Post-remission therapy for acute myeloid leukemia

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ABSTRACT

Induction followed by post-remission therapy including intensive chemotherapy with high-dose cytarabine, autologous and allogeneic hematopoietic stem cell transplantation is recognized as the main road towards cure in acute myeloid leukemia. In recent years, also a renaissance of maintenance therapy after completion of intensive consolidation has been observed with the introduction of kinase inhibitors and demethylating agents in clinical trials. Greater insight into the genetic background of the disease fostered the extension of disease classification and pre-treatment risk-categorization by gene mutations. In addition, the pre-treatment risk-defining parameters have been supplemented by markers evaluated at distinct time points during treatment and follow up. In this context, minimal residual disease assessment is increasingly used to dynamically fine tune treatment recommendations. Currently, the gold standard to counterbalance a higher risk of relapse by treatment strategies based on hematopoietic stem cell transplantation with grafts from matched related or unrelated donors is still valuable, whereas autologous hematopoietic stem cell transplantation showed promising results especially in patients categorized as low-risk. Nonetheless, more targeted approaches including kinase inhibitors and demethylating agents in combination with or sequentially before or after intensive chemotherapy are currently in clinical evaluation and may lead to more genotype- instead of purely risk-adapted treatment strategies.

Introduction

Acute myeloid leukemia (AML) is the most common acute leukemia in adults with an incidence of 3-4 per 100,000 men and women per year. AML is a genetically very heterogeneous disorder characterized by the accumulation of somatically acquired genetic changes in hematopoietic progenitor cells altering normal mechanisms of self-renewal, proliferation, and differentiation.

Recently, the Cancer Genome Atlas Research Network reported on the genomic and epigenomic landscapes of adult *de novo* AML based on next generation sequencing data performed on 200 AML patients.[1] The investigators identified 23 significantly mutated genes, and another 237 gene mutations found in 2 or more samples. The authors proposed a classification of gene mutations into 9 categories based on their biological function with 199 of the 200 analyzed patients having at least one mutation in one of these categories (Table 1). These findings will probably influence the future disease classification system.

To date, AML is categorized on the basis of the 2008 revised WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues[2] in several distinct disease entities which are preponderantly defined by the underlying cyto- and molecular-genetic aberrations.[3] From a more practical clinical perspective, AML can be grouped into 4 risk groups according to the recommendations of an international expert panel on behalf of the European LeukemiaNet (Table 2).[4] Of note, the prognostic value of the sub-classification of intermediate risk group into two subcategories, intermediate-1 and intermediate-2, is still not completely clear. For example, in a retrospective study of the Cancer and Leukemia Study Group B (CALGB), evaluating the impact of the ELN classification on outcome, revealed that intermediate-I and intermediate-II groups in older patients (>60 years) had similar outcomes, whereas the intermediate-II group in younger patients had better survival but not better remission rates or disease-free survival than the intermediate-I group.[5]

Outcome is influenced by patient features such as age, comorbidities and performance status, as well as disease characteristics including type of AML (*de novo*, treatment-related, secondary after myelodysplastic/myeloproliferative disease) and, by far the most important, the genetic profile. The median age at diagnosis of patients with AML ranges from 66 to 71 years (SEER Cancer Statistics Review 1975-2009)[6] and the proportion of patients receiving intensive chemotherapy decreases with increasing age.[7]

The combination of an anthracycline and cytarabine ('3+7') remains the standard of care of intensive induction therapy in patients considered medically fit, and complete remission (CR) rates ranges from 65% to 75% in younger adult patients (≤ 60 years) and from 40% to 60% in older patients (>60 years).[4] In patients ineligible for intensive chemotherapy, treatment options are limited with low-dose cytarabine and the hypomethylating agents decitabine or azacitidine (azacitidine limited to patients with 20-30% bone marrow blasts) resulting in CR rates of between 10% and 30%.[8-10]

After achieving a first CR, post-remission therapy is mandatory to prevent relapse. The goal of this review is to highlight: i) the current standard of intensive post-remission chemotherapy; ii) prognostic and predictive pre-treatment markers guiding the choice of post-remission treatment strategy (i.e. intensive chemotherapy, autologous or allogeneic HSCT) in first CR; and iii) minimal residual disease (MRD) measurement dur-

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ing treatment and follow up and its predictive value for treatment adaptation.

Post-remission therapy with intensive chemotherapy

The concept of intensive post-remission chemotherapy is based on the observation that after achievement of a first CR virtually all patients relapse in the absence of further treatment.[11] In addition, intensive post-remission chemotherapy using a very intensive high-dose cytarabine-based regimen was superior to prolonged low-dose maintenance therapy in younger patients.[12] Furthermore, a landmark study of the Cancer and Leukemia Group B (CALGB) established the current standard of post-remission chemotherapy for patients aged 60 years and younger. In the prospective up-front randomized study, four repeated cycles of high-dose cytarabine (3 g/m², bid, Days 1,3,5) had been superior to intermediate- (400 mg/m² cont. Days 1-5) or standard-dose cytarabine (100 mg/m² cont. Days 1-5) with respect to relapse free survival (RFS) and overall survival (OS).[13] In all patients, maintenance therapy was programmed for after completion of intensive consolidation therapy with four monthly cycles of cytarabine (100 mg/m², s.c., bid, Days 1-5) and daunorubicin (45 mg/m², Day 1). Nonetheless, optimal dose of cytarabine, number of cycles, and benefit of additional chemotherapeutic agents have remained open issues.

The French ALFA-group showed no beneficial effect on survival end points of one cycle of high-dose cytarabine (3 g/m², bid, Days 1,3,5,7) plus amsacrine (100 mg/m², Days 1-3) followed by one cycle of a timed-sequential post-remission chemotherapy with mitoxantrone (12 mg/m², Days 1-3), cytarabine (500 mg/m², cont., Days 1-3, 8-10), and etoposide (200 mg/m², Days 8-10) compared to treatment according to the high-dose cytarabine CALGB arm including maintenance therapy.[14] Furthermore, no superiority of four courses of a multi-agent post-remission chemotherapy including standard dose cytarabine (200 mg/m², cont., Days 1-5) combined alternating with mitoxantrone, daunorubicine, aclarubicin, etoposide plus vincristine over three cycles of high-dose cytarabine (2 g/m², bid, Days 1-5) could be shown.[15] Schaich *et al.* reported on the comparison of three cycles of high-dose cytarabine based on the CALGB regimen but without further maintenance therapy versus three cycles combination chemotherapy with MAC (cytarabine 1 g/m², bid, Days 1-6; mitoxantrone, 10 mg/m², Days 4-6), MAMAC (cytarabine 1 g/m², bid, Days 1-5; amsacrine 100 mg/m², Days 1-5) followed by MAC.[16] Again, no beneficial effect of the combination therapy on survival end points could be demonstrated on an intention-to-treat basis. Of note, a per protocol analysis even favored the single agent high-dose cytarabine arm. In the recently published Medical Research Council MRC-15 trial, combination post-remission therapy with MACE (amsacrine, 100 mg/m², Days 1-5; cytarabine, 200 mg/m², cont., Days 1-5; etoposide, 100 mg/m², Days 1-5) followed by MidAC (mitoxantrone, 10 mg/m², Days 1-5; cytarabine, 1.0 g/m², bid, Days 1-3) was compared to two cycles of single agent high-dose cytarabine in two different doses (3 g/m² and 1.5 g/m², single dose) applied according to the CALGB regimen.[17] After two cycles of intensive consolidation therapy, patients were additionally randomized to either no further treatment versus one additional cycle of intensive consolidation therapy with high-dose cytarabine (1.5 g/m², bid, Days 1,3,5). Again no beneficial effect on survival end points of combination intensive post-remission chemotherapy could be shown compared to the two arms based on single-agent high-dose cytarabine. Interestingly, the comparison of the two single-agent high-dose cytarabine consolidation arms revealed that a lower dose (1.5 g/m², single dose) was associated with a strong trend towards a higher cumulative incidence of relapse compared to the standard dose (3 g/m², single dose). After two induction and two consolidation cycles, a third intensive consolidation cycle was not superior to no further treatment.[17] Taken together, four up-front randomized prospective multicenter trials comparing combination post-remission therapy to single agent high-dose cytarabine in younger patients (<60 years) with AML failed to show an improvement in any survival end point.[14-17] Thus, the CALGB established consolidation post-remission chemotherapy with single-agent high-dose cytarabine (3 g/m², bid Days 1,3,5)13 for at least two cycles after two induction cycles or three to four cycles while only one induction cycle remains the standard for younger adult patients. Of note, halving dosage from 3 g/m² to 1.5g/m² single dose was associated with a strong trend towards a higher cumulative incidence of relapse[17] which is in some contrast to pharmacological studies suggesting a saturation of arabinosylcytosine-5'triphosphate formation by the transport system in leukemic blasts already at dosages of 200 to 250 mg/m²/h corresponding to a single dose of 1.0 g/m² IV infusion over three hours.[18] Although not formally evaluated in a prospective randomized trial, chemotherapy-based maintenance treatment after completion of intensive consolidation therapy according the high-dose cytarabine CALGB regimen does not seem to influence survival end points and is nowadays not recommended.

Subsequent subgroup analyses according to cytogenetics in the initial CALGB study revealed that the beneficial effect of high-dose cytarabine compared to intermediate- and standard-dose cytarabine was restricted to core binding factor (CBF)-AML including t(8;21) and inv(16)/t(16;16) as well as cytogenetically normal (CN)-AML, whereas patients exhibiting other cytogenetic aberrations had a dismal outcome irrespective of the cytarabine dose administered.[19] Further subgroup analyses had also been performed in all four randomized trials comparing single agent high-dose cytarabine to combination consolidation therapy according to the cyto- and molecular-genetic risk profile.[14-17] The overall picture is not completely consistent; in two trials,[15,17] single agent high-dose cytarabine was superior in CBF-AML compared to combination post-remission chemotherapy whereas combination therapy was superior in the German SAL trial.[16] In patients with intermediate-risk AML, a beneficial effect of single agent high-dose cytarabine had been shown in the ALFA study[14] supported by a trend for positive results for *NPM1*-mutated AML in the German SAL study.[16] Moreover, in the MRC-15 study, combination therapy was better in patients with an unfavorable risk.[17] Thus, single agent high-dose chemotherapy (3 g/m², bid, Days 1,3,5) remains the preferable post-remission chemotherapy in younger adults with CBF- and intermediate-risk AML including CN-AML, whereas combination post-remission therapy may be considered in high-risk patients.

In contrast to younger patients, high-dose cytarabine appears to be too toxic in patients over 60 years of age and therefore the use of high-dose cytarabine is generally dis-

couraged in older patients.[13] Two studies comparing intensive consolidation post-remission therapy to low-dose chemotherapy delivered in an outpatient setting showed contradictory results. The first study compared a single additional intensive cycle of chemotherapy to six repeated cycles of lower-dose out-patient combination chemotherapy.[20] There was a significant beneficial effect of the lower-dose out-patient combination chemotherapy compared to one course of intensive post-remission therapy with a better overall and relapse-free survival. In contrast, the results of a second study suggested that after a first intensive consolidation therapy a second intensive cycle was superior compared to a one-year oral schedule of combination chemotherapy.[21] In contrast to the little progress made in attempts to optimize post-remission therapy in older patients, great progress has been achieved in middle aged and older patients (age 50-70 years) by using gemtuzumab ozogamicin (GO) as adjunct to intensive induction and consolidation therapy.[22] In this study, two post-remission consolidation cycles with a combination of daunorubicin (60 mg/m^2, Day 1 first cycle and Days 1-2 second cycle) and cytarabine (1 g/m^2, bid, Days 1-4) had been administered in patients achieving a first CR with acceptable toxicity with or without GO (3 mg/m^2, Day 1). In this study, GO as an adjunct to induction and consolidation therapy did not improve CR rate but did improve all survival end points. However, the administration of GO in a dosage of 3 mg/m^2 per administration given either as a single shot or on Days 1, 4 and 7 as adjunct and in parallel to induction therapy resulted in an improvement in the survival end points, whereas GO given during consolidation therapy seems to have no impact (for review see Thol *et al.*,[23]). Of note, the addition of three cycles of GO (6 mg/m^2) after a first intensive cytarabine-based consolidation therapy showed no beneficial effect with regard to survival end points.[24] Thus, the value of intensive post-remission chemotherapy in older patients continues to be a subject of debate. However, regimens including intermediate-dose cytarabine as in the French ALFA study[22] may serve as a backbone for the addition of novel drugs. Maintenance treatment with an oral azacitidine formulation is currently evaluated in older patients (≥55years) in first CR in a prospective randomized phase III trial (Eudra-CT n. 2012-003457-28).

Future strategies of improving post-remission therapy will include standard HiDAC consolidation in combination with novel drugs (e.g. kinase inhibitors) followed by single agent maintenance in patients fit for intensive chemotherapy, whereas in patients not eligible for intensive chemotherapy, as well as in older patients, an immediate start of maintenance therapy with novel drugs once first CR is achieved can be envisaged. Advantages in using HiDAC as a consolidation base on which novel drugs can be added are the expected lower rate of drug-drug interactions compared to regimens including anthracyclines or anthracendiones, the limited and well known hematologic toxicity, and the very good comparability to historical data in terms of toxicity and clinical outcome.

Prognostic and predictive pre-treatment markers guiding the choice of post-remission treatment strategy

Post-remission therapies with high-dose cytarabine, autologous and allogeneic HSCT have been evaluated with the aim of preventing relapse and of improving overall survival. To date, allogeneic HSCT is considered to be the intensive post-remission therapy with the strongest anti-leukemic effect. However, the benefit of allogeneic HSCT on overall survival may be compromised by non-relapse, treatment-related mortality (TRM). The European LeukemiaNet AML Working Party has proposed an integrated risk-adapted approach for younger patients with AML in first CR taking into account: i) the risk of relapse after intensive chemotherapy versus allogeneic HSCT; ii) TRM of allogeneic HSCT; and iii) patient and transplant-specific parameters such as comorbidity, donor type, and age as reflected by HCT-CI and EBMT scores (Table 3).[25] According to this recommendation, AML with *RUNX1-RUNX1T1* (only with pre-treatment white blood cell count ≤20/nL), *CBFB-MYH11*, *NPM1*mut/*FLT3*-ITDneg and *CEBPA*dm were grouped into the good-risk category,

Table 1. Categorization and frequency of gene mutations according to functional properties based on next-generation sequencing in 200 *de novo* AML patients. Modified according to the Cancer Genome Atlas Research Network.[1]

Category	Frequency
Transcription factor fusions	18%
PML-RARA	
CBFB-MYH11	
RUNX1-RUNX1T1	
PICALM-MLLT10	
NPM1 mutations	27%
Tumor suppressor genes	16%
TP53	
WT1	
PHF6	
DNA methylation	44%
DNMT3A	
DNMT3B	
DNMT1	
TET1	
TET2	
IDH1	
IDH2	
Activated signaling	59%
FLT3	
KIT	
Other tyrosine kinases	
Serin–threonine Kinases	
KRAS/NRAS	
PTPs (protein tyrosin phosphatases)	
Myeloid transcription factors	22%
RUNX1	
CEBPA	
Other myeloid transcription factors	
Chromatin modifiers	30%
MLL fusions	
MLL-PTD	
NUP98-NSD1	
ASXL1	
EZH2	
KDM6A	
other	
Cohesin complex*	13%
Spliceosome complex#	14%

**Cohesin is a protein complex regulating the separation of sister chromatids during cell division (mitosis or meiosis). #Spliceosome is a complex of snRNA and protein subunits removing introns from a transcribed pre-mRNA (hnRNA) segment.*

whereas AML with monosomal karyotype, abn(3q), and those with high EVI1 expression were grouped into the very poor risk category.[25] For the two remaining categories in between (i.e. intermediate and poor risk), a combination of cytogenetics and response to initial chemotherapy is used for grouping. Based on the integrated approach, allogeneic HSCT represents the most appropriated post-remission therapy in patients with low HCT-CI and EBMT scores in the intermediate, poor and very poor categories. However, the recommendations become more complex especially in the intermediate and poor risk group with rising HCT-CI and EBMT scores. The risk groups of the integrated risk classification according to the European LeukemiaNet AML Working Party are discussed in more detail below.

Good risk (Table 3)

In patients with core binding factor (CBF) AML, KIT mutations have been associated with an increased relapse rate.[26,27] However, based on a recent report on AML with inv(16)/t(16;16), this unfavorable impact on relapse rate does not translate into an inferior survival. In contrast, AML with inv(16)/t(16;16) harboring additional *FLT3* mutations including *FLT3*-ITD and *FLT3*-TKD as well as those with the second most frequent secondary cytogenetic aberration, trisomy 8, were associated with a strong negative impact in multivariable analysis on OS.[27] However, these results need further confirmation and thus co-operating gene mutations as well as secondary cytogenetic aberrations in CBF-AML should not be used to guide treatment decisions. Based on a large meta-analysis, no beneficial effect on survival end points could be shown for an allogeneic HSCT in first CR compared to intensive post-remission chemotherapy in CBF-AML.[28] In a retrospective study, Gorin *et al.* showed comparable results in CBF-AML treated in first CR with an autologous or allogeneic HSCT for all survival end points.[29] Thus, autologous HSCT may be a treatment option in CBF-AML with additional risk factors in first CR. In phase II clinical trials, dasatinib as a potent KIT-inhibitor has been evaluated in combination with induction and consolidation as well as single agent maintenance therapy (e.g. *clinicaltrials.gov identifier* 01238211 and 00850382); final results are awaited.

In AML exhibiting the genotype *NPM1*-mut/*FLT3*-ITDneg, two reports from co-operative study groups showed a negative impact of cooperating IDH1/2 mutations on relapse-free survival and OS.[30,31] In contrast, Patel *et al.* reported on a favorable impact of the genotype *NPM1*-mut/*FLT3-ITD*neg only if co-operating *IDH1/2* mutations were present.[32] Such opposed effects of genotypes on outcome highlights statistical shortcomings of retrospective molecular studies.

Further conflicting results have been reported on the prognostic value of *TET2* mutations in AML with *NPM1*-mut/*FLT3*-ITDneg or *CEBPA*dm.[33,34] Metzeler *et al.* demonstrated that in ELN favorable risk patients with CN-AML who have a *CEBPA*dm and or *NPM1*mut/*FLT3*-ITDneg, *TET2* mutated patients did poorly on all survival end points.[34] In this analysis, *TET2* mutations were significantly more frequent in older compared to younger patients. Although multivariable analysis revealed an independent impact of *TET2* mutations, age may be an important confounding factor. This is supported by the report from Gaidzik *et al.* focusing on a large cohort of homogeneously treated younger adults.[33] In this study, *TET2* mutations had no prognostic impact on the whole group but also no prognostic impact on all subgroups, including that defined by the genotypes *NPM1*-mut/*FLT3*-ITDneg and *CEBPA*dm. Thus, the prognostic value of *TET2* mutations at least in younger patients is limited; in older patients, a confirmatory study of the results from Metzeler et al. is needed.

Based on a large individual patient data-based meta-analysis, no beneficial effect on survival end points for an allogeneic HSCT in first CR compared to intensive post-remission chemotherapy in AML with the genotype *NPM1*-mut/*FLT3*-ITDneg could be shown.[35]

In AML with mutated *CEBPA*, a provisional WHO 2008 entity, several studies have convincingly shown that AML with double mutant *CEBPA* (*CEBPA*dm) can be distinguished from AML with single mutant *CEBPA* with respect to biological and prognostic features. The favorable prognostic impact of mutant *CEBPA* that was previously demonstrated in several studies can be attributed to the subtype of AML with *CEBPA*dm.[36-39] Therefore several investigators have suggested restricting the provisional entity "AML with *CEBPA* mutations" to those with biallelic mutations. In addition, a favorable prognosis of AML with *CEBPA*dm could not only be shown on the background of a normal karyotype but also of intermediate-risk cytogenetics whereas del(9q) and del(11q) had been identified as the most frequent secondary cytogenetic aberrations.[40] Allogeneic and autologous HSCT compared very favorably with intensive post-remission chemotherapy on RFS, whereas the unfavorable RFS after intensive post-remission chemotherapy could be made up after relapse by a high rate of second CR followed by allogeneic HSCT.[40] Thus, although AML with CEBPAdm is categorized in the good risk group, an autologous or an allogeneic HSCT can be considered in first CR taking into account the patient's personal profile.

Intermediate risk (Table 3)

The intermediate risk category of the recommendations

Table 2. Standardized reporting for correlation of cytogenetic and molecular genetic data in AML with clinical data according to Döhner *et al.*[4]

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

*'t(8;21) and inv(16)/t(16;16) are frequently denoted as core binding factor AML (CBF-AML). *Cytogenetically normal AML (CN-AML). #Includes all AMLs with normal karyotype except for those included in the favorable subgroup. +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, i.e. t(15;17), t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23), t(6;9), inv(3) or t(3;3).*

Table 3. Patient-specific, integrated risk-based application of allogeneic hematopoietic stem cell transplantation in AML CR1 according to Cornelissen *et al*.[25]

AML-risk Group**	AML Risk assessment, including response to induction-I	Risk of relapse following consolidation by		Prognostic scores for nonrelapse mortality that would indicate allogeneic HSCT as preferred consolidation		
		Chemo/auto BSCT	alloHSCT	EBMT-score	HCT-CI score	NRM
Good	t(8;21) with WBC ≤20 inv(16)/t(16;16) Mutated *CEBPA* (double mutated) Mutated *NPM1* without *FLT3*–ITD Early first complete remission and no MRD	35-40%	15-20%	NA (≤ 1)	NA (<1)	10-15%
Intermediate	t(8;21) with WBC >20 Cytogenetically normal (or with loss of X and Y chromosomes), WBC count ≤100 and early first complete remission (after first cycle of chemotherapy)	50-55%	20-25%	≤ 2	≤ 2	<20-25%
Poor	Otherwise good or intermediate, but no complete remission after first cycle of chemotherapy Cytogenetically normal and WBC >100 Cytogenetically abnormal	70-80%	30-40%	≤ 3/4	≤ 3/4	<30%
Very poor	Monosomal karyotype abn(3q26) High *EVI1* expression	>90%	40-50%	≤ 5	≤ 5	<40%

**The proposed patient-specific application of allogeneic HSCT in patients with AML in their first complete remission integrates the individual risks for relapse and non-relapse mortality and aims for a disease-free survival (DFS) benefit of at least 10% for the individual patient compared with consolidation by a non-allogeneic HSCT approach. †The categorization of AML is based on cytogenetic, molecular and clinical parameters (including white blood cell count) into good, intermediate and (very) poor subcategories and is subject to continuing study and debate. Here, categories are arbitrarily presented according to the latest policy of the Dutch-Belgian Cooperative Trial Group for Hematology Oncology and Swiss Group for Clinical Cancer Research (HOVON–SAKK) consortium. Relapse percentages were derived from published reports.[25] §Includes response to first induction. Categorization requires one of the parameters indicated. AML: acute myeloid leukemia; EBMT: European Group For Blood and Marrow Transplantation; DFS: disease-free survival; EVI1: Ecotropic viral integration site 1; HCT–CI: hematopoietic cell transplantation comorbidity index; HSCT: hematopoietic stem cell transplantation; CEBPA: gene encoding CCAAT enhancer-binding protein α; FLT3: gene encoding fms-like tyrosine kinase receptor–3; ITD: internal tandem duplication; NA: not advocated; NPM1: gene encoding nuclear matrix protein; MRD: minimal residual disease; WBC: white blood cell count.*

comprises mainly AML with CN-AML who achieve a CR after induction therapy.

DNMT3A have been found to be frequently mutated in AML with normal karyotype (30-35%).[41-44] Two studies have demonstrated that *DNMT3A* mutations are independently associated with poor OS.[41,42] However, patients exhibiting a *DNMT3A* mutation were significantly older in both studies and thus again age may be an important confounding factor in these analyses. Marcucci *et al*. reported on a differential prognostic effect of *DNMT3A* mutations in older versus younger patients according to the affected codon; older patients with *DNMT3A* mutations in codon R882 in exon 23 had an inferior outcome, whereas in younger patients, those with *DNMT3A* mutations other than R882 did worse.[42] In the largest analysis so far published on 1770 young adults, *DNMT3A* mutations had no consistent impact on survival end points in the whole group.[44] However, in subgroup analyses, *DNMT3A* mutations were found to be associated with an unfavorable prognosis in the ELN molecular unfavorable subgroup (Table 2) of CN-AML.

Approximately two-thirds of *RUNX1* mutations are found in CN-AML and have been associated with a very unfavorable prognosis in both young and elderly patients.[45,46] Gaidzik *et al*. reported a dismal outcome for all survival end points in patients with *RUNX1* mutations after consolidation chemotherapy when compared to allogeneic HSCT in first CR.[45]

Based on the recommendations made according to the integrated risk classification of the ELN-AML Working Party, an allogeneic HSCT in this risk group is preferable in cases for which a matched donor is available and in the absence of relevant comorbidities reflected by an EBMT and HCT-CI score equal to or below 2 (Table 3). An exemption may be AML with a *FLT3*-ITD in the presence of an *NPM1* mutation and a low *FLT3*-ITD mutant to wild-type ratio (<0.5) with a reported favorable outcome also after intensive chemotherapy.[47] However these results need further confirmation. Furthermore, FLT3-inhibitors are in clinical evaluation,[48] where maintenance after intensive consolidation therapy but also after allogeneic HSCT has been evaluated in ongoing clinical trials (e.g. *clinicaltrials.gov identifier* 00651261 and 01477606).

Poor and very poor risk (table 3)

Patients categorized in the poor or very poor risk group have per se a dismal prognosis and most of these patients should be offered an allogeneic HSCT if a CR is achieved[25] and even if a CR is not achieved.[49] *TP53* alterations are closely associated with a complex karyotype and in particular also with a monosomal karyotype,[50] and thus most are already categorized in the very poor risk group. However, if a CR is achieved, again an allogeneic HSCT should be offered if possible. Whether maintenance therapy with hypomethylating agents after an allogeneic HSCT improves RFS is currently under evaluation in several clinical trials

(e.g. *clinicaltrials.gov identifier* 01168219, 01995578, and 01541280). However, azacitidine in combination with donor lymphocytes is an active treatment in high-risk patients who have relapsed after allogeneic HSCT.[51]

Minimal residual disease assessment during treatment and follow up

Beyond pre-treatment risk stratification, measurement of the disease burden during treatment and follow up emerges as a tool to fine tune the risk assessment on an individual basis with dynamic adaptation of post-remission treatment strategy. Minimal residual disease (MRD) can be evaluated by polymerase chain reaction (PCR) and multiparameter flow cytometry (MPFC); approaches using next generation sequencing are currently evaluated.

Leukemia fusion genes and gene mutations can be detected by RT-PCR or the currently more widely used real time quantitative (RQ)-PCR. By using this technique, MRD can be measured with high sensitivity (10^{-4} to 10^{-6}) in CBF-AML (*RUNX1-RUNX1T1*[52] and *CBFβ-MYH11*[53]) as well as in AML with t(9;11), MLL-AF9.[54] Consistently, either negativity or marked reduction in transcript level at different time points were associated with a lower risk of relapse. After first induction therapy, a more than 3 log reduction in AML with t(8;21) or an absolute reduction to copy numbers below 10 in AML with inv(16)/t(16;16) were associated with a low relapse probability of 4% and 21% at two years, respectively, whereas patients with intermediate reduction, 1-3 log reduction in AML with t(8;21) and 10-500 copy numbers in AML with inv(16)/t(16;16) had significantly higher relapse rates of 30%-42% and 52%, respectively.[52] Patients with only a marginal reduction in MRD levels after induction therapy virtually all relapsed. In a study of the German-Austrian AMLSG focusing on AML with inv(16)/t(16;16), RQ-PCR negativity during consolidation and early follow up (first 3 months) was associated with an RFS of 91% compared to only 35% in the MRD-positive group after two years.[53] Similarly, in AML with *NPM1* mutation, RQ-PCR negativity after completion of consolidation therapy was associated with a low cumulative incidence of relapse of 15.5% compared to 66.5% in the RQ-PCR positive group after four years.[55] Thus, intensification of post-remission therapy with an allogeneic HSCT in first CR can be envisaged if MRD levels stay either positive or above a distinct level in CBF-AML and AML with *NPM1* mutation. However, such an approach with MRD-directed treatment intensification has so far not been studied prospectively in a controlled manner. The concept of the HOVON/SAKK 132 AML study integrates this approach based on MPFC MRD assessment. For this purpose, the prognostic value of MPFC MRD assessment has been established in the HOVON/SAKK AML-42A study.[56] After two cycles of induction treatment, an MRD level of less than 0.1% was associated with a lower cumulative incidence (CIR) of relapse of 37% compared to patients with an MRD level of more than 0.1% with a CIR of 68%.[56] Similar results had been reported by the MRC in older patients receiving intensive treatment.[57] After the second course of induction therapy, patients with an MRD level less than 0.1% had a CIR of 73% compared to 82% in those with MRD levels of more than 0.1%. Although the differences were statistically significant, the positive predictive value was limited in both studies. Therefore, a prospective evaluation of this important clinical question is mandatory. Consistently in all studies, a steady increase in MRD levels during the follow-up period was closely associated with hematologic relapse. Thus, the initiation of a pre-emptive salvage therapy intervention during molecular relapse may be advantageous; but again, this has to be evaluated prospectively.

Conclusions

1.It is still strongly recommended that, if informed consent is given, patients with AML should be treated in clinical trials.

2.High-dose cytarabine in a dosage of 2-3 g/m², bid, Days 1,3,5, remains the standard for intensive post-remission chemotherapy.

3.It is key to weigh the risk of relapse and non-relapse mortality in post-remission therapy with allogeneic HSCT against intensive chemotherapy to identify the best treatment option for each individual patient.

4.Minimal residual disease assessment during treatment and follow up allows post-remission treatment and pre-emptive salvage treatment to be adapted before overt hematologic relapse occurs.

Authorship and Disclosures

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