Risk factors for relapse after allogeneic transplantation in acute myeloid leukemia

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ABSTRACT

Acute myeloid leukemia is a clonal neoplasm derived from myeloid progenitor cells with a varying outcome. The initial goal of treatment is the achievement of complete remission, defined for over 40 years by morphology. However, without additional post-remission treatment the majority of patients relapse. In many cases of acute myeloid leukemia, allogeneic stem cell transplantation offers the best prospects of cure. In 2013, 5608 stem cell transplantations in acute myeloid leukemia were performed in Europe (5228 allogeneic and 380 autologous stem cell transplantations). Most stem cell transplantations are performed in first complete remission. However, despite a considerable reduction in the chance of relapse, in most studies, overall survival benefit of allogeneic stem cell transplantation is modest due to substantial non-relapse mortality. Here we discuss the many factors related to the risk of relapse after allogeneic stem cell transplantation.

Introduction

Acute myeloid leukemia (AML) is a clonal neoplasm derived from myeloid progenitor cells with a varying outcome. The initial goal of treatment is the achievement of complete remission (CR), defined for over 40 years by morphology.[1] Without additional post-remission treatment, however, the majority of patients relapse. In many cases of AML, allogeneic stem cell transplantation (alloSCT) offers the best prospects of cure. Apart from the conditioning regimen, the anti-leukemic potential is mainly based on the immunological graft-*versus*-leukemia effect. Indeed, AML is the most frequent indication for alloSCT, as indicated by data from both the European Group for Blood and Marrow Transplantation (EBMT) and the International Bone Marrow Transplant Registry (IBMTR), and the number of patients transplanted for this indication is growing year by year.[2] In 2013, 5608 stem cell transplants in AML in Europe were performed [alloSCT: 5228 and autologous (auto) SCT: 380]. The rise in frequency in recent years is due to the application of reduced intensity conditioning (RIC) regimens and the expansion of alternative donor stem cell sources derived from mismatched relatives and unrelated volunteers. The total number of family donors was 2354 (1913 HLA identical and 441 non-identical) while 2863 unrelated transplants were performed, among which there were 211 cord blood transplantations. The majority of patients were transplanted in first complete remission (CR1). A meta-analysis of prospective trials and trials reporting relapse-free survival (RFS) and/or overall survival (OS) outcomes after assigning adult patients with AML in CR1 to undergo alloSCT *versus* non-alloSCT treatment, based on donor availability (donor *vs.* no-donor comparisons), showed that, except for good risk AML in CR1, alloSCT gives a significant survival benefit for intermediate and poor risk AML.[3] However, despite a considerable reduction in the possibility of relapse, in most studies, OS benefit of alloSCT is modest due to substantial non-relapse mortality (Table 1).[4-9] Now we are approaching a situation in which we can identify a suitable donor, either matched unrelated, haplo-identical or cord, for nearly every patient, This means that both disease-related and transplant-related factors should be carefully balanced before proceeding to an allogeneic or non-allogeneic approach after achieving CR.[10]

Unfortunately, even after alloSCT, a substantial number of AML patients will ultimately relapse, and in these cases survival is very poor.[11] Hematologists are now facing the question "which patient should get a transplant in first remission?" Predicting relapse in an individual patient still remains a challenge. Here, we will mainly focus on factors predicting for relapse after allogeneic transplant in AML.

Transplant-related factors in relation to relapse risk after transplantation

Dose intensity is a main determinant for relapse. With increasing dosage, even at the myeloablative (MA) level, the chance of relapse decreases.[12] RIC have become popular and these are being increasingly adopted. Although no prospective randomized trials have been completed, these lower intensity conditioning regimens seem to be associated with a higher rate of relapse. A retrospective EBMT comparison of conditioning regimens showed an increase in relapse rate of 23%-39% after MA *versus* RIC.[13] Nevertheless OS benefits for MA are, at best, modest due to the increased non-relapse mortality (NRM) in comparison with RIC. Although the only prospective randomized study comparing RIC *versus* MA was stopped early because of slow accrual of patients, there was no significant difference in RFS, NRM and OS.[14] In general, it is clear that increasing the intensity of the conditioning regimen results in more acute graft-*versus*-host disease (GvHD) and increased NRM. In several, but not all, studies, more intensive GvHD prophylaxis, for example by pre-transplant administration of anti-thymocyte globulin or T-cell depletion of the graft, resulted in a higher relapse rate, probably due to less GvL. Whether early donor lymphocyte infusion after T-cell depleting strategies or high-dose cyclophosphamide post transplant counterbalance NRM *versus* relapse is the subject of much debate and investigation.[15,16] Chronic GvHD reduced the chance of relapse in many studies; however, in a recent registry study of the Center for International Blood and Marrow Transplant Research (CIBMTR), its impact seemed only clinically relevant for CML after myeloablative alloSCT and not for AML.[17] In this study, cGvHD was primarily associated with a higher transplant-related mortality (TRM).

Disease-related characteristics in relation to relapse risk after transplantation

Patient-specific biological factors associated with risk of relapse after transplantation are basically the same as those in patients treated with chemotherapy only. Based on cytogenetic analysis, three risk groups for relapse can be identified, as described by Grimwade *et al*.[18] New categories such as the monosomal karyotype associated with a very poor outcome are also used to estimate the possibility of relapse by various AML trial groups.[19] Novel genomic technology has paved the way for the detection of numerous new molecular aberrations, further underlining the heterogeneity of AML, and these are also helpful to establish a prognosis. Some of these molecular aberrancies (mutations in the *NPM1, CEBPA* and *FLT3* genes) have already been incorporated into the widely applied recommendations for standardized reporting of genetic abnormalities by the European LeukemiaNet panel of experts published in 2010 (Table 2).[20] Since then, several new molecular aberrancies have been identified, some of which are clearly associated with outcome, while others are less so. The assumption that most of these aberrancies are equally important for risk of relapse after chemotherapy, as well as after transplantation, still has to be proved. Some of these novel genetic data will be discussed below.

Mutations of CCAAT/enhancer binding protein alpha

Mutations of CCAAT/enhancer binding protein alpha (CEBPA) encodes a transcription factor essential for differentiation along the neutrophil lineage. Although already incorporated into the ELN recommendations, it has become clear that only double and not single mutated CEBPA is associated with a favorable outcome.[21]

Mutations of the nucleophosmin gene

The nucleophosmin gene (*NPM1*) gene encodes for a protein that shuttles between nucleus and cytoplasm, acting, amongst other things, as a molecular chaperone of histone proteins. In addition, it is involved in other critical cellular functions, such as ribosome biogenesis and transport, and centrosome duplication during the cell cycle. Mutations consist of a 4-base pair insertion that alters the C-terminal end of the protein, leading to a nuclear export signal of the encoded protein, and thus to loss of shuttling activity. Several studies have shown a favorable impact of the *NPM1* mutation on outcome in the absence of *FLT3* gene mutations. In a donor *versus* no donor analysis, Rollig *et al*. recently showed that alloSCT resulted in a significantly prolonged RFS in patients with $NPM1^+$ mutated AML. However, OS was not improved, most probably due to the fact that relapsed $NPM1^+$ AML patients responded well to salvage treatment.[22] $NPM1^+$FLT3 ITD-AMLs are now grouped together with the core binding

Table 1. Allogeneic stem cell transplantation for acute myeloid leukemia in first complete remission in comparison to autologous stem cell transplantation and chemotherapy.

AML Trial Group	Relapse rate			Overall survival		
	Allo	Auto	Chemo	Allo	Auto	Chemo
EORTC/AML-8	24%	41%	57%	59%	56%	46%
GOELAM	37%	45%	55%	55%	52%	58%
ECOG/CALGB/SWOG	29%	48%	61%	46%	43%	52%
EORTC AML-10	30%	52%		58%	50%	
UK MRC AML-10	36%	52%*		55%	42%*	
HOVON-SAKK	32%	59%*		54%	46%*	

AML: acute myeloid leukemia; ALLO: allogeneic stem cell transplantation; AUTO: autologous stem cell transplantation; CHEMO: chemotherapy. Table modified from Kanate et al.[51]
**No separate data of autoSCT and chemotherapy available.*

factor (CBF) leukemias and the CEBPA double mutants and have a good prognosis.

Internal tandem duplications of the FMS-like tyrosine kinase 3 gene

Internal tandem duplications (ITDs) of the FMS-like tyrosine kinase 3 gene occur in 20%-30% of AML cases, predominantly in those with normal cytogenetics. They are associated with a poor outcome, especially when the ratio between mutated and non-mutated *FLT3* gene is more than 0.51. This is not only due to an increased risk of relapse, but also due to refractoriness to induction treatment.[23,24]

Expression of the ecotropic viral integration-1 oncogene

Ten percent of AML cases show high ecotropic viral integration-1 (EVI-1) oncogene expression, which predicts for a particularly poor outcome. Besides inv(3)/t(3;3), EVI1(+) is significantly associated with the chromosome abnormalities monosomy 7 and t(11q23). EVI1+ is virtually absent in favorable-risk AML and AML with NPM1 mutations.[25]

Mutations in DNA-methyltransferase-3A

The DNA-methyltransferase-3A (DNMT3A) enzyme plays a role in DNA methylation by transferring methyl-groups to DNA CpG islands. Mutations in this gene are detected in approximately 20% of AML cases and enriched in normal karyotype AML (CN-AML) with an incidence of between 30%-37%. Various studies have proved to be inconclusive as to the prognostic value for outcome. A recent meta-analysis showed a slightly poor prognostic impact on OS.[26,27]

Mutations in the additional sex combs-like 1 gene

The additional sex combs-like (ASXL)1 gene is an epigenetic scaffolding protein that assembles epigenetic regulators and transcription factors to specific genomic loci with histone modifications. The incidence of truncating mutations in ASXL1 is between 5%-12% and is always associated with a poor outcome.[28]

Mutations in the ten-eleven translocation-2 gene

The ten-eleven translocation-2 (TET2) gene is a key enzyme for DNA demethylation and a critical regulator for hematopoietic stem cell homeostasis, whose functional impairment leads to hematologic malignancies. Mutations are frequently found in AML. Although in most studies it is associated with a poor outcome, the prognostic relevance has not been definitively established.[29,30]

TP53 *mutations and 17p abnormalities*

The Study Alliance Leukemia (SAL) and other groups reported poor survival data in patients with abn(17p) or TP53-mutated AML.[31] In a large retrospective repository-based analysis published by Grossmann *et al.*, patients with TP53-mutated AML had the worst prognosis of all molecularly defined risk groups, with a median OS of 4.6 months and event-free survival (EFS) of 0% at three years.[32]

Isocitrate dehydrogenase1 and -2 mutations

Isocitrate dehydrogenase1 and -2 (IDH1/2) mutations are found in AML as well in gliomas, both with an incidence of approximately 15%-20%. IDH1 and IDH 2 are critical enzymes in the citric acid cyclus, converting isocitrate to α-ketoglutarate. Due to the mutation, 2 hydroxyglutarate (2-HG) instead of α-ketoglutarate is formed. 2-HG inhibits TET enzymes, which results in hypermethylation. This is thought to suppress expression of tumor suppressor genes. These mutations are mutually exclusive with *TET2* mutations. Although the prognostic significance has still not been established, some studies have shown a poor outcome.[33,34]

RUNX1 *mutations*

RUNX1 mutations are considered to be associated with a poor prognosis. The incidence (8%-16%) is higher in elderly AML and in secondary AML.[35]

Mutations in other genes

C-KIT mutations especially have poor prognostic implications in t(8;21) AML and predict for higher relapse rates than unmutated cases; however, MRD status before alloSCT was more predictive than c-kit status.[36] The clinical impact of expression levels of numerous other genes such as *BAALC, ERG, MN1* etc., still has to be determined. How these new molecular aberrancies could be integrated

Table 2. Standardized reporting for correlation of cytogenetic and molecular genetic data in acute myeloid leukemia with clinical data.[2]

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

**Includes all AMLs with normal karyotype except for those included in the favorable subgroup; most of these cases are associated with poor prognosis, but they should be reported separately because of the potential different response to treatment. †For most abnormalities, adequate numbers have not been studied to draw firm conclusions regarding their prognostic significance. ‡Three or more chromosome abnormalities in the absence of one of the WHO designated recurring translocations or inversions, that is, t(15;17), t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23), t(6;9), inv(3) or t(3;3), indicate how many complex karyotype cases have involvement of chromosome arms 5q, 7q, and 17p.*

Table 3. Recommendation for allogeneic SCT in AML CR1 based on integrated risk profiles. Adapted by HOVON-SAKK from the ELN recommendation by adding new molecular markers and MRD.[10]

AML risk group‡	AML risk assessment criteria at diagnosis and early/late CR	MRD after cycle2 +/-	Risk of relapse following consolidation approach		Prognostic scores for nonrelapse mortality that would indicate allogeneic HSCT as preferred consolidation		
			Chemotherapy or autologous HSCT (%)	Allogeneic HSCT (%)	EBMT score[52]	HCT-CI score[53]	Nonrelapse mortality risk (%)
Good	t(8;21) or AML1-ETO, WBC≤20 inv16/t(16;16) or CBFB-MYH11 CEBPA-biallelic mutant+ *FLT3*TD-/NMP1+,	+or-	35–40	15–20	NA (≤1)	NA (<1)	10–15
Intermediate	CN –X –Y, WBC≤100, CRe t(8;21) or AML1-ETO, plus WBC>20 or mutant KIT	- -	50–55	20–25	≤2	≤2	<20–25
Poor	CN –X –Y, WBC≤100, CRe t(8;21) or AML1-ETO, WBC>20 and/or mutant KIT CN –X –Y, WBC≤100, not CRe CN –X –Y, WBC>100, CA, but non-CBF, MK-, no abn3q26	+ + + +or- _ _	70–80	30–40	≤3–4	≤3–4	<30
Very poor	CN –X –Y, WBC>100 CA, but non CBF, MK-, no abn3q26, EVI1-neg MK+ abn3q26 Non CBF, EVI1+ Non CBF with mutant p53, or mutant RUNX1, or mutant ASXL1 or bi-allelic FLT3-ITD with *FLT3*-ITD/*FLT3*wt ratio of >0.6	+or-	>90	40–50	≤5	≤5	<40

The proposed patient-specific application of allogeneic HSCT in AML CR1 integrates the individual risks for relapse and non-relapse mortality and aims for a 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 WBC) into good, intermediate and (very) poor subcategories as currently applied by the Dutch–Belgian Cooperative Trial Group for Hematology Oncology and Swiss Group for Clinical Cancer Research (HOVON–SAKK) consortium.

into a new prognostic algorithm was illustrated by Patel *et al*. who applied high throughput sequencing of TET2, ASXL1, DNMT3A, CEBPA, PHF6, WT1, TP53, EZH2, RUNX1, PTEN, FLT3, NPM1, HRAS, KRAS, NRAS, KIT, IDH1 and IDH2 in over 500 patients from the ECOG E1900 trial. They showed that the mutational analysis of 9 of these genes could be used to retrospectively classify patients into more precise subgroups with favorable-risk, intermediate-risk, or unfavorable-risk profiles, with marked differences in the overall outcome.[37] Another example of how molecular profiling may be helpful has been shown by Grosmann *et al*.[38] In a large cohort of AML patients for whom cytogenetic data was available, they investigated the following molecular alterations: PML-RARA, RUNX1-RUNX1T1, CBFB-MYH11, FLT3-ITD, and MLL-PTD, as well as mutations in NPM1, CEPBA, RUNX1, ASXL1, and TP53. Five distinct prognostic subgroups were identified: 1) very favorable: PML-RARA rearrangement or CEPBA double mutations (OS at 3 years: 82.9%); 2) favorable: RUNX1-RUNX1T1, CBFB-MYH11, or NPM1 mutation without FLT3-ITD (OS at 3 years: 62.6%); 3) intermediate: none of the mutations leading to assignment into groups 1, 2, 4, or 5 (OS at 3 years: 44.2%); 4) unfavorable: MLL-PTD and/or RUNX1 mutation and/or ASXL1 mutation (OS at 3 years: 21.9%); and 5) very unfavorable: TP53 mutation (OS at 3 years: 0%). This profile, based only on molecular abnormalities, provided a more powerful model for prognostication than cytogenetics.[38] Taken together, these data indicate that more detailed genetic analysis may lead to improved risk stratification

Clinical features in relation to risk of relapse after transplantation

Apart from cytogenetic and molecular prognostic markers identified at diagnosis, a number of clinical variables that can be assessed either at diagnosis or during induction and consolidation treatment might offer additional prognostic information. These include high WBC count, time to complete remission, day 15 presence of blasts, extramedullary disease, and quantifiable levels of minimal residual disease (MRD) after induction or consolidation therapy. Here we will focus on the prognostic value of MRD.

Minimal residual disease

Despite a multitude of prognostic factors at diagnosis, the outcome of patients is still highly variable and not individually predictable. It thus seems that prognosticators at diagnosis will not enable clinicians to reach the ultimate goal of truly individualized risk assessment. One important issue is that the prognostic impact of these factors in the present risk groups does not take into account the contribution of several cellular resistance mechanisms at diagnosis, and also post-diagnosis factors, which include

dosage, compliance, pharmacological resistance, and probably other unknown features. These factors, which corroborate proper risk classification, are only partly covered by inclusion of CR status. However, the ability to define residual disease far below the level of 5% blast cells is changing the landscape of risk classification. This so-called minimal residual disease (MRD) approach currently enables detection of leukemia cells down to levels of 1:1,000–1:106 white blood cells, compared to only 1:20 for morphology.[39,40]

Methods for detection of minimal residual disease

The most sensitive method at present is real-time quantitative polymerase chain reaction (RQ-PCR). Chimeric fusion genes like *PML-RARA*, *RUNX1-RUNXT1*, and *CBFB-MYH11* are reliable markers for MRD evaluation; however these genetic abnormalities are only present in approximately 20% of AML cases. NPM1 mutation is an abnormality that occurs more frequently for which mutation-specific RQ-PCR assays have been developed to monitor MRD.[41] Another approach for assessment of MRD has gained increasing attention: the use of aberrant (i.e. leukemia-associated) immunophenotypes (LAIP) with flow cytometry. Currently, with this approach, aberrancies may be detected in over 90% of AML cases at diagnosis. These LAIPs consist of normally occurring markers, present in aberrant combinations in AML bone marrow (BM), while only at very low frequencies, or even absent, in normal and regenerating BM.[42]

Minimal residual disease in clinical studies

During the last 15 years, numerous single institute studies have been carried out, both in adult and pediatric AML, which have established the independent prognostic value of "Immunophenotypic MRD." The results of the first multicenter (31 sites) multinational prospective MRD study in adult AML (aged 18-60 years) were recently reported by the HOVON/SAKK investigators. In this study, BM MRD was assessed at five different sites in samples obtained after one and 2 cycles of induction therapy and after consolidation therapy.[43] MRD measurements were performed in a blinded fashion, i.e. without knowing the patients' performance, while patients were treated according to protocol also without knowledge of MRD-related data. After all treatment cycles, low MRD values distinguished patients with relatively favorable outcome from those with adverse RFS and OS. In the whole patient group, and in the clinically most interesting subgroup with intermediate risk cytogenetics, MRD was an independent prognostic. After all treatment courses, low MRD values distinguished patients with relatively favorable outcome from those with adverse RFS and OS. Multivariate analysis after cycle 2I, when decisions about consolidation treatment have to be made, confirmed that high MRD values (>0.1% of WBC) was associated with a considerably higher risk of relapse even after adjustment for consolidation treatment time-dependent co-variate risk score and early or later CR. Similar results were obtained by Freeman *et al*. who used MRD assessments in a group of elderly patients. In addition, MRD determination by means of quantitative PCR of PML-RARA, AML1-ETO, CBFB-MYH11 transcripts and of mutations in NPM1 has also proven to be of value in the clinical arena.[44-46]

Before transplantation, MRD status in CR1 AML patients was demonstrated to be a highly valuable marker of disease recurrence and shorter OS after transplant. Several studies in patients undergoing myeloablative conditioning all show that the presence of MRD has a negative impact on post-transplant relapse risk.[47-49] Recently, Walter *et al*. showed that the same holds true for non-myeloablative conditioning.[50] An integrated risk-adapted approach on allogeneic transplantation for patients with AML is given in Table 3. This is currently being followed in the HOVON-SAKK trials and follows the ELN consensus statement, now further refined by introducing new molecular markers and MRD determination after cycle 2.

Conclusion

Risk of relapse after allogeneic stem cell transplantation is a composite of many factors, as described here. An estimation of this risk together with an estimation of expected treatment-related mortality should be used as a guide to determine which patient should be offered an alloSCT as post-remission treatment. It is clear that this must be an individualized, well-balanced clinical decision, which cannot be made from a single recommendation that fits all.

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