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Optimization of the indications for allogeneic stem cell transplantation in Acute Myeloid Leukemia based on interactive diagnostic strategies

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Summary

The indications for allogeneic stem cell transplantation (SCT) in Acute Myeloid Leukemia (AML) represent a real challenge due to the clinical and genetic heterogeneity of the disorder. Therefore, an optimized indication for SCT in AML first requires the determination of the individual relapse risk based on diverse chromosomal and molecular prognosis-defining aberrations. A broad panel of diagnostic methods is needed to allow such subclassification and prognostic stratification: cytomorphology, cytogenetics, molecular genetics, and immunophenotyping by multiparameter flow cytometry. These methods should not be seen as isolated techniques but as parts of an integral network with hierarchies and interactions. Examples for a poor risk constellation as a clear indication for allogeneic SCT are provided by anomalies of chromosome 7, complex aberrations, or FLT3-length mutations. In contrast, the favorable reciprocal translocations such as the t(15;17)/PML-RARA or t(8;21)/AML1-ETO are not indications for SCT in first remission due to the rather good prognosis after standard therapy. Further, the indication for SCT should include the results of minimal residual disease (MRD) diagnostics by polymerase chain reaction (PCR) or flow cytometry. New aspects for a safe and fast risk stratification as basis for an optimized indication for SCT in AML might be provided by novel technologies such as microarray-based gene expression profiling.

Keywords: Acute Myeloid Leukemia (AML), Allogeneic Stem Cell Transplantation (SCT), Indication, Cytogenetics, Polymerase Chain Reaction (PCR)

Introduction

Acute Myeloid Leukemia (AML) represents as highly heterogeneous disorder with very variable clinical courses and response to chemotherapy. Long term survival ranges from >80% in Acute Promyelocytic Leukemia (APL) with the (15;17)/*PML-RARA* translocation to <10% in cases with complex aberrations (≥3 chromosomal abnormalities). Additionally, the internal tandem duplications/length mutations within the *FLT3*-gene (*FLT3*-ITD/LM) confer an extremely adverse prognostic impact (Thiede *et al*, 2002; Schnittger *et al*, 2002), whereas isolated mutations in the *Nucleophosmin* (*NPM1*) gene in normal karyotype cases are predictive for a more favorable prognosis (Falini *et al*, 2005).

Due to these variances in outcome, the prospective determination of the intensity of treatment in AML is a major task. This applies especially to the indications for allogeneic stem cell transplantation (SCT), as the benefit of the graft *versus* leukemia (GvL) effect has to be balanced against the risks of transplant-associated morbidity and mortality (TRM) in each individual case.

A broad panel of diagnostic methods is necessary to meet the demands of an optimized risk stratification which forms basis for the decision for SCT: Cytogenetic abnormalities are identified by chromosomal banding analyses in ~55% of patients with AML and represent the strongest known prognostic parameters in AML (Bloomfield *et al*, 1997; Swansbury *et al*, 1994). In the remaining 45% of patients where no chromosomal abnormalities can be identified, molecular strategies based on diverse polymerase chain reaction (PCR) techniques allow a more detailed risk stratification in >80% of all cases (Marcucci *et al*, 2005).

Early cytomorphological assessment of bone marrow blast reduction after induction therapy contributes additional prognostic

information (Kern *et al*, 2003a). This can be combined with multiparameter flow cytometry (MFC), as the quantification of cells with a leukemia associated immunophenotype (LAIP) before and after induction therapy allows an early and very sensitive evaluation of the response to treatment (Kern *et al*, 2003b). Quantitative PCR can also be helpful in evaluating the response to therapy at an early timepoint (Schnittger *et al*, 2003). During follow-up of the disease, the quantification of the minimal residual disease (MRD) load by PCR and MFC permits the detection of impending relapse on a molecular level before clinical or morphological manifestation (Grimwade *et al*, 1996; Lo *et al*, 1999).

Thus, the indication for allogeneic SCT in AML requires not only a broad panel of laboratory methods but also has high demands for the knowledge and interpretation of a variety of cytogenetic and molecular markers. To further increase insights into this complex panel of criteria that are relevant for the decision for allogeneic SCT in AML, this work intends to give an overview of the relevant diagnostic methods and markers which can support this complex decision process.

Cytomorphological criteria

The performance of bone marrow cytomorphology shortly after the end of induction allows an assessment of early blast clearance in AML patients. A reduction of blasts <10% on day 16 after the start of induction (**"day 16 blasts"**) was demonstrated to represent a favorable prognostic parameter. In contrast, the persistence of higher blast percentages at this time-point is a negative prognostic sign and should always provoke the question whether there might be an indication for allo-SCT (Preisler *et al*, 1986; Kern *et al*, 2003a).

Cytogenetic criteria

Chromosome banding analyses still play a central role for sub-classification and determination of prognosis in AML (Byrd *et al*, 2002; Slovak *et al*, 2000). To verify the results obtained by chromosome banding and to further clarify more complex aberrations, several fluorescence *in situ* hybridization (FISH) techniques (e.g. interphase FISH, metaphase FISH, 24-color FISH/SKY) can additionally be performed. Further, interphase FISH provides a higher sensitivity, as 100-200 cells can be evaluated without problems in comparison to 20-25 metaphases by chromosomal banding (Haferlach *et al*, 2007).

The karyotypes allow separation of AML patients into three prognostic relevant groups: The **favorable subgroup** is represented by the **recurrent reciprocal translocations** t(15;17)/*PML-RARA,* t(8;21)/*AML1-ETO,* and inv(16)/*CBFB-MYH11* from the first hierarchy of the WHO classification (Jaffe *et al*, 2001). Due to the favorable outcome which is achieved by standard therapy in these cytogenetic subgroups, allogeneic SCT is not performed in first complete remission anymore. However, in the case of relapse, allogenic SCT also remains an option in these subgroups (de Labarthe *et al*, 2005; Grimwade *et al*, 1998; Yanada *et al*, 2005a).

The **second prognostically intermediate subgroup** contains patients with a **normal karyotype** or certain distinct aberrations—e.g. trisomy 8—which do not confer a specific prognostic impact. However, the subgroup of patients with a normal karyotype can be separated into several subentities on the basis of diverse molecular markers, so the indication for SCT can be further determined and differentiated even in this heterogeneous group.

The **third prognostically unfavorable subgroup** includes **unbalanced karyotypes**, characterized by gain or loss of whole chromosomes or chromosomal regions. Patients with anomalies of chromosomes 3—e.g. an inversion inv(3)/t(3;3)(q21q26)—or structural or numerical **abnormalities of chromosome 7** are also part of this group. **Complex aberrant karyotypes** which are defined as >3 chromosomal anomalies are found in 10-15% of all AML cases. Conventional chemotherapy achieves stable remissions only rarely (Büchner *et al*, 2004). Complex aberrations are interpreted as result of multistep leukemogenesis and show similarities to solid tumors with respect to the pathomechanisms and the inferior response to cytotoxic therapy (Schoch *et al*, 2001). Another example are the **11q23/***MLL***-rearrangements**, which occur often in therapy induced AML (t-AML) after treatment with topoisomerase-II inhibitors such as Etoposide.

All these prognostically unfavorable subgroups are characterized by relapse rates of up to 80%. Whereas allogeneic SCT was shown to result in survival of >40%, intensive chemotherapy or high dose chemotherapy followed by autologous stem cell support results in long time survival of only 15-20% in these subgroups. Therefore, diagnosis of the respective karyotypes should in all cases be followed by early planning of allogeneic SCT if possible (Suciu *et al*, 2003).

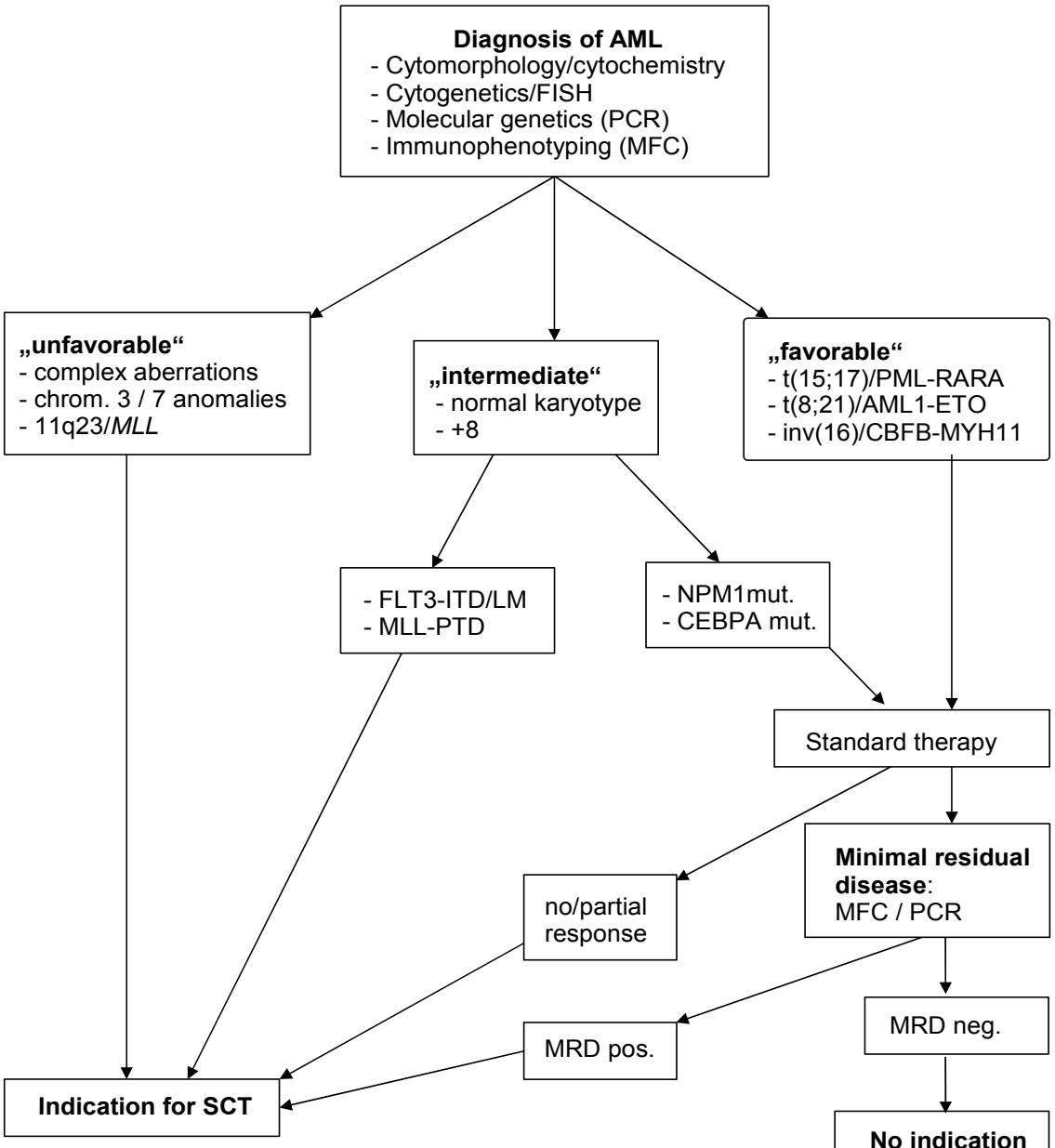
Previously, it had been thought that **secondary AML** (s-AML) after MDS and **therapy associated AML** are *per se* associated with inferior outcome. However, recent studies showed that prognostically unfavorable karyotypes are more frequent in these subgroups, but that prognosis of the individual karyotypes does not differ from the corresponding cytogenetic alterations in *de novo* AML (Messner, 2006; Larson, 2007). However, stable disease free survival of >30% has been achieved in s-AML after MDS by allogeneic SCT in some studies (de Witte *et al*, 2000), and dose reduced conditioning protocols might further improve these results (Kröger *et al*, 2003).

Molecular criteria

From molecular the aspect, the subgroup of patients with a normal karyotype is composed of a large spectrum of diverse mutations that are associated with distinct prognostic profiles: Length mutations/internal tandem duplications of the *FLT3* gene (*FLT3***-LM/ITD**), which are represented by insertions of a few hundred base pairs, are found in ~40% of all patients with normal karyotype (Schnittger *et al*, 2004;Yanada *et al*, 2005b). Prognosis is dismal, and stable remissions after standard chemotherapy protocols are only seen occasionally (de Labarthe *et al*, 2005). With allogenic SCT, survival could be improved from 20-25% up to 45-50% in some studies (Bornhäuser *et al*, 2007; Schlenk, ASH annual meeting abstracts 2006, 108).

In contrast, isolated **mutations of the** *NPM1* **gene** are prognostically favorable. They are detected in ~50% of all patients and represent the most frequent molecular marker in AML with a

Figure 1. Algorithm for the decision process towards allogeneic SCT in AML according to the genetic results and to the response to therapy



FISH: Fluorescence in situ hybridization; mut.: mutations;
MFC: multiparameter flow cytometry; PCR: polymerase chain reaction;
MRD: minimal residual disease;
SCT: allogeneic stem cell transplantation.

specific association to normal karyotype. The respective mutation is represented by diverse subtypes of a 4 base pair insertion and results in a disturbed function of a tumor suppressor pathway (Falini *et al*, 2005).

Recently, Schlenk *et al.* demonstrated that patients with isolated *NPM1*-mutations without evidence of *FLT3*-length mutations and with a normal karyotype do not benefit from allogeneic SCT in first remission. However, when the *FLT3*-LM and the *NPM1*-mutation occur in coincidence, outcome was improved when allogeneic SCT was performed (Schlenk, ASH annual meeting abstracts 2006, 108).

Other mutations are relevant as well, e.g. **mutations of the** *CEPBA***-gene**. Due to the favorable prognosis their isolated presence should exclude SCT from first-line treatment concepts in first remission (Schlenk, ASH annual meeting abstracts 2006, 108).

The spectrum of molecular markers being able to allow a more differentiated indication for SCT in normal karyotype AML is continuously increasing: Mutations within the *MLL*-gene (partial tandem duplications, *MLL***-PTD**) are prognostically unfavorable (Dohner *et al*, 2002) and represent a further indication for SCT (Schlenk, ASH annual meeting abstracts 2006, 108). Thus, molecular screening in patients with a normal karyotype is of high priority for the decision for SCT. (*Table 1* provides an overview on the prognostic relevant subgroups in AML on the basis of cytogenetic and molecular markers.)

Table 1. Prognostic subgroups in AML according to the genetic results

Prognostic subgroup	Cytogenetic/molecular results
Favorable	- t(15;17)/*PML-RARA* - t(8;21)/*AML-ETO* - inv(16)/*CBFB-MYH11* - isolated *NPM1*-mutations (normal karyotype) - isolated *CEPBA*-mutations (normal karyotype)
Intermediate	- Normal karyotype - trisomy 8
Unfavorable	- complex aberrations (≥3 chrom. anomalies) - monosomy 7 - anomalies of chromosome 3 - *FLT3* length mutations (*FLT3*-LM/*FLT3*-ITD) - *MLL*-PTD

Minimal residual disease criteria

Minimal residual disease (MRD) diagnostics are of increasing importance for the definition of therapeutic strategies in AML. The highest level of sensitivity (up to 10^{-5}-10^{-6}) is provided by quantitative PCR or nested PCR (Shimoni & Nagler, 2004).

In the reciprocal transcript fusions t(15;17)/*PML-RARA*, t(8;21)/*AML1-ETO*, and inv(16)/*CBFB-MYH11* **quantitative real-time-PCR** can be used to assess the reduction of the leukemic cell load after therapy. A persistence of the transcript (Grimwade *et al*, 1998) or a minor decrease (Schnittger *et al*, 2003) are predictive for a significantly enhanced relapse risk. Although these balanced translocations play a minor role in SCT nowadays, as patients can be cured by standard chemotherapy in many cases, increases of the particular molecular markers might be detected 3-6 months before the cytomorphological manifestation of relapse and can still represent an indication for allogeneic SCT.

So far quantitative PCR is available for part of the known molecular mutations only, but efforts are being made to develop such quantification strategies for other markers also. In some studies of limited size it was shown that the *NPM1* mutations represent a stable MRD parameter which can be followed quantitatively by real-time PCR (Chou *et al*, 2007; Gorello *et al*, 2006). For patients with the *FLT3*-LM, follow-up monitoring can be performed by semi-quantitative PCR (Schnittger *et al*, 2004) or by quantitative methods after design of patient-specific primers due to the heterogeneity of the mutations (Scholl *et al*, 2005b). For some markers, e.g. for mutations within the loop of the *FLT3* tyrosine kinase domain (TKD), assays for quantitative monitoring (Scholl *et al*, 2005a) are being developed, so the spectrum of molecular markers being suitable for MRD is continuously expanding.

Another useful method for MRD studies in AML is provided by **multiparameter flow cytometry** (MFC), as a leukemia associated immunphenotype (LAIP) can be determined in 95% of all patients (Campana, 2003; Kern *et al*, 2004; Griesinger *et al*, 1999; Kern *et al*, 2007). Sensitivities of up to 10^{-2}-10^{-4} are achieved, (Feller *et al*, 2004) which also allows MRD monitoring in patients where there are no molecular markers for MRD studies available. Numerous studies demonstrated that the LAIP positive cells show an increase before morphological relapse occurs. Therefore, an increase of LAIP positive cells after therapy should always raise concern and can represent an indication for allogeneic SCT (Laane *et al*, 2006). (*Figure 1* shows an algorithm for the decision process to allogeneic SCT in AML.)

Hierarchy of diagnostic methods

To allow a most efficient flow of methods and an optimized risk stratification in the decision process towards allogeneic SCT, the diverse methods should be seen in the context of the whole panel, and hierarchies between the diverse methods should be used to guide the more specific techniques. Cytomorphological results raising suspicion for the balanced transcripts t(15;17)/*PML-RARA*, t(8;21)/*AML1-ETO*, or inv(16)/*CBFB-MYH11* should immediately be followed by the corresponding interphase FISH or PCR analyses for confirmation of subtypes.

When chromosomal banding shows numerical or structural aberrations, the appropriate interphase FISH probes for confirmation and clarification of the results should be selected accordingly. Additionally, interphase FISH can be integrated in the MRD panel due to the higher sensitivity of 1:100–1:200 cells (Bacher *et al*, 2006). In normal karyotype cases, molecular screening, e.g., for the *NPM1* and *FLT3*-LM, should be initiated. This might be completed by analyses for the *CEBPA* mutations,

MLL-PTD, or *FLT3*-TKD, as these markers all are associated with normal karyotype and are essential for risk stratification in the indication to SCT (Schlenk *et al*, 2004). The determination of the individual LAIP provides a solid basis for later follow-up to detect relapse at the earliest possible timepoint for eventual early planning of SCT.

Conclusions

Therapeutic concepts in AML try to adapt the intensity of therapy to the individual relapse risk. In poor-risk patients, allogeneic SCT is the therapy of choice, whereas in patients with a good prognosis such as the favorable reciprocal translocations, allogeneic SCT is restricted to impending or manifest relapse (de Labarthe *et al*, 2005). This risk stratification is possible only on the basis of patient-specific biological parameters and an exact subclassification of AML cases according to distinct cytogenetic and molecular markers. Further, indications for allogeneic SCT should include the results of MRD diagnostics, as persistence or increase of molecular markers might be an indication for a change of therapy towards SCT.

Thus, therapeutic decisions and the indications for allogeneic SCT require a multimodal diagnostic approach composed by a combination of cytogenetics, FISH, molecular genetics, and MRD diagnostics based on real time PCR and MFC.

However, many questions still require clarification. The combination of diverse markers might be relevant, as prognosis might differ from patients with isolated mutations. Examples are provided by the coincidence of the *PML-RARA* mutation with the *FLT3*-LM where prognosis is more unfavorable than in patients with an isolated t(15;17) (Gilliland, 2003), or by the coincidence of *FLT3*-LM and *NPM1*mutations (Falini *et al*, 2005). These overlaps between the diverse genetic subgroups can be responsible for variations in the clinical outcome which are seen in distinct AML subentities and need further investigation.

Further, results should be provided as soon as possible after diagnosis of AML to pave the way to allogeneic SCT in poor-risk cases. Novel methods such as gene expression profiling with microarrays, which allow the simultaneous analysis of thousands of genes, might allow an even more detailed risk stratification and prognostication within the shortest time in the near future, (Haferlach *et al*, 2003) which would also facilitate the indication for allogeneic SCT. Drug specific sensitivity assays based on gene expression analyses might soon offer more exact predictions concerning the expected success of the planned chemotherapy (Messner, 2006).

In conclusion, an optimized indication for allogeneic SCT in AML requires the interaction of a broad panel of diagnostic methods, which should be open for new developments to pave the way to an easier, safer, and faster risk stratification in this complex disorder.

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Оптимизация показаний к аллогенной трансплантации стволовых клеток при остром миелобластном лейкозе (ОМЛ), основанная на интерактивных диагностических стратегиях

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Резюме

Показания к аллогенной трансплантации стволовых клеток (алло-ТГСК) при остром миелобластном лейкозе (ОМЛ) представляют серьезные трудности из-за клинической и генетической гетерогенности данного заболевания. Поэтому оптимизация показаний к ГАСК при ОМЛ требует, прежде всего, определения индивидуального риска рецидива. При этом прогноз основывается на различных хромосомных и молекулярных аберрациях. Необходима широкая панель диагностических приемов, чтобы обеспечить такую субклассификацию и стратификацию по прогнозу, а именно: цитоморфологические, цитогенетические, молекулярно-генетические методы и иммунофенотипирование посредством мультипарамерической проточной цитометрии. Эти методы должны рассматриваться не как изолированные технологии, а как часть интегральной сети иерархий и взаимодействий. Неблагоприятным прогностическим маркером считается наличие несбалансированных кариотипов с утратой или наличием лишних хромосом или их крупных фрагментов. Приводятся примеры сочетаний маркеров высокого риска для обоснования четких показаний к алло-ТГСК, например аномалий хромосомы 7, сложных аберраций (>3 хромосомных аномалий) или мутаций по протяженности FLT3. Напротив, относительно благоприятные реципрокные транслокации, такие, как t(15;17)/PML-RARA или t(8;21)/AML1-ETO и inv(16)/CBFB-MYH11, мутации гена NPM1. Не являются показанием к ТГСК в первой ремиссии, в связи с достаточно хорошим прогнозом при проведении стандартной терапии. В дальнейшем показания к ТГСК должны включать в себя результаты оценки минимальной остаточной болезни (МОБ) посредством полимеразной цепной реакции (ПЦР) или проточной цитометрии. Новые аспекты безопасной и быстрой стратификации по риску для оптимизации показаний к ТГСК при ОМЛ могут быть выработаны на основе новых технологий, таких, как оценка профилей экспрессии множества генов с помощью микроэрреев (биочипов).

Ключевые слова: Острый миелобластный лейкоз (ОМЛ), Аллогенная трансплантация стволовых клеток (алло-ТГСК), Показания, Цитогенетика, Полимеразная цепная реакция (ПЦР)