (>6 months) CR1. Patients with short initial first remissions may be candidates for an investigational drug.

10.3 Salvage consolidation treatment including stem cell transplantation

Allogeneic HSCT is the preferred consolidation therapy once a new remission has been attained.¹⁷⁶,²³⁴ This can be a transplant from an HLA identical sibling donor, or a matched unrelated donor. If such a donor is not available, an alternative donor can be considered, ie, an umbilical cord blood unit, or a haplo-identical donor. In this situation, the risk of the disease has to be balanced with the risk of the transplant.²³⁴ The long-term value of allogeneic HSCT following RIC regimens remains to be evaluated but current experience suggests a greater relapse incidence as compared with transplants following high-dose preparative regimens. If an allogeneic HSCT is not possible (eg, due to lack of a suitable donor), an autologous HSCT is often regarded as the second best option.¹⁵⁰,²⁴⁰ However, often it is not possible to collect an adequate leukemia-free autograft at this phase of the
disease. Retrospective studies show that patients who achieve second CR and proceed to autologous HSCT have a probability of long-term survival in the range of 20-50%. The outcome obviously applies to a highly selected minority of patients. Patients who have already undergone an allogeneic HSCT prior to relapse generally have immunoprophylaxis discontinued, and they may receive donor lymphocyte infusions. These interventions are not an option in patients who already have active GVH disease. Finally, transplant recipients with a late relapse (>1 year after allogeneic HSCT) may be offered a second transplant.

11. Molecularly targeted therapy

Progress has been made in deciphering the molecular pathogenesis of AML, and in a few instances this has led to the development of molecularly targeted approaches. Genetic alterations comprise mutations in genes that activate signal transduction cascades (eg, FLT3, KIT, RAS), gene fusions or mutations resulting in enhanced or repressed transcriptional activity (eg, PML-RARA, RUNX1-RUNX1T1, CBFB-MYH11, CEBPA), or altered function of genes involved in nuclear cytoplasmic shuttling (eg, NPM1, NUP98, NUP214). Inhibition of deregulated transcriptional activity has, with the exception of the use of all-trans retinoic acid and arsenic trioxide in APL, proved to be a less tractable pharmacological goal compared with inhibition of constitutive tyrosine kinase activity. Finally, targeting the myeloid-associated antigen CD33 by the use of anti-CD33 monoclonal antibody has become an interesting new approach.

It is commonly accepted that the AML phenotype results from multiple genetic/epigenetic lesions affecting differentiation, proliferation, and apoptosis. Consequently, targeting of a single aberrant protein is unlikely to eradicate the leukemic clone. Furthermore, although several molecularly targeted therapies have been shown to be active in AML, it is clear from early clinical studies that most of these novel agents will need to be used in combination with conventional cytotoxic therapy. In the following sections, only three modalities are considered that have reached phase III clinical trial development for front-line therapy of AML.

11.1 Gemtuzumab ozogamicin

GO is a humanized anti-CD33 antibody chemically linked to the cytotoxic agent calicheamicin that inhibits DNA synthesis and induces apoptosis. GO is approved for relapsed AML (currently in the United States and Japan, but not in Europe) in older patients who are not considered candidates for other cytotoxic therapies. GO can produce remissions in 15-35% of older patients in first relapse. In a feasibility study in younger adults, the addition of GO to standard induction therapy led to a promising 91% CR rate. Randomized trials evaluating the addition of GO to conventional chemotherapy have been
completed (eg, MRC AML 15 trial; final results are pending) or are ongoing (eg, SWOG Protocol S0106; ClinicalTrials.gov Identifier: NCT00085709).

11.2 FLT3 inhibitors

A number of FLT3-selective tyrosine kinase inhibitors (eg, midostaurin [PKC412], lestaurtinib [CEP-701], sunitinib [SU11248]) have in vitro cytotoxicity to leukemia cells. These inhibitors have activity as a single agent in relapsed AML with FLT3 mutations, although usually only a transient reduction of blasts in blood, and, to a lesser extent, in marrow, has been observed. Pilot studies combining intensive induction and consolidation therapy with FLT3 inhibitors have shown promising response rates in patients with FLT3 mutations. A randomized phase III trial has been initiated evaluating midostaurin as front-line therapy of younger adult AML patients with activating FLT3 mutations (ClinicalTrials.gov Identifier: NCT00651261).

11.3 Demethylating agents

Two demethylating agents, the cytosine analogs azacitidine and decitabine, have been approved for the treatment of MDS, azacitidine by the Federal Drug Administration (FDA) and the European Medicine Agency (EMEA) and decitabine by the FDA. In a phase III randomized trial, azacitidine prolonged OS compared with conventional care regimens in patients with intermediate-2 or high-risk MDS. Approximately one-third of these patients (n=113) were classified as having AML under current WHO criteria (blast counts 20-30%). Of note, in these patients, the 2-year OS was 50% with azacitidine compared with 16% with conventional treatment regimens. Based on these results, azacitidine has been approved for older AML with 20-30% blasts. While these results are promising, data for more proliferative AML are currently not available. Randomized trials comparing demethylating agents with conventional cytotoxic agents in older AML patients with blast percentages >30% are underway.

12. Management of special situations

12.1 Hyperleukocytosis

Hyperleukocytosis, generally defined as a WBC >100x10^9/L, is associated with increased induction mortality mainly due to hemorrhagic events, tumor lysis syndrome, and infections. Hyperleukocytosis with leukostasis and for example pulmonary infiltrates or retinal and cerebral hemorrhages requires immediate medical treatment. Leukapheresis is an option for the initial management of hyperleukocytosis; however, no impact on long-term outcome has been shown. In general, the recommended therapy to lower WBC is hydroxyurea, given at dosages up to 50-60 mg/kg/d, until WBCs are <10-20x10^9/L. Until the WBC has been reduced, excessive red blood cell transfusions can lead to increased blood viscosity. Special attention should be given to the prevention of tumor lysis syndrome (eg, hydration, control of uric acid production using allopurinol or rasburicase, control of urine pH).
12.2 Central nervous system involvement

Initial involvement of the central nervous system (CNS) in AML occurs in <5% of patients. There is no indication for intrathecal prophylaxis in patients without CNS symptoms, although it may be considered in special situations (eg, hyperleukocytosis).

In patients with CNS involvement, 40-50 mg cytarabine should be administered intrathecally, 2- to 3-times per week until clearance of blasts, followed by 3 further injections with the same dosage. Alternatively, liposomal cytarabine (50 mg every other week) may be given for approximately 6 cycles. For prevention of arachnoiditis, dexamethasone (4 mg tid p.o.) may be considered on the days of intrathecal application. Prolonged application of intrathecal therapy does not appear to be justified, given that such therapy carries the risk of complications (eg, leukencephalopathy). In patients with a CNS recurrence, craniospinal irradiation with or without intrathecal chemotherapy has also been shown to be effective; however, its impact on long-term outcome is unknown.

12.3 Myeloid sarcoma

Myeloid sarcoma (synonyms: extramedullary myeloid tumor, granulocytic sarcoma, chloroma) is a tumor mass consisting of myeloid blasts in which the tissue architecture is effaced, occurring at an anatomical site other than the bone marrow, most commonly in skin, lymph nodes, gastrointestinal tract, bone, soft tissue, and testis. Myeloid sarcoma may present de novo preceding AML, concurrently with AML, or as blastic transformation of MDS, MPN, or MDS/MPN. Diagnosis is ascertained by cytochemical and/or immunohistochemical analyses. Often myeloid sarcomas have myelomonocytic or monoblastic morphology. Myeloid sarcomas should be evaluated for genetic and phenotypic features that allow for their classification in the appropriate WHO entity. Myeloid sarcomas have been associated with hyperleukocytosis, t(8;21) and CD56 positivity.

Myeloid sarcoma occurring de novo should be considered as AML and treated as such. Data on the prognostic impact of myeloid sarcoma are limited. Whereas some studies reported a negative impact in selected subgroups, others suggest that outcome of patients with myeloid sarcoma after conventional chemotherapy or allogeneic HSCT may not be inferior. Involved field radiation therapy may be considered to enhance local tumor control.

12.4 Pregnancy

Acute leukemia during pregnancy should be managed jointly by the hematologist, obstetrician, and neonatologist. Consideration must be given to both the health of the mother and to the immediate as well as long-term consequences for the fetus exposed to cytotoxic agents. Pregnancy does not appear to alter the course of AML, with more than 75% of patients achieving CR after standard chemotherapy. Treatment should be started immediately since delays may compromise maternal outcome.
Leukemia in a pregnant woman bears an increased risk for abortion, intrauterine growth restriction, and perinatal mortality.\textsuperscript{276-278} The earlier in gestation the diagnosis of leukemia, the higher the incidence of spontaneous abortion, prematurity and low birthweight. The risk of teratogenicity is highest during weeks 2-8 after conception, the period of organogenesis.\textsuperscript{278} Malformations after exposure to anthracyclines and cytarabine in the first trimester have been reported.\textsuperscript{278} Idarubicin differs from the other anthracyclines since it is more lipophilic favoring an increased placental transfer and has a higher DNA affinity; thus, daunorubicin should be given rather than idarubicin.\textsuperscript{279} Given the risk of teratogenicity, the option of therapeutic termination during the first trimester of pregnancy should be submitted for consideration by the mother. Chemotherapy delivered during the second and third trimester of pregnancy has been reported as safe, although stillbirths and low birthweight have also been observed.\textsuperscript{273,275-277} Delivery while patient and fetus may be cytopenic should be avoided.

13. Supportive care
13.1 Prophylactic anti-infectious treatment

For prophylaxis and treatment of infectious diseases, prevailing institutional infectious organisms and their drug resistance pattern should primarily be considered. Personal hygiene, dental care, and vigorous hand washing (the latter also for family and caregivers) are very important for prevention of infections. Reasonable precautions should be undertaken to protect patients from bacteria or fungi in their environment. Although eating fresh fruits and/or vegetables is often discouraged, there is little evidence that adherence to such a “neutropenic diet” prevents infections.\textsuperscript{280}

Fungal prophylaxis. Invasive fungal infections are a major cause of morbidity and mortality in patients with prolonged neutropenia.\textsuperscript{281} A review of randomized trials in AML found a significant reduction in fungal infection-related mortality and invasive fungal infection in patients given anti-fungal prophylaxis rather than placebo while the difference in all-cause mortality was borderline significant.\textsuperscript{282} Prophylaxis with itraconazole, posaconazole, or amphotericin, ie, drugs with anti-mold activity, reduced the risk of documented aspergillus infection and likely had some effect on all-cause mortality. A recent trial found that patients randomized to posaconazole had fewer invasive fungal infections than patients randomized to either fluconazole or itraconazole according to institutional practice.\textsuperscript{283} Survival was longer in the posaconazole group, although it is unclear how much of the difference was due to the superiority of posaconazole to itraconazole rather than the superiority of posaconazole to fluconazole. Also, prophylaxis may not be as efficacious if other modes of prophylaxis are used, eg, high efficiency particulate (HEPAR) air filtration. Nonetheless, the limited data suggest that patients should receive an anti-mold agent, rather than fluconazole, during remission induction therapy.
**Antibiotic prophylaxis.** Bacterial infections are an important cause of morbidity and mortality in neutropenic patients following chemotherapy for AML.\textsuperscript{284} A Cochrane review compared antibiotic prophylaxis with placebo or no intervention in afebrile neutropenic patients.\textsuperscript{285} Antibiotic prophylaxis significantly decreased the risk of death and the risk of infection-related death. The most significant reduction in risk for all-cause mortality was observed in trials testing prophylaxis with quinolones, despite the occurrence of adverse effects and development of resistance.\textsuperscript{286} Thus, antibiotic prophylaxis should be given following chemotherapy for AML with a preference for a quinolone.

### 13.2 Growth factors

Numerous studies\textsuperscript{138,185,287-303} have shown that myeloid growth factors, either GM-CSF or G-CSF, accelerate neutrophil recovery by 2-5 days, can reduce antibiotic use, duration of fever, and number of days spent in hospital, and do not retard platelet recovery, or have a detrimental effect by stimulation of leukemic cell growth. However, the use of growth factors does not translate into a survival benefit. The cost effectiveness of growth factors is difficult to assess and has been inconsistently reported, but there does not seem to be significant cost savings. Thus, the general use of growth factors in AML cannot be recommended. However, in individual cases (eg, severe infection prior to expected neutrophil recovery), growth factor use can be considered.

### 13.3 Transfusion support

**Platelet transfusion.** The introduction of platelet transfusions has dramatically reduced mortality from hemorrhage in AML.\textsuperscript{304} For many years, platelet transfusions were given to keep platelet counts above 20x10\(^9\)/L. However, in three randomized studies, no significant differences in severe bleeding were shown if a threshold of <10x10\(^9\)/L rather than <20x10\(^9\)/L for prophylactic platelet transfusion was employed.\textsuperscript{305-307} American Society of Clinical Oncology guidelines recommend a threshold of 10x10\(^9\)/L for prophylactic platelet transfusions.\textsuperscript{308} Besides the platelet count, mucosal bleeding, infection, severe mucositis, and fever should be considered in the assessment of bleeding risk and should increase the transfusion threshold.

To prevent alloimmunization, removal of contaminating leucocytes is advised. Nevertheless, alloimmunization remains a major obstacle to effective transfusion due to antibodies to HLA class I antigens in many women with prior pregnancies or patients with prior transfusions.\textsuperscript{309} Such alloimmunized patients are transfused with either HLA-matched platelets or crossmatch-compatible platelets. Both are equally effective.\textsuperscript{310} Although single-donor platelets are often thought preferable to platelets obtained from multiple donors, a randomized trial showed that they were no more effective in reducing alloimmunization or refractoriness to transfusions.\textsuperscript{311} The antifibrinolytic agent tranexamic acid can be useful in reducing bleeding and platelet transfusion.\textsuperscript{312} Anecdotal reports suggest a role for the use of
recombinant human activated factor VII in the treatment of bleeding in AML patients refractory to platelet transfusions; however, well designed clinical trials are lacking.313

Red blood cell transfusion. Although evidence is lacking, it is generally accepted to keep the hemoglobin level above 8 g/dl, especially in thrombocytopenic patients.

Granulocyte transfusion. No good evidence exists to recommend granulocyte transfusions in the treatment of AML. A multicenter randomized trial to address the utility of such transfusions in the setting of infections is being conducted in the United States (ClinicalTrials.gov Identifier: NCT00627393).

Transfusion practice before, during and after HSCT. Only leukocyte-depleted erythrocyte and platelet components should be given to decrease the risk of HLA-associated alloimmunization and reduce the risk of CMV transmission.314 Both autologous and allogeneic stem cell recipients are at risk for transfusion-associated graft-versus-host disease (GVHD). Gamma-irradiation (at least 25 Gy) is the only reliable method to prevent transfusion-associated GVHD. There is no general consensus when to begin with the transfusion of irradiated blood products. Commonly used practice is to transfuse irradiated blood products from start of conditioning until 6 months after transplant (in the absence of chronic GVHD) (allogeneic HSCT), and from 7 days prior to stem cell harvest until 3 months post transplant for autologous HSCT. Some centers start as soon as a patient is identified to be a potential HSCT candidate. All CMV seronegative HSCT recipients with seronegative donors should receive CMV-seronegative blood products.
Acknowledgements

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Author’s Contribution

All authors reviewed the literature and wrote first drafts of specific sections. HD and CDB assembled the sections and wrote the final version of the manuscript. All authors reviewed and approved the final version of the manuscript.

Conflict of Interest Disclosure

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14. References


147. Büchner T, Hiddemann W, Berdel WE, et al. 6-thioguanine, cytarabine, and daunorubicin (TAD) and high-dose cytarabine and mitoxantrone (HAM) for induction, TAD for consolidation, and either prolonged maintenance by reduced monthly TAD or TAD-HAM-TAD and one course of intensive consolidation by sequential HAM in adult patients at all ages with de novo acute myeloid leukaemia (AML): a randomized trial of the German AML Cooperative Group. J Clin Oncol. 2003;21(24):4496-4504.


165. Byrd JC, Dodge RK, Carroll A, et al. Patients with t(8;21)(q22;q22) and acute myeloid leukemia have superior failure-free and overall survival when repetitive cycles of high-dose cytarabine are administered. J Clin Oncol. 1999;17(12):3767-3775.


Table 1. Acute myeloid leukemia and related precursor neoplasms, and acute leukemias of ambiguous lineage (WHO 2008)

<table>
<thead>
<tr>
<th>Acute myeloid leukemia with recurrent genetic abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML with t(8;21)(q22;q22); RUNX1-RUNX1T1</td>
</tr>
<tr>
<td>AML with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); CBFβ-MYH11</td>
</tr>
<tr>
<td>APL with t(15;17)(q22;q12); PML-RARA*</td>
</tr>
<tr>
<td>AML with t(9;11)(p22;q23); MLLT3-MLL†</td>
</tr>
<tr>
<td>AML with t(6;9)(p23;q34); DEK-NUP214</td>
</tr>
<tr>
<td>AML with inv(3)(q21q26.2) or t(3;3)(q21q26.2); RPN1-EVI1</td>
</tr>
<tr>
<td>AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1</td>
</tr>
<tr>
<td>Provisional entity: AML with mutated NPM1</td>
</tr>
<tr>
<td>Provisional entity: AML with mutated CEBPA</td>
</tr>
</tbody>
</table>

| Acute myeloid leukemia with myelodysplasia-related changes‡                                     |
| Therapy-related myeloid neoplasms§                                                            |
| Acute myeloid leukemia, not otherwise specified (NOS)                                          |
| Acute myeloid leukemia with minimal differentiation                                             |
| Acute myeloid leukemia without maturation                                                      |
| Acute myeloid leukemia with maturation                                                         |
| Acute myelomonocytic leukemia                                                                  |
| Acute monoblastic/monocytic leukemia                                                           |
| Acute erythroid leukemia                                                                      |
| Pure erythroid leukemia                                                                        |
| Erythroleukemia, erythroid/myeloid                                                             |
| Acute megakaryoblastic leukemia                                                                |
| Acute basophilic leukemia                                                                      |
| Acute panmyelosis with myelofibrosis (syn.: acute myelofibrosis; acute myelosclerosis)         |
| Myeloid sarcoma (syn.: extramedullary myeloid tumor; granulocytic sarcoma; chloroma)           |
| Myeloid proliferations related to Down syndrome                                                |
| Transient abnormal myeloipoiesis (syn.: transient myeloproliferative disorder)                 |
| Myeloid leukemia associated with Down syndrome                                                  |
| Blastic plasmacytoid dendritic cell neoplasm                                                   |
| Acute leukemias of ambiguous lineage                                                           |
| Acute undifferentiated leukemia                                                                |
| Mixed phenotype acute leukemia with t(9;22)(q34;q11.2); BCR-ABL1                              |
| Mixed phenotype acute leukemia with t(v;11q23); MLL rearranged                                |
| Mixed phenotype acute leukemia, B/myeloid, NOS                                                  |
| Mixed phenotype acute leukemia, T/myeloid, NOS                                                  |
| Provisional entity: Natural killer (NK) cell lymphoblastic leukemia/lymphoma                   |

Adopted from reference 3; for a diagnosis of AML, a marrow blast count of ≥20% is required, except for AML with the recurrent genetic abnormalities t(15;17), t(8;21), inv(16) or t(16;16) and some cases of erythroleukemia.

* Other recurring translocations involving RARA should be reported accordingly: eg, AML with t(11;17)(q23;q12)/ZBTB16-RARA; AML with t(11;17)(q13;q12); NUMA1-RARA; AML with t(5;17)(q35;q12); NPM1-RARA; or AML with STAT5B-RARA (the latter having a normal chromosome 17 on conventional cytogenetic analysis)

† Other translocations involving MLL should be reported accordingly: eg, AML with t(6;11)(q27;q23); MLLT4-MLL; AML with t(11;19)(q23;p13.3); MLL-MLLT; AML with t(11;19)(q23;p13.1); MLL-ELL; AML with t(10;11)(p12;q23); MLLT10-MLL.

‡ >20% blood or marrow blasts AND any of the following: previous history of myelodysplastic syndrome (MDS), or myelodysplastic/myeloproliferative neoplasm (MDS/MPN); myelodysplasia-related cytogenetic abnormality (see below); multilineage dysplasia; AND absence of both prior cytotoxic therapy for unrelated disease and aforementioned recurring genetic abnormalities; cytogenetic abnormalities sufficient to diagnose AML with myelodysplasia-related changes are:
- complex karyotype (defined as 3 or more chromosomal abnormalities)
- unbalanced changes: -7 or del(7q); -5 or del(5q); (i(17q) or t(17p); -13 or del(13q); del(11q); del(12p) or del(12p); del(9q);
- balanced changes: t(11;16)(q23;p13.3); t(3;21)(q26.2;q22.1); t(1;3)(p36.3;q21.1); t(2;21)(p21;q23); t(5;12)(q33;p12); t(5;7)(p22;q32); t(5;17)(q33;p13); t(5;10)(q32;q21); t(3;5)(q25;q44).

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

‖ BCR-ABL1 positive leukemia may present as mixed phenotype acute leukemia, but should be treated as BCR-ABL1 positive acute lymphoblastic leukemia.
Table 2. Expression of cell surface and cytoplasmic markers for the diagnosis of acute myeloid leukemia and mixed phenotype acute leukemia

<table>
<thead>
<tr>
<th>Diagnosis of acute myeloid leukemia (AML)*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Precursor stage</td>
<td>CD34, CD38, CD117, CD133, HLA-DR</td>
</tr>
<tr>
<td>Granulocytic markers</td>
<td>CD13, CD15, CD16, CD33, CD65, cytoplasmic myeloperoxidase (cMPO)</td>
</tr>
<tr>
<td>Monocytic markers</td>
<td>nonspecific esterase (NSE), CD11c, CD14, CD64, lysozyme, CD4, CD11b, CD36, NG2 homologue†</td>
</tr>
<tr>
<td>Megakaryocytic markers</td>
<td>CD41 (glycoprotein IIb/IIIa), CD61 (glycoprotein IIIa), CD42 (glycoprotein 1b)</td>
</tr>
<tr>
<td>Erythroid marker</td>
<td>CD235a (glycophorin A)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diagnosis of mixed phenotype acute leukemia (MPAL)‡</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloid lineage</td>
<td>MPO or evidence of monocytic differentiation (at least 2 of the following: NSE, CD11c, CD14, CD64, lysozyme)</td>
</tr>
<tr>
<td>B-lineage</td>
<td>CD19 (strong) with at least 1 of the following: CD79a, cCD22, CD10, or CD19 (weak) with at least 2 of the following: CD79a, cCD22, CD10</td>
</tr>
<tr>
<td>T-lineage</td>
<td>cCD3, or surface CD3</td>
</tr>
</tbody>
</table>

* For the diagnosis of AML, the table provides a list of selected markers rather than a mandatory marker panel.
† Most cases with 11q23 abnormalities express the NG2 homologue (encoded by CSPG4) reacting with the monoclonal antibody 7.1.
‡ Requirements for assigning more than one lineage to a single blast population adopted from the WHO classification. Note that the requirement for assigning myeloid lineage in MPAL is more stringent than for establishing a diagnosis of AML. Note also that MPAL can be diagnosed if there are separate populations of lymphoid and myeloid blasts.
Table 3. Test / procedures in the initial work-up of a patient with AML

<table>
<thead>
<tr>
<th>Test / Procedure</th>
<th>General Practice</th>
<th>Clinical Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tests to establish the diagnosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete blood counts and differential count</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Bone marrow aspirate</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Bone marrow trephine biopsy</td>
<td>Optional</td>
<td>Optional</td>
</tr>
<tr>
<td>Immunophenotyping</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Cytogenetics</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>RUNX1-RUNXI, CBFB-MYH11, PML-RARA, or other gene fusion screening</td>
<td>Optional</td>
<td>Optional</td>
</tr>
<tr>
<td><strong>Additional tests / procedures at diagnosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Demographics and medical history</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Performance status (ECOG/WHO score)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Analysis of comorbidities</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Biochemistry, coagulation tests, urine analysis</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Serum pregnancy test</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Information on oocyte and sperm cryopreservation</td>
<td>Optional</td>
<td>Optional</td>
</tr>
<tr>
<td>Eligibility assessment for allogeneic HSCT</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hepatitis A, B, C; HIV-1 testing</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Chest X-ray, 12-lead ECG; echocardiography (on indication)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Lumbar puncture</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Biobanking</td>
<td>Optional</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Prognostic / predictive marker assessment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPM1, CEBPA, FLT3 gene mutation</td>
<td>Optional</td>
<td>Yes</td>
</tr>
<tr>
<td>WT1, RUNX1, MLL, KIT, RAS, TP53, TET2, IDH1 gene mutation</td>
<td>No</td>
<td>Investigational</td>
</tr>
<tr>
<td>ERG, MN1, EVI1, BAALC gene expression</td>
<td>No</td>
<td>Investigational</td>
</tr>
<tr>
<td>Detection of minimal residual disease</td>
<td>No</td>
<td>Investigational</td>
</tr>
</tbody>
</table>

**Notes:**
- a Mandatory in patients with a dry tap (punctio sicca).
- b Should be performed if chromosome morphology is of poor quality, or if there is typical morphology but the suspected cytogenetic abnormality is not present.
- c Including race or ethnicity, family history, prior exposure to toxic agents, prior malignancy, therapy for prior malignancy, information on smoking.
- d Biochemistry: glucose, sodium, potassium, calcium, creatinine, aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase, lactate dehydrogenase, bilirubin, urea, total protein, uric acid, total cholesterol, total triglycerides, creatinine phosphokinase (CPK)
- e Coagulation tests: prothrombin time (PTT), international normalized ratio (INR) where indicated, activated partial thromboplastin time (aPTT)
- f Urine analysis: pH, glucose, erythrocytes, leukocytes, protein, nitrite.
- g HLA typing and CMV testing should be performed in those patients eligible for allogeneic stem cell transplantation.
- h Required in patients with clinical symptoms suspicious of central nervous system involvement; patient should be evaluated by imaging study for intracranial bleeding, leptomeningeal disease, and mass lesion; lumbar puncture considered optional in other settings (e.g., high WBC).
- i Pretreatment leukemic bone marrow and blood sample; for further optional storing see section 4.7.
- j Biobanking should also be performed in general practice if at all possible.
- k Strongly encouraged in AML with normal karyotype.
Table 4. Standardized reporting for correlation of cytogenetic and molecular genetic data in AML with clinical data*

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

* Frequencies, response rates and outcome measures should be reported by genetic group, and, if sufficient numbers are available, by specific subsets indicated; excluding cases of acute promyelocytic leukemia.
† 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 chromosome abnormalities in the absence of one of the WHO designated recurring translocations or inversions, ie, 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)/t(3;3); indicate how many complex karyotype cases have involvement of chromosome arms 5q, 7q, and 17p.
Table 5. Response criteria in AML

<table>
<thead>
<tr>
<th>Category</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete remission (CR)*</td>
<td>Bone marrow blasts &lt;5%; absence of blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count &gt;1.0 x 10^9/L (1,000/µL); platelet count &gt;100 x 10^9/L (100,000/µL); independence of red cell transfusions</td>
</tr>
<tr>
<td>CR with incomplete recovery (CRi)†</td>
<td>All CR criteria except for residual neutropenia [&lt;1.0 x 10^9/L (1,000/µL)] or thrombocytopenia [&lt;100 x 10^9/L (100,000/µL)]</td>
</tr>
<tr>
<td>Morphologic leukemia-free state‡</td>
<td>Bone marrow blasts &lt;5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required</td>
</tr>
<tr>
<td>Partial remission (PR)</td>
<td>Relevant in the setting of phase I and II clinical trials only; all hematologic criteria of CR; decrease of bone marrow blast percentage to 5% to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%</td>
</tr>
<tr>
<td>Cytogenetic CR (CRc)§</td>
<td>Reversion to a normal karyotype at the time of morphologic CR (or CRi) in cases with an abnormal karyotype at the time of diagnosis; based on the evaluation of 20 metaphase cells from bone marrow</td>
</tr>
<tr>
<td>Molecular CR (CRm)¹</td>
<td>No standard definition; depends on molecular target</td>
</tr>
</tbody>
</table>

**Treatment failure**

- **Resistant disease (RD)**
  - Failure to achieve CR or CRi (general practice; phase II/III trials), or failure to achieve CR, CRi or PR (phase I trials); only includes patients surviving ≥7 days following completion of initial treatment, with evidence of persistent leukemia by blood and/or bone marrow examination

- **Death in aplasia**
  - Deaths occurring ≥7 days following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 days of death, without evidence of persistent leukemia

- **Death from indeterminate cause**
  - Deaths occurring before completion of therapy, or <7 days following its completion; or deaths occurring ≥7 days following completion of initial therapy with no blasts in the blood, but no bone marrow examination available

**Relapse**#

- Bone marrow blasts ≥5%; or reappearance of blasts in the blood; or development of extramedullary disease

Definitions of response criteria are based primarily on those given by Cheson et al.²

* All criteria need to be fulfilled; marrow evaluation should be based on a count of 200 nucleated cells in an aspirate with spicules; if ambiguous, consider repeat exam after 5-7 days; flow cytometric evaluation may help to distinguish between persistent leukemia and regenerating normal marrow; a marrow biopsy should be performed in cases of dry tap, or if no spicules are obtained; no minimum duration of response required.

† The criterion of CRi is of value in protocols using intensified induction or double induction strategies, in which hematologic recovery is not awaited, but intensive therapy will be continued. In such protocols, CR may even not be achieved in the course of the entire treatment plan. In these instances, the overall remission rate should include CR and CRi patients. Some patients may not achieve complete hematologic recovery upon longer observation times.

‡ This category may be useful in the clinical development of novel agents within phase I clinical trials, in which a transient morphologic leukemia-free state may be achieved at the time of early response assessment.

§ Four studies showed that failure to convert to a normal karyotype at the time of CR predicts inferior outcome.¹¹²-¹¹⁵

† As an example, in CBF AML low-level PCR-positivity can be detected in patients even in long-term remission. Normalizing to 10^4 copies of ABL1 in accordance with standardized criteria, transcript levels below 12 to 10 copies appear to be predictive for long-term remission.¹⁰⁸-¹¹⁰

#In cases with low blast percentages (5-10%), a repeat marrow should be performed to confirm relapse. Appearance of new dysplastic changes should be closely monitored for emerging relapse. In a patient who has been recently treated, dysplasia or a transient increase in blasts may reflect a chemotherapy effect and recovery of hematopoiesis. Cytogenetics should be tested to distinguish true relapse from therapy-related MDS/AML.
Table 6. Outcome measures in AML

<table>
<thead>
<tr>
<th>Category</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall survival</td>
<td>Defined for all patients of a trial; measured from the date of entry into a study to the date of death from any cause; patients not known to have died at last follow-up are censored on the date they were last known to be alive</td>
</tr>
<tr>
<td>Relapse-free survival*</td>
<td>Defined only for patients achieving CR or CRi†; measured from the date of achievement of a remission until the date of relapse or death from any cause; patients not known to have relapsed or died at last follow-up are censored on the date they were last examined</td>
</tr>
<tr>
<td>Event-free survival</td>
<td>Defined for all patients of a trial; measured from the date of entry into a study to the date of induction treatment failure, or relapse from CR or CRi†, or death from any cause; patients not known to have any of these events are censored on the date they were last examined</td>
</tr>
<tr>
<td>Cumulative incidence of relapse (CIR)‡</td>
<td>Defined for all patients achieving CR or CRi† measured from the date of achievement of a remission until the date of relapse; patients not known to have relapsed are censored on the date they were last examined; patients who died without relapse are counted as a competing cause of failure</td>
</tr>
</tbody>
</table>

* Relapse-free and disease-free survival have been used with the same definition
† In studies where the criterion CRi is used, relapse-free survival should be defined for all patients achieving CR or CRi; for event-free survival, relapse should be considered from CR and CRi.
‡ It is important to provide estimates of cumulative incidence of death (CID) as well, since just considering the results of CIR may be misleading if for instance CIR is lower for one group but CID is actually higher for that same group.
Table 7. Prognostic index for younger adults with AML in relapse*  

<table>
<thead>
<tr>
<th>Risk Category</th>
<th>Index (score in points)</th>
<th>Survival probability at 1 y</th>
<th>Survival probability at 5 y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable risk (9% of patients)</td>
<td>0 – 6</td>
<td>70%</td>
<td>46%</td>
</tr>
<tr>
<td>Intermediate risk (25% of patients)</td>
<td>7 – 9</td>
<td>49%</td>
<td>18%</td>
</tr>
<tr>
<td>Unfavorable risk (66% of patients)</td>
<td>10 – 14</td>
<td>16%</td>
<td>4%</td>
</tr>
</tbody>
</table>

* Evaluated in patients 15 to 60 years; APL with t(15;17) excluded; prognostic relapse score is estimated according to the following factors:
  - Duration of remission prior to relapse: more than 18 months (0 points); 7-18 months (3 points); and 6 months or less (5 points)
  - Cytogenetics at initial diagnosis: inv(16) or t(16;16) (0 points); t(8;21) (3 points); other (5 points)
  - Prior hematopoietic stem cell transplantation: no (0 points); yes (2 points)
  - Age at time of relapse: 35 years or less (0 points); 36-45 years (1 point); more than 45 years (2 points).
Figure 1. Pie chart illustrating the molecular heterogeneity of cytogenetically normal AML based on mutations in the \textit{NPM1}, \textit{CEBPA}, \textit{MLL}, \textit{FLT3} [internal tandem duplication (ITD) and tyrosine kinase domain (TKD) mutations at codons D835 and I836], \textit{NRAS}, and \textit{WT1} genes. The bluish colors denote \textit{NPM1} mutated subsets, the orange/red colors \textit{CEBPA} mutated subsets, and the yellow/green colors \textit{MLL} mutated subsets; the grey colors depict subsets without hypothetical class II mutations, and the white sector shows the subset without any mutation in the above mentioned genes. Data are derived from mutational analysis of 485 younger adult patients with cytogenetically normal AML from AMLSG.
Figure 1