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SPECIAL REPORT

Relapse of AML after hematopoietic stem cell transplantation: methods of monitoring and preventive strategies. A review from the ALWP of the EBMT

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Allogeneic hematopoietic stem cell transplantation (allo-SCT) remains the therapeutic method with the most potent anti-leukemic activity mediated by the graft versus leukemia effect. However, a significant proportion of patients with AML will relapse after allo-SCT. The prognosis for these patients is dismal, with a probability of long-term survival of $< 20\%$. Data from previous studies have shown that disease-specific prognostic factors, are in general, the same as those in patients treated with conventional chemotherapy. Minimal residual disease (MRD) and chimerism status monitoring after allo-SCT may be used as predictors of impending relapse and should be part of routine follow-up for AML patients. A significant number of studies have shown that pre-emptive administration of donor lymphocyte infusion (DLI) based on MRD and chimerism monitoring, as well as prophylactic DLI in AML patients at high risk of relapse is effective in preventing relapse. In this review, we discuss strategies for the identification of high-risk patients, review current therapeutic options and provide our recommendations for the management of post-SCT AML.

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-SCT) results in the most durable remissions for patients with high-risk AML. Transplant-related mortality (TRM) and disease relapse, however, remain two of the most significant barriers to long-term survival for these patients. Approximately 40% of post-SCT AML patients will relapse and face a dismal prognosis with a 2-year survival of $< 20\%$. Salvage treatment options include intensive chemotherapy followed by donor lymphocyte infusion (DLI), second allo-SCT, clinical trial enrollment or best supportive care.[1,2] The efficacy of hypomethylating agents and targeted therapies in this setting has been reported in several small case series.[3] Additional poor-risk factors include relapse within 6 months of allo-SCT, active GvHD at relapse and age > 40 years.[1] Due to the near-uniformly poor prognosis, and challenges in providing optimal therapies to these patients, strategies directed at the prevention of relapse are highly desirable. An approach that couples the pre-emptive identification of high-risk patients with diligent post-transplant monitoring and strategies for early intervention is necessary to improve the disease outcomes for these patients.

AML is a biologically aggressive disease. Even among patients with favorable risk, core-binding factor (CBF) AML, 58% will die by 10 years.[4] In the setting of poor-risk AML, allogeneic SCT favorably alters the disease course for some; however, a significant number of these adverse-risk patients will relapse and face a shortened overall survival (OS). To some extent, many of these relapses are predictable. Pre-transplant markers that are used to identify patients with biologically aggressive disease, and to guide consolidation therapy recommendations, may predict for relapse after SCT. Other factors, including reduced intensity conditioning (RIC), utilization of bone marrow (BM) allografts and other technical components of allo-SCT are associated with relapse in the post-SCT period. Post-SCT changes, including the development of low-volume disease or a mixed chimera, may also precede occult relapse.

Several groups have reported that the early identification of these high-risk patients, coupled with the prescription of aggressive immunotherapy and/or chemotherapy, may improve disease outcomes.[5–9] In this manuscript, we outline our recommendations for an individualized, risk-adapted strategy for the early identification and prevention of relapsed AML in the post-SCT period. It should be emphasized that several of these strategies fall outside of the current standard of care.

IDENTIFICATION OF PATIENTS AT HIGH RISK FOR RELAPSE AFTER ALLO-SCT

Disease-related parameters

Many of the disease-specific risk factors used to identify high-risk disease in the pre-SCT setting are validated predictors of post-SCT relapse (Table 1).[10–15] Therefore, the basic prognostic scheme

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Table 1. Risk factors for relapse after allogeneic hematopoietic stem cell transplantation in patients with AML

Parameter		*Relapse incidence*	*Ref*
Disease-related risk factors			
Cytogenetic risk group	Favorable risk	Low	10,11
	Intermediate risk	Intermediate	17
	Poor risk	High	
Other cytogenetic abnormalities	Monosomal karyotype	High	12,13
Molecular markers	*NPM1*mut, FLT3-WT	Low	14–16
	Bialleleic CEBPA-mut	Low	28–30
	FLT3-ITD	High	
	NPM1-WT, FLT3-WT, CEBPA-WT	Intermediate	
	Additional myeloid mutations	Indeterminate	
CR status	CR1	Low	18–20
	Beyond CR1	High	
MRD status	MRD positivity at the time of allo-SCT	High	21–25
Transplant-related risk factors			
Conditioning regimen	RIC regimens	High	31–33
GvHD prophylaxis regimen	Intensive regimens containing anti-T-cell antibodies or ATG, T-cell-depleted grafts	Controversial	41,43–45
GvHD	Absence of chronic GvHD	High	42

Abbreviations: ATG = anti-thymocyte globulin; RIC = reduced intensity conditioning; SCT = stem cell transplantation; WT = wild type.

proposed by the European Leukemia Net (ELN) working group also has applicability in the allo-SCT setting.[16] In addition, advanced disease status at transplant is a significant adverse-risk factor for post-SCT relapse. Patients that undergo allo-SCT with advanced disease are also prone to poor outcomes.[17–20]

To maximize the likelihood of a long-term remission or cure, most clinicians prefer that, if feasible, AML patients are in morphologic CR prior to allo-SCT. New technologies have led to improvements in the sensitivity of molecular assays and the detection of low-volume leukemic populations. The emergence of minimal residual disease (MRD) monitoring has gained momentum at many centers as a marker of disease risk and is now an important component of the risk stratification process. The presence of MRD is associated with an increased risk of relapse in the post-SCT setting. In several studies, pre-transplant MRD positivity is strongly associated with inferior outcomes.[21–25]

A recent, large retrospective study of 359 consecutive adult patients with AML after myeloablative allo-SCT demonstrated superior 3-year relapse free survival (RFS) and OS for patients that tested negative for MRD using multiparameter flow cytometry (MFC). Patients in MRD-positive morphologic remissions, and those with active leukemia, had comparable relapse rates at 3 years (67% vs 65%, respectively) and OS (26% vs 23%, respectively).[26] The strength of these results was underscored in an accompanying editorial confirming that all CRs can no longer be viewed as equal.[27]

The heterogeneity of AML is emphasized by the variable disease outcomes observed within the conventional risk classification system. Recognizing these challenges, investigators have sequenced several key genes in an effort to advance our understanding of the disease biology. Recently, Bejar *et al.*[28] evaluated 87 myelodysplastic syndrome (MDS) patients using massive parallel sequencing and detected genetic mutations in $>90\%$. The most frequently encountered mutations were ASXL1, TP53, DNMT3A and RUNX1. Mutations in TP53, TET2 and DNMT3A were associated with a shortened OS after allo-SCT.[28] Recent work, presented at the American Society of Hematology Annual Meeting, also demonstrated poor OS for MDS and AML patients with TET2 mutations.[29] A second abstract, reporting the results of 308 MDS or secondary AML patients, showed a median of 2 mutations in 82% of patients studied. Mutations in IDH2 and NRAS were linked to disease relapse, whereas PTPN11 and PHF6 predicted for a more indolent disease course.[30]

Transplant-related parameters

Retrospective studies, including registry analyses from the European Society for Blood and Marrow Transplantation (EBMT), have demonstrated an inverse relationship between the intensity of the conditioning regimen and the cumulative incidence of post-SCT relapse.[31–33] Recently, the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) presented the results of a randomized study comparing myeloablative conditioning (MAC) and RIC. This study was stopped early after a significantly higher relapse rate was discovered in the RIC group.[34] A similar prospective study, stopped early due to poor enrollment, demonstrated comparable outcomes between MAC and RIC.[35] Importantly, in the setting of MRD positivity, MAC is superior to RIC as it is associated with lower rates of relapse, superior RFS and OS.[36] Based on these data, MAC is preferable for patients who are candidates for this approach and should be considered for patients with MRD positivity.

Recently, the Acute Leukemia Working Party (ALWP) of the EBMT compared PBSC with BM grafts in patients that underwent RIC prior to allografting. Nearly 90% of these patients had AML. A higher incidence of chronic GvHD, improved RFS and better OS was seen in the PBSC group.[37] This is likely secondary to the potent graft versus leukemia (GVL) effects associated with the T-cell-rich PBSC graft.[38]

The primary mechanism by which RIC maintains long-term disease control is via a potent GVL effect. Chronic GvHD and GVL are intrinsically linked and several studies have established a correlation between these two parallel processes.[39,40] Some studies have reported that aggressive GvHD prophylaxis, either by the use of anti-T-cell antibodies during conditioning or by *in vitro* T-cell depletion (TCD) results in a higher relapse rate.[41,42]

The influence of TCD on transplant outcomes is an area of controversy. In 2012, the BMT CTN compared CD34+ cell selection with standard immunosuppressive therapy (IST) in patients with AML. CD34+ selection reduced the incidence of chronic GvHD but did not adversely affect the risk of relapse.[43] Earlier this year, two prospective, multicenter, randomized studies were published comparing anti-thymocyte globulin (ATG) versus no ATG. The primary endpoint in the Canadian/Australian study was freedom from IST. Patients who received ATG were significantly more likely to be free from IST at 12 months than those who did not (37 vs 16%). Epstein–Barr virus reactivation was more common in the ATG group.[44] Alternatively, the study by Kroger *et al.*

demonstrated a significantly lower rate of chronic GvHD with ATG but no difference in two-year RFS or OS between the groups.[45] Patients that received ATG had an improved chronic GvHD/RFS.[45]

Summary of recommendations

In an effort to reduce the risk of relapse, patients with features of high-risk disease, particularly MRD positivity, should be considered for MAC regimens whenever possible. When RIC is required, we recommend the use of PBSC grafts for high-risk patients due to the lower risk of relapse and improved OS. Based on the results of two, large prospective studies, the use of ATG is safe, does not adversely affect disease outcomes and should not be regarded as a risk factor for relapse. In the presence of high-risk features, we advocate for close disease surveillance in the post-SCT period with early intervention as outlined below.

MONITORING PATIENTS AFTER ALLO-SCT

Mounting evidence indicates that the natural history of high-risk, post-SCT AML can be altered with early detection of low-volume disease and aggressive intervention. Although the treatment of Ph-positive ALL is different from that of AML, prior work in this disease provides important lessons in the efficacy of pre-emptive management. Early use of the tyrosine kinase inhibitor imatinib in the upfront and MRD-positive setting results in favorable outcomes in post-SCT Ph+ ALL patients.[46] In AML, intensive monitoring of high-risk patients with the objective of early intervention is a developing niche within the treatment landscape of post-SCT AML.

The evaluation of MRD has been previously discussed as a validated methodology for identifying high-risk patients prior to allo-SCT. This technique is also effective in the post-transplant period to identify high-risk patients for early intervention. In this setting, the quantitative follow-up of MRD at regular intervals, in conjunction with other metrics, should be applied in patients with AML after allo-SCT.[47] Quantifying leukemic load may aid in the selection of high-risk patients, permit individualized decision-making and reduce morphologic relapse.

Several MRD assays have been employed to identify residual leukemic cells in a background of normal hematopoietic cells. To be deemed reliable, MRD assays must be reproducible, standardized across different laboratories, and of sufficiently high sensitivity and specificity to ensure clinical applicability.

The minimum threshold of sensitivity should be at least 1×10^{-3} (1 leukemic in a background of 1000 normal hematopoietic cells) and ideally should approach 1×10^{-6}.[48]

MRD by MFC

Currently AML MRD monitoring in standard clinical practice is based on MFC among other approaches. MFC relies on the identification of cells carrying leukemia-associated immunophenotypes (LAIPs) and can be applied in up to 90% of AML patients. LAIPs result from altered antigen expression patterns on normal hematopoietic cells. Using LAIPs to study MRD results in sensitivities of 1×10^{-3} to 1×10^{-4}.[49] The prognostic significance of MRD by MFC in the post-SCT setting is well-documented in the medical literature. In all studies published to date, the presence of MRD is associated with a significantly higher risk of relapse compared with MRD-negative patients.[50–53]

Despite the obvious favorable impact these findings will have on patient care, MFC remains an imperfect technique. Disease heterogeneitiy, coupled with variations in instruments and fluorophores, operator technique/gating and spectral overlap all contribute to significant differences between institutions, limit reproducibility and hamper standardization. As of yet, there is no standardized method for analyzing MFC data and no universally agreed upon cutoff, both of which make reproducibility challenging.

MRD by PCR

PCR assays for MRD evaluation are based on the detection of a unique leukemic transcript such as a fusion gene, mutated or overexpressed gene. Real-time PCR (RT-PCR) assays represent an appealing method for MRD monitoring since they offer a sensitive and accurate estimation of the leukemic cell burden.

Quantitative PCR for detection of fusion gene transcripts. Fusion gene transcripts can be detected in up to 20% of patients with non-acute promyelocytic leukemia AML. The bulk of these transcripts are CBF leukemias which manifest the RUNX1-RUNX1T1 and CBFB-MYH11 fusion-genes. Several other fusion transcripts exist, including MLL-v, DEK-NUP214, BCR-ABL, RPN1-EVI1 and RBM15-MKL1, all of which can be used as targets for molecular monitoring in high-risk AML patients.[54]

The prognostic significance of MRD estimated by using quantitative PCR in patients with CBF leukemias after conventional chemotherapy has been reported.[55] A recent study including patients with t(8;21) showed the prognostic significance of MRD persistence after allo-SCT. In a multivariate analysis, MRD positivity was the most important predictor of relapse.[54] Elmaagacli, *et al*.[56] monitored MRD by RT-PCR in patients with inv(16) AML and showed that MRD negativity after allo-SCT is associated with a decreased incidence of relapse.

Quantitative PCR for detection of mutated genes. Mutations of the nucleophosmin gene (*NPM1*) are present in 50% of AML patients with normal karyotype. *NPM1*-gene mutations are stable during the disease course and therefore represent an ideal marker for MRD monitoring. By using quantitative PCR, prior work has demonstrated the feasibility of MRD monitoring as a tool that predicts for relapse. A recent study showed that the persistence or increase of $>10\%$ of *NPM1*mut/ABL1 copies (corresponding to 1000 copies *NPM1*mut/10 000 copies of ABL1), predicts for relapse after allo-SCT.[57]

Molecular testing for the Fms-like Tyrosine Kinase 3 Internal Tandem Duplication (FLT3-ITD) mutation is commonly performed at centers around the world in the diagnostic setting. A high FLT3-ITD to wild-type (WT) allelic ratio ($\geqslant0.51$) is associated with shortened RFS and OS compared with patients with a low-allelic ratio.[58] Studying FLT3-ITD mutations in the surveillance setting is more challenging. Two studies have demonstrated important differences between diagnostic and relapse FLT3-ITD mutations. In a retrospective study of 50 patients, 22% had a different FLT3-ITD mutation at relapse.[59] In a second study, out of 108 relapsed AML patients, 16 patients had a FLT3-ITD mutation at both diagnosis and relapse, 8 relapsed patients acquired the mutation at relapse and 1 relapsed with FLT3-ITD negative disease. Interestingly, six out of six patients with a FLT3-ITD mutation at diagnosis showed changes in the mutation patterns at relapse.[60] These data demonstrate the complexity of using molecular techniques to monitor for relapsed FLT3-ITD+ AML.

Finally, in a recent study by Brambati *et al*.,[61] the quantitative post-SCT monitoring of mutations in DNMT3A, IDH1 and IDH2, performed using droplet digital PCR, provided promising results in predicting subsequent relapse.

Quantitative PCR for overexpressed genes. In the study by Candoni *et al*.,[62] post-SCT patients had Wilms tumor 1 (WT1) transcript levels monitored. All except one patient in continuous-CR displayed normal WT1 copy numbers whereas patients that relapsed had high-WT1 copy numbers. Other studies have demonstrated the clinical utility of serial WT1 transcript level monitoring by RT-PCR as a method of MRD assessment.[62]

Disease monitoring by chimerism

Chimerism analysis is considered standard practice for monitoring the post-transplant engraftment of donor cells. These studies are useful in predicting disease relapse; however, many of the same limitations that beleaguer MFC also apply to chimerism studies.[47] Most notably, variability in the assays leads to differences in the sensitivity of chimerism studies. PCR of short tandem repeats is the most widely adopted method, with a sensitivity of 1×10^{-2} to 1×10^{-3}.[63] Using quantitative PCR for the detection of donor/recipient specific polymorphisms, or by detecting sequences unique to the Y-chromosome in sex-mismatched donors/recipients, can increase the sensitivity by two to three log-fold.[64]

The sensitivity and specificity of chimerism studies can be further increased by evaluating specific cell subsets. In MDS and AML, chimerism analysis of the CD34+ cell compartment increases the sensitivity and specificity of the assay. In a study of 85 patients who were evaluated for CD34+ chimerism in PB samples, the loss of the CD34+ donor cells to $< 80\%$ was uniformly associated with relapse.[65] Monitoring PB T-cell chimerism also has predictive value in this patient population. With this approach, the complete chimerism (CC) of the T-cell subset predicts for a higher incidence of GvHD and a reduced incidence of relapse.[66] The presence of a stable, or increasing mixed chimerism (MC), in the lymphoid lineage may reflect an reduced risk of developing GvHD, loss of the favorable GVL effect and an increased risk of disease relapse. Recently, a phase II study of AML patients that received RIC allo-SCT called this into question. There was no correlation between CD3+ chimerism and disease-specific endpoints including RFS and OS.[67]

Chimerism studies are less specific and, in the absence of other markers, arguably less effective in predicting for relapse compared with MRD monitoring.[48] It should be emphasized that MC is not equivalent to disease recurrence. Recrudescence of host material may represent either normal hematopoiesis or return of the leukemic clone. Therefore, the presence of a MC by itself is not indicative of an active hematologic neoplasm. Despite these limitations, routine chimerism monitoring should be performed in conjunction with other, more sensitive MRD monitoring tools to identify patients at increased risk of relapse who might benefit from early intervention.

Summary of recommendations

High-risk AML patients should undergo routine, post-SCT monitoring with more than one modality, if possible, to ensure sensitivity in detecting recurrence of the leukemic clone. Due to the polyclonal nature of AML, patients may relapse with new clones that evade prior molecular testing. For this reason, we recommend the use tailored molecular monitoring, and routine chimerism studies during the surveillance of high-risk AML patients. The addition of MFC can be considered on BM aspirates during surveillance.

Chimerism studies should routinely be monitored for patients conditioned with both MAC and RIC regimens. These studies pose minimal risk to patients and have the potential to change therapy. We recommend unsorted chimerism studies at all BM aspirations and biopsies, evaluation of myeloid and lymphoid chimerism studies at routine clinic visits and in instances where relapsed disease is questioned. After a sustained remission of $\geqslant 2$ years, the frequency of monitoring may be reduced. Chimeric abnormalities, such as MC or increasing MC, should be interpreted in the context of the patients' laboratory studies, history and physical exam.

PREVENTION OF POST-TRANSPLANT RELAPSE

The intensive, widespread monitoring of these patients is associated with significant resources and anxiety for patients and their caregivers. To justify this cost, there must be evidence to support the idea that routine disease surveillance will identify patients with impending relapses and that, with the appropriate therapy, a proportion of these relapses can be averted. A growing body of data supports the use of immunotherapy and/or chemotherapy to alter the natural history of these post-SCT patients' disease course. These methods are divided into immunotherapeutic and drug-based approaches.

To date, the simplest and most well-studied immune interventions are the withdrawal of IST and administration of DLI. In some studies, the early withdrawal of IST, even in the absence of DLI, can prevent overt morphologic relapse in high-risk patients.[5] Prophylactic or pre-emptive DLI has been associated with improvement in disease-specific endpoints.

It should also be considered, however, that in the context of haploidentical SCT a considerable proportion of relapses are due to leukemic variants characterized by the genomic loss of the mismatched HLA haplotype, which through this mechanism become resistant to donor T cells.[68,69] Thus, in the haploidentical context, testing eventual HLA loss at the time of relapse might be warranted before employing predictably inefficacious DLIs.

A brief overview on the rationale and current use of DLIs is presented below.

Pre-emptive DLI based on chimerism monitoring

The term 'pre-emptive' is used for early intervention based on the results of laboratory evaluations, suggesting that disease relapse is imminent. In a prospective trial, 81 children with AML after allo-SCT were followed by serial chimerism monitoring. MC was defined as the presence of host DNA above 1%. Patients with MC and a spontaneous decrease in host DNA were labeled as having decreasing MC. Alternatively, those in whom the proportion of host DNA is rising are said to have increasing MC. The patients with CC or decreasing MC had a lower relapse rate compared with patients with increasing MC. DLI administration in patients with increasing MC decreased the relapse rate and favorably affected outcomes.[6]

A subsequent, prospective multicenter study in 71 children with AML demonstrated similar results. Immunosuppression was stopped in children with MC after allo-SCT, and DLI was administered if no GvHD occurred after 3–4 weeks. Repeated DLI at an increased dose was allowed if MC persisted in the absence of GvHD. Thirteen out of 20 children with MC received DLI. The EFS was 80% and 30% for patients with CC and MC, respectively. Patients with MC who received DLI had an EFS of 46%, while 100% of MC patients relapsed without DLI.[7] Collectively, these data indicate that early intervention with DLI may avert disease relapse and improve outcomes.

Pre-emptive intervention including DLI based on MRD monitoring

Dominieto *et al*.[8] reported the results of a retrospective analysis of pre-emptive DLI administration in a group of 80 patients with acute leukemia (36 AML, 44 ALL) after allo-SCT. MRD monitoring was performed on monthly BM samples using RT-PCR for detection of WT1 transcripts. DLI was administered to all patients with measurable MRD who had an available donor and no evidence of GvHD. The cumulative incidence of relapse was 16% in MRD-negative patients, compared with 6% of MRD-positive patients treated with DLI and 63% of MRD-positive patients without DLI.[8] The benefit of early intervention with DLI in the prevention of morphologic relapse was presented in a prospective, multicenter study that evaluated *RUNX1/RUNX1T1* transcript levels for MRD assessments. Again, patients treated with DLI had a reduced cumulative incidence of relapse and improved RFS at 2 years.[54]

The largest prospective trial testing the efficacy of pre-emptive DLI in acute leukemia patients after allo-SCT was performed by the Chinese. MRD monitoring was performed on BM samples by using

MFC and RT-PCR for detection of WT1 in 814 standard risk acute leukemia patients (AML or high-risk MDS in 70%). Patients were classified into three groups; the cumulative incidence of relapse at 3 years was 22% for the entire cohort. A total of 18% of MRD-negative patients relapsed compared with 65% of MRD-positive patients that did not receive DLI. In comparison, the administration of DLI was associated with a 2.3-fold reduction in relapse risk as only 28% of MRD-positive patients who received DLI relapsed. In these patients, the infusion of donor lymphocytes was associated with the development of GvHD but did not alter TRM.[9]

Prophylactic administration of DLI

Prophylactic DLI has been largely tested in the setting of TCD allo-SCT. In many studies, the transplantation of a TCD graft was subsequently followed by the infusion of standard doses of T cells that occurred between day +30 and 22 weeks after allo-SCT. T-cell doses range from 1×10^6 to 1×10^7/kg. The TRM and relapse rate differed significantly between studies and, therefore, the optimal approach for DLI in this setting remains uncertain.[70–75] In spite of the variability of these outcomes, an important theme that emerged from these studies is that DLI administration before day +100 is associated with an unacceptably high rate of GvHD.

The role of prophylactic DLI has been tested in high-risk AML. In a prospective study of 75 patients with high-risk MDS or AML, DLI was administered after day +120 in patients who were free of GvHD and whose IST was successfully weaned >30 days prior to DLI. After conditioning and initiation of GvHD prophylaxis, patients received a starting dose of CD3+ cells that was 2×10^5 in the unrelated and 1×10^6/kg in the matched-related donor setting. Escalating cell doses were administered at 4–6 week intervals. The administration of DLI improved outcomes, with a 3-year OS of 42% and RFS of 40%. Patients who developed limited chronic GvHD had superior OS, confirming prior data that link chronic GvHD with superior disease outcomes.[76] The efficacy of the same approach has been proven in another prospective trial of high-risk AML patients with a complex karyotype.[77] Finally, the use of prophylactic DLI is associated with favorable long-term outcomes. In a recent report of 46 AML patients treated with DLI (pre-emptively due to increasing MC (4), molecular MRDpositive (1), and adjuvant/prophylaxis (41), patients that received DLI had a superior 7-year OS compared with those who did not receive DLI (67 vs 31%).[78]

Other approaches for the administration of low-dose DLI ($0.5–1.5\times10^6$ CD3+ cells per kg) have been reported. In 42 patients with high-risk acute leukemias, prophylactic DLI was effective in reducing the relapse rate and converting MC to CC in the vast majority of patients.[79] Finally, a 'modified' protocol pioneered by the Chinese has proven effective as prophylaxis in patients with advanced leukemia after a matched sibling donor and haploidentical SCT.[80,81]

Summary of recommendations

In aggregate, a growing body of data suggests that pre-emptive DLI in MRD-positive AML patients is a safe and effective method for preventing relapse. We recommend aggressively weaning IST in all high-risk patients with measurable AML. After the discontinuation of IST, we advocate for the administration of DLI after D+100 in the setting of measurable disease and/or increasing MC. Clinicians should utilize their clinical judgement in identifying high-risk patients who are suitable candidates for this approach. Close observation for the development of GvHD is essential.

POST-TRANSPLANT THERAPY

A large, and growing number of novel agents have been tested in pre-clinical and clinical trials with the aim of obtaining disease control in aggressive AML.[82] To date, there are limited data in the post-SCT maintenance setting.

The most commonly used agent azacitidine has known activity in patients with MDS.[83] Retrospective data, as well data from the RELAZA trial, showed that maintenance of azacitidine after allo-

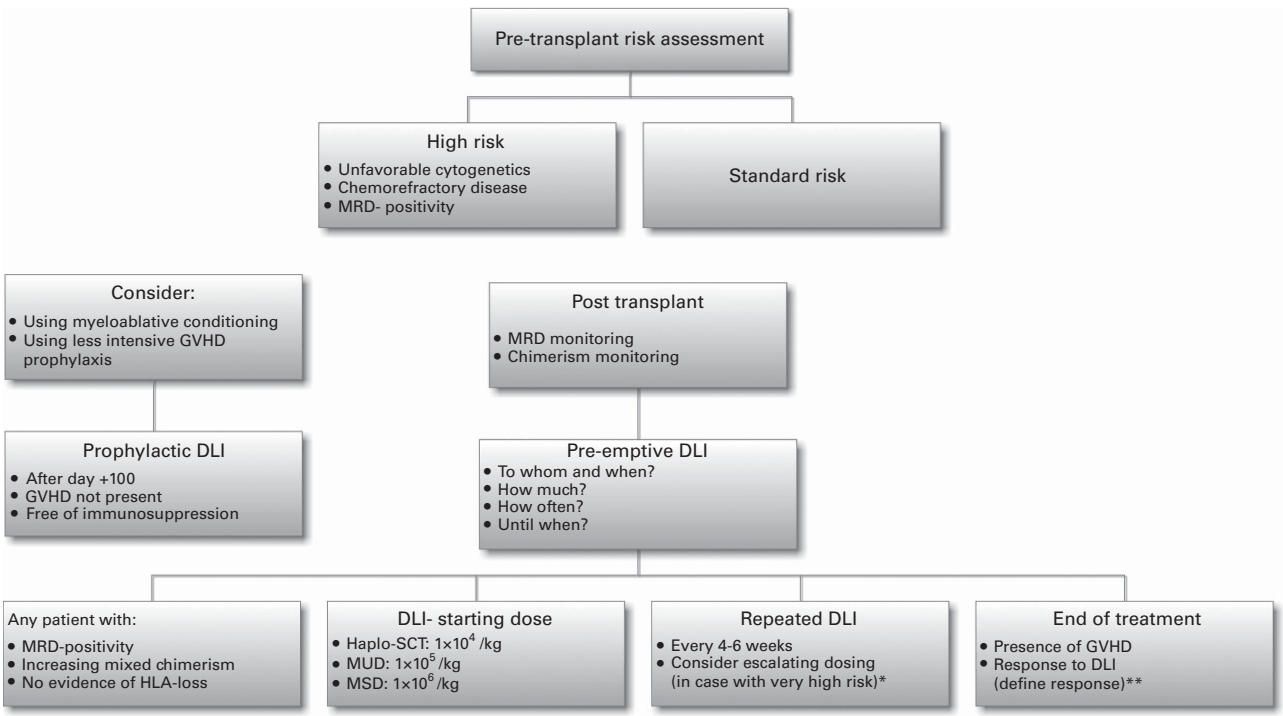

Figure 1. Prophylactic and pre-emptive DLI in patients with high-risk AML after allo-SCT: a proposed treatment algorithm. *Patients with increasing mixed chimerism or leukemic burden despite administration of DLI. **Response is defined either as conversion to complete donor chimerism in case of previous mixed chimerism, or as conversion to MRD negativity in case of previous MRD positivity.

SCT in high-risk AML patients is safe and likely effective.[84,85] Similarly, the RICAZA trial demonstrated that azacitidine is well-tolerated and likely to reduce the risk of relapse, particularly in patients with a CD8+ T-cell response.[86] Several prospective clinical trials designed to answer this question are ongoing.

Patients with FLT3-ITD AML are candidates for allo-SCT due to the poor prognosis conferred by this mutation and are at increased risk of developing post-transplant relapse compared with FLT3-WT patients.[15,16] A phase I trial designed to identify the maximum tolerated dose of sorafenib in the post-SCT maintenance period for FLT3-ITD+ AML demonstrated a 1-year RFS of 85% and a 1-year OS of 95%. In patients that were in CR1/CR2 at SCT, the 1-year RFS and OS were 95% and 100%, respectively.[87] A large number of FLT3-inhibitors have undergone pre-clinical testing, and clinical trials examining the efficacy in preventing relapse when administering as maintenance after allo-SCT are currently underway.[88–91]

CONCLUSIONS AND THERAPEUTIC ALGORITHM

In conclusion, pre-transplant patient assessment should be based on an estimation of relapse, as well as non-relapse mortality risk by using the EBMT and CCC-I scoring systems.[92,93]

In the setting of high-risk, post-SCT AML, we propose that surveillance and early intervention should follow an individualized, risk-adapted strategy (Figure 1).

- Patients with high-relapse risk and low risk of TRM: consider MAC and/or a less-intensive GvHD prophylaxis regimen.
- Patients with high risk of relapse defined by pre-transplant assessment: consider prophylactic DLI administration if active GvHD is not present. This should occur after day +100, unless otherwise indicated by post-transplant monitoring of MRD and chimerism status.
- Post-transplant follow-up should be based on serial MRD, as well as chimerism monitoring.
- Patients with persistent MRD positivity or increasing MC are at high risk of relapse and should be treated with withdrawal of IST and pre-emptive DLI administration.
- DLI starting doses should be at the order of 1×10^4, 1×10^5, 1×10^6/kg after haploidentical, matched unrelated and matched sibling donor allo-SCT, respectively.
- Repeated DLI should be administered at 4–6-week intervals in non-responding and GvHD-free patients. DLI administration should be repeated until response or presence of GvHD whichever occurred first.
- Prophylactic intervention should be administered in the context of a clinical trial.
- There is a need for future studies exploring the role of prophylactic azacitidine and of other novel agents in the post-SCT setting.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1 Bejanyan N, Weisdorf DJ, Logan BR, Wang HL, Devine SM, de Lima M *et al.* Survival of patients with acute myeloid leukemia relapsing after allogeneic hematopoietic cell transplantation: a center for international blood and marrow transplant research study. *Biol Blood Marrow Transplant* 2015; **21**: 454–459.
2 Schmid C, Labopin M, Nagler A, Niederwieser D, Castagna L, Tabrizi R *et al.* Treatment, risk factors, and outcome of adults with relapsed AML after reduced intensity conditioning for allogeneic stem cell transplantation. *Blood* 2012; **119**: 1599–1606.
3 Metzelder SK, Schroeder T, Finck A, Scholl S, Fey M, Götze K *et al.* High activity of sorafenib in FLT3-ITD-positive acute myeloid leukemia synergizes with allo-immune effects to induce sustained responses. *Leukemia* 2012; **26**: 2353–2359.
4 Appelbaum FR, Kopecky KJ, Tallman MS, Slovak ML, Gundacker HM, Kim HT *et al.* The clinical spectrum of adult acute myeloid leukaemia associated with core binding factor translocations. *Br J Haematol* 2006; **135**: 165–173.
5 Rosenow F, Berkemeier A, Krug U, Müller-Tidow C, Gerss J, Silling G *et al.* CD34(+) lineage specific donor cell chimerism for the diagnosis and treatment of impending relapse of AML or myelodysplastic syndrome after allo-SCT. *Bone Marrow Transplant* 2013; **48**: 1070–1076.
6 Rujkijyanont P, Morris C, Kang G, Gan K, Hartford C, Triplett B *et al.* Risk-adapted donor lymphocyte infusion based on chimerism and donor source in pediatric leukemia. *Blood Cancer J* 2013; **3**: e137.
7 Rettinger E, Willasch AM, Kreyenberg H, Borkhardt A, Holter W, Kremens B *et al.* Preemptive immunotherapy in childhood acute myeloid leukemia for patients showing evidence of mixed chimerism after allogeneic stem cell transplantation. *Blood* 2011; **118**: 5681–5688.
8 Dominietto A, Pozzi S, Miglino M, Albarracin F, Piaggio G, Bertolotti F *et al.* Donor lymphocyte infusions for the treatment of minimal residual disease in acute leukemia. *Blood* 2007; **109**: 5063–5064.
9 Yan CH, Liu DH, Liu KY, Xu LP, Liu YR, Chen H *et al.* Risk stratification-directed donor lymphocyte infusion could reduce relapse of standard-risk acute leukemia patients after allogeneic hematopoietic stem cell transplantation. *Blood* 2012; **119**: 3256–3262.
10 Cornelissen JJ, Breems D, van Putten WL, Gratwohl AA, Passweg JR, Pabst T *et al.* Comparative analysis of the value of allogeneic hematopoietic stem-cell transplantation in acute myeloid leukemia with monosomal karyotype versus other cytogenetic risk categories. *J Clin Oncol* 2012; **30**: 2140–2146.
11 Middeke JM, Beelen D, Stadler M, Göhring G, Schlegelberger B, Baurmann H *et al.* Outcome of high-risk acute myeloid leukemia after allogeneic hematopoietic cell transplantation: negative impact of abnl(17p) and -5/5q-. *Blood* 2012; **120**: 2521–2528.
12 van Gelder M, de Wreede LC, Schetelig J, van Biezen A, Volin L, Maertens J *et al.* Monosomal karyotype predicts poor survival after allogeneic stem cell transplantation in chromosome 7 abnormal myelodysplastic syndrome and secondary acute myeloid leukemia. *Leukemia* 2013; **27**: 879–888.
13 Fang M, Storer B, Estey E, Othus M, Zhang L, Sandmaier BM *et al.* Outcome of patients with acute myeloid leukemia with monosomal karyotype who undergo hematopoietic cell transplantation. *Blood* 2011; **118**: 1490–1494.
14 Pfeiffer T, Schleuning M, Mayer J, Haude KH, Tischer J, Buchholz S *et al.* Influence of molecular subgroups on outcome of acute myeloid leukemia with normal karyotype in 141 patients undergoing salvage allogeneic stem cell transplantation in primary induction failure or beyond first relapse. *Haematologica* 2013; **98**: 518–525.
15 Brunet S, Labopin M, Esteve J, Cornelissen J, Socié G, Iori AP *et al.* Impact of FLT3 internal tandem duplication on the outcome of related and unrelated hematopoietic transplantation for adult acute myeloid leukemia in first remission: a retrospective analysis. *J Clin Oncol* 2012; **30**: 735–741.
16 Schmid C, Labopin M, Socie G, Daguindau E, Volin L, Huynh A *et al.* Outcome and risk factor analysis of molecular subgroups in cytogenetically normal AML treated by allogeneic transplantation. *Blood* 2015; **126**: 2062–2069.
17 Cornelissen JJ, Gratwohl A, Schlenk RF, Sierra J, Bornhäuser M, Juliusson G *et al.* The European LeukemiaNet AML Working Party consensus statement on allogeneic HSCT for patients with AML in remission: an integrated-risk adapted approach. *Nat Rev Clin Oncol* 2012; **9**: 579–590.
18 Duval M, Klein JP, He W, Cahn JY, Cairo M, Camitta BM *et al.* Hematopoietic stem-cell transplantation for acute leukemia in relapse or primary induction failure. *J Clin Oncol* 2010; **28**: 3730–3738.
19 Armistead PM, de Lima M, Pierce S, Qiao W, Wang X, Thall PF *et al.* Quantifying the survival benefit for allogeneic hematopoietic stem cell transplantation in relapsed acute myelogenous leukemia. *Biol Blood Marrow Transplant* 2009; **15**: 1431–1438.
20 Craddock C, Labopin M, Pillai S, Finke J, Bunjes D, Greinix H *et al.* Factors predicting outcome after unrelated donor stem cell transplantation in primary refractory acute myeloid leukaemia. *Leukemia* 2011; **25**: 808–813.
21 Leung W, Pui CH, Coustan-Smith E, Yang J, Pei D, Gan K *et al.* Detectable minimal residual disease before hematopoietic cell transplantation is prognostic but does not preclude cure for children with very-high-risk leukemia. *Blood* 2012; **120**: 468–472.
22 Laane E, Derolf AR, Björklund E, Mazur J, Everaus H, Söderhäll S *et al.* The effect of allogeneic stem cell transplantation on outcome in younger acute myeloid leukemia patients with minimal residual disease detected by flow cytometry at the end of post-remission chemotherapy. *Haematologica* 2006; **91**: 833–836.
23 Walter RB, Gooley TA, Wood BL, Milano F, Fang M, Sorror ML *et al.* Impact of pretransplantation minimal residual disease, as detected by multiparametric flow

cytometry, on outcome of myeloablative hematopoietic cell transplantation for acute myeloid leukemia. *J Clin Oncol* 2011; **29**: 1190–1197.
24 Walter RB, Gyurkocza B, Storer BE, Godwin CD, Pagel JM, Buckley SA *et al.* Comparison of minimal residual disease as outcome predictor for AML patients in first complete remission undergoing myeloablative or nonmyeloablative allogeneic hematopoietic cell transplantation. *Leukemia* 2015; **29**: 137–144.
25 Zhou Y, Othus M, Araki D, Wood BL, Radich JP, Halpern AB *et al.* Pre- and post-transplant quantification of measurable ('minimal') residual disease via multiparameter flow cytometry in adult acute myeloid leukemia. *Leukemia* 2016; **30**: 1456–1464.
26 Araki D, Wood BL, Othus M, Radich JP, Halpern AB, Zhou Y *et al.* Allogeneic hematopoietic cell transplantation for acute myeloid leukemia: time to move toward a minimal residual disease-based definition of complete remission? *J Clin Oncol* 2016; **34**: 329–336.
27 Bassan R. Using minimal residual disease to improve treatment response definitions and hematopoietic cell transplantation strategy in acute leukemia. *J Clin Oncol* 2016; **34**: 300–302.
28 Bejar R, Stevenson KE, Caughey B, Lindsley RC, Mar BG, Stojanov P *et al.* Somatic mutations predict poor outcome in patients with myelodysplastic syndrome after hematopoietic stem-cell transplantation. *J Clin Oncol* 2014; **32**: 2691–2698.
29 Hamilton B, Majhail N, Hirsch C, Przychodzen B, Rybicki L, DeLima M *et al. Prognostic Impact of Molecular Mutations in Acute Myeloid Leukemia (AML) and Myelodysplastic Syndromes (MDS) on Allogeneic Hematopoietic Cell Transplant (HCT) Outcomes: Adverse Impact of TET2 Mutations.* American Society of Hematology 57th Annual Meeting and Exposition: Orlando, FL, USA, 2015.
30 Heuser M, Koenecke C, Gabdoulline R, Loffeld P, Dobbernack V, Panagiota V *et al. Molecular Predictors of Outcome in Patients with MDS and AML Following MDS After Allogeneic Hematopoietic Stem Cell Transplantation.* American Society of Hematology 57th Annual Meeting and Exposition: Orlando, FL, USA, 2015.
31 Aoudjhane M, Labopin M, Gorin NC, Shimoni A, Ruutu T, Kolb HJ *et al.* Comparative outcome of reduced intensity and myeloablative conditioning regimen in HLA identical sibling allogeneic haematopoietic stem cell transplantation for patients older than 50 years of age with acute myeloblastic leukaemia: a retrospective survey from the Acute Leukemia Working Party (ALWP) of the European group for Blood and Marrow Transplantation (EBMT). *Leukemia* 2005; **19**: 2304–2312.
32 Luger SM, Ringdén O, Zhang MJ, Pérez WS, Bishop MR, Bornhauser M *et al.* Similar outcomes using myeloablative vs reduced-intensity allogeneic transplant preparative regimens for AML or MDS. *Bone Marrow Transplant* 2012; **47**: 203–211.
33 Martino R, de Wreede L, Fiocco M, van Biezen A, von dem Borne PA, Hamladji RM *et al.* Comparison of conditioning regimens of various intensities for allogeneic hematopoietic SCT using HLA-identical sibling donors in AML and MDS with $<$ 10% BM blasts: a report from EBMT. *Bone Marrow Transplant* 2013; **48**: 761–770.
34 Scott BL, Pasquini MC, Logan B, Wu J, Devine S, Porter DL *et al. Results of a Phase III Randomized, Multi-Center Study of Allogeneic Stem Cell Transplantation after High Versus Reduced Intensity Conditioning in Patients with Myelodysplastic Syndrome (MDS) or Acute Myeloid Leukemia (AML): Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 0901*. American Society of Hematology 57th Annual Meeting and Exposition: Orlando, FL, USA, 2015.
35 Bornhäuser M, Kienast J, Trenschel R, Burchert A, Hegenbart U, Stadler M *et al.* Reduced-intensity conditioning versus standard conditioning before allogeneic haemopoietic cell transplantation in patients with acute myeloid leukaemia in first complete remission: a prospective, open-label randomised phase 3 trial. *Lancet Oncol* 2012; **13**: 1035–1044.
36 Ustun C, Courville EL, DeFor T, Dolan M, Randall N, Yohe S *et al.* Myeloablative, but not reduced-intensity, conditioning overcomes the negative effect of flow-cytometric evidence of leukemia in acute myeloid leukemia. *Biol Blood Marrow Transplant* 2016; **22**: 669–675.
37 Savani BN, Labopin M, Blaise D, Niederwieser D, Ciceri F, Ganser A *et al.* Peripheral blood stem cell graft compared with bone marrow after reduced intensity conditioning regimens for acute leukemia: a report from the ALWP of the EBMT. *Haematologica* 2016; **101**: 256–262.
38 Byrne M, Savani BN, Mohty M, Nagler A. Peripheral blood stem cell versus bone marrow transplantation: a perspective from the acute leukemia working party of the european society for blood and marrow transplantation. *Exp Hematol* 2016; pii: S0301–472X(16)30077-7 (epub ahead of print).
39 Kolb HJ. Graft-versus-leukemia effects of transplantation and donor lymphocytes. *Blood* 2008; **112**: 4371–4383.
40 Gustafsson Jernberg A, Remberger M, Ringdén O, Winiarski J. Graft-versus-leukaemia effect in children: chronic GvHD has a significant impact on relapse and survival. *Bone Marrow Transplant* 2003; **31**: 175–181.
41 Valcárcel D, Martino R, Piñana JL, Sierra J. Allogeneic stem cell transplantation after reduced-intensity conditioning for acute myeloid leukaemia: impact of chronic graft-versus-host disease. *Curr Opin Oncol* 2009; **21**: S35–S37.
42 Stern M, de Wreede LC, Brand R, van Biezen A, Dreger P, Mohty M *et al.* Sensitivity of hematological malignancies to graft-versus-host effects: an EBMT megafile analysis. *Leukemia* 2014; **28**: 2235–2240.
43 Pasquini MC, Devine S, Mendizabal A, Baden LR, Wingard JR, Lazarus HM *et al.* Comparative outcomes of donor graft CD34+ selection and immune suppressive therapy as graft-versus-host disease prophylaxis for patients with acute myeloid leukemia in complete remission undergoing HLA-matched sibling allogeneic hematopoietic cell transplantation. *J Clin Oncol* 2012; **30**: 3194–3201.
44 Walker I, Panzarella T, Couban S, Couture F, Devins G, Elemary M *et al.* Pretreatment with anti-thymocyte globulin versus no anti-thymocyte globulin in patients with haematological malignancies undergoing haemopoietic cell transplantation from unrelated donors: a randomised, controlled, open-label, phase 3, multicentre trial. *Lancet Oncol* 2016; **17**: 164–173.
45 Kröger N, Solano C, Wolschke C, Bandini G, Patriarca F, Pini M *et al.* Antilymphocyte globulin for prevention of chronic graft-versus-host disease. *N Engl J Med* 2016; **374**: 43–53.
46 Pfeifer H, Wassmann B, Bethge W, Dengler J, Bornhäuser M, Stadler M *et al.* Randomized comparison of prophylactic and minimal residual disease-triggered imatinib after allogeneic stem cell transplantation for BCR-ABL1-positive acute lymphoblastic leukemia. *Leukemia* 2013; **27**: 1254–1262.
47 Shimoni A, Nagler A. Non-myeloablative stem cell transplantation (NST): chimerism testing as guidance for immune-therapeutic manipulations. *Leukemia* 2001; **15**: 1967–1975.
48 Kröger N, Bacher U, Bader P, Böttcher S, Borowitz MJ, Dreger P *et al.* NCI First international workshop on the biology, prevention, and treatment of relapse after allogeneic hematopoietic stem cell transplantation: report from the committee on disease-specific methods and strategies for monitoring relapse following allogeneic stem cell transplantation. part i: methods, acute leukemias, and myelodysplastic syndromes. *Biol Blood Marrow Transplant* 2010; **16**: 1187–1211.
49 Buckley SA, Appelbaum FR, Walter RB. Prognostic and therapeutic implications of minimal residual disease at the time of transplantation in acute leukemia. *Bone Marrow Transplant* 2013; **48**: 630–641.
50 Díez-Campelo M, Pérez-Simón JA, Pérez J, Alcoceba M, Richtmon J, Vidriales B *et al.* Minimal residual disease monitoring after allogeneic transplantation may help to individualize post-transplant therapeutic strategies in acute myeloid malignancies. *Am J Hematol* 2009; **84**: 149–152.
51 Rossi G, Carella AM, Minervini MM, di Nardo F, Waure C, Greco MM *et al.* Optimal time-points for minimal residual disease monitoring change on the basis of the method used in patients with acute myeloid leukemia who underwent allogeneic stem cell transplantation: a comparison between multiparameter flow cytometry and Wilms' tumor 1 expression. *Leuk Res* 2015; **39**: 138–143.
52 Bastos-Oreiro M, Perez-Corral A, Martínez-Laperche C, Bento L, Pascual C, Kwon M *et al.* Prognostic impact of minimal residual disease analysis by flow cytometry in patients with acute myeloid leukemia before and after allogeneic hemopoietic stem cell transplantation. *Eur J Haematol* 2014; **93**: 239–246.
53 Zhao XS, Yan CH, Liu DH, Xu LP, Liu YR, Liu KY *et al.* Combined use of WT1 and flow cytometry monitoring can promote sensitivity of predicting relapse after allogeneic HSCT without affecting specificity. *Ann Hematol* 2013; **92**: 1111–1119.
54 Wang Y, Wu DP, Liu QF, Qin YZ, Wang JB, Xu LP *et al.* In adults with t(8;21)AML, posttransplant RUNX1/RUNX1T1-based MRD monitoring, rather than c-KIT mutations, allows further risk stratification. *Blood* 2014; **124**: 1880–1886.
55 Yin JA, O'Brien MA, Hills RK, Daly SB, Wheatley K, Burnett AK. Minimal residual disease monitoring by quantitative RT-PCR in core binding factor AML allows risk stratification and predicts relapse: results of the United Kingdom MRC AML-15 trial. *Blood* 2012; **120**: 2826–2835.
56 Elmaagacli AH, Beelen DW, Kroll M, Trzensky S, Stein C, Schaefer UW. Detection of CBFbeta/MYH11 fusion transcripts in patients with inv(16) acute myeloid leukemia after allogeneic bone marrow or peripheral blood progenitor cell transplantation. *Bone Marrow Transplant* 1998; **21**: 159–166.
57 Shayegi N, Kramer M, Bornhäuser M, Schaich M, Schetelig J, Platzbecker U *et al.* The level of residual disease based on mutant NPM1 is an independent prognostic factor for relapse and survival in AML. *Blood* 2013; **122**: 83–92.
58 Schlenk RF, Kayser S, Bullinger L, Kobbe G, Casper J, Ringhoffer M *et al.* Differential impact of allelic ratio and insertion site in FLT3-ITD-positive AML with respect to allogeneic transplantation. *Blood* 2014; **124**: 3441–3449.
59 McCormick SR, McCormick MJ, Grutkoski PS, Ducker GS, Banerji N, Higgins RR *et al.* FLT3 mutations at diagnosis and relapse in acute myeloid leukemia: cytogenetic and pathologic correlations, including cuplike blast morphology. *Arch Pathol Lab Med* 2010; **134**: 1143–1151.
60 Shih LY, Huang CF, Wu JH, Lin TL, Dunn P, Wang PN *et al.* Internal tandem duplication of FLT3 in relapsed acute myeloid leukemia: a comparative analysis of bone marrow samples from 108 adult patients at diagnosis and relapse. *Blood* 2002; **100**: 2387–2392.
61 Brambati C, Galbiati S, Xue E, Toffalori C, Crucitti L, Greco R *et al.* Droplet digital polymerase chain reaction for DNMT3A and IDH1/2 mutations to improve early

detection of acute myeloid leukemia relapse after allogeneic hematopoietic stem cell transplantation. *Haematologica* 2016; **101**: e157–e161.
62 Candoni A, Toffoletti E, Gallina R, Simeone E, Chiozzotto M, Volpetti S *et al.* Monitoring of minimal residual disease by quantitative WT1 gene expression following reduced intensity conditioning allogeneic stem cell transplantation in acute myeloid leukemia. *Clin Transplant* 2011; **25**: 308–316.
63 Thiede C, Bornhäuser M, Oelschlägel U, Brendel C, Leo R, Daxberger H *et al.* Sequential monitoring of chimerism and detection of minimal residual disease after allogeneic blood stem cell transplantation (BSCT) using multiplex PCR amplification of short tandem repeat-markers. *Leukemia* 2001; **15**: 293–302.
64 Alizadeh M, Bernard M, Danic B, Dauriac C, Birebent B, Lapart C *et al.* Quantitative assessment of hematopoietic chimerism after bone marrow transplantation by real-time quantitative polymerase chain reaction. *Blood* 2002; **99**: 4618–4625.
65 Hoffmann JC, Stabla K, Burchert A, Volkmann T, Bornhäuser M, Thiede C *et al.* Monitoring of acute myeloid leukemia patients after allogeneic stem cell transplantation employing semi-automated CD34+ donor cell chimerism analysis. *Ann Hematol* 2014; **93**: 279–285.
66 Bader P, Kreyenberg H, Hoelle W, Dueckers G, Kremens B, Dilloo D *et al.* Increasing mixed chimerism defines a high-risk group of childhood acute myelogenous leukemia patients after allogeneic stem cell transplantation where pre-emptive immunotherapy may be effective. *Bone Marrow Transplant* 2004; **33**: 815–821.
67 Devine SM, Owzar K, Blum W, Mulkey F, Stone RM, Hsu JW *et al.* Phase II study of allogeneic transplantation for older patients with acute myeloid leukemia in first complete remission using a reduced-intensity conditioning regimen: results from cancer and leukemia group B 100103 (alliance for clinical trials in oncology)/blood and marrow transplant clinical trial network 0502. *J Clin Oncol* 2015; **33**: 4167–4175.
68 Vago L, Perna SK, Zanussi M, Mazzi B, Barlassina C, Stanghellini MT *et al.* Loss of mismatched HLA in leukemia after stem-cell transplantation. *N Engl J Med* 2009; **361**: 478–488.
69 Crucitti L, Crocchiolo R, Toffalori C, Mazzi B, Greco R, Signori A *et al.* Incidence, risk factors and clinical outcome of leukemia relapses with loss of the mismatched HLA after partially incompatible hematopoietic stem cell transplantation. *Leukemia* 2015; **29**: 1143–1152.
70 Barrett AJ, Mavroudis D, Tisdale J, Molldrem J, Clave E, Dunbar C *et al.* T cell-depleted bone marrow transplantation and delayed T cell add-back to control acute GvHD and conserve a graft-versus-leukemia effect. *Bone Marrow Transplant* 1998; **21**: 543–551.
71 Schaap N, Schattenberg A, Bär B, Preijers F, van de Wiel van Kemenade E, de Witte T. Induction of graft-versus-leukemia to prevent relapse after partially lymphocyte-depleted allogeneic bone marrow transplantation by pre-emptive donor leukocyte infusions. *Leukemia* 2001; **15**: 1339–1346.
72 Montero A, Savani BN, Shenoy A, Read EJ, Carter CS, Leitman SF *et al.* T-cell depleted peripheral blood stem cell allotransplantation with T-cell add-back for patients with hematological malignancies: effect of chronic GvHD on outcome. *Biol Blood Marrow Transplant* 2006; **12**: 1318–1325.
73 Lee CK, de Magalhaes-Silverman M, Hohl RJ, Hayashi M, Buatti J, Wen BC *et al.* Prophylactic T cell infusion after T cell-depleted bone marrow transplantation in patients with refractory lymphoma. *Bone Marrow Transplant* 2002; **29**: 615–620.
74 Ferrá C, Rodríguez-Luaces M, Gallardo D, Encuentra M, Martín-Henao GA, Peris J *et al.* Individually adjusted prophylactic donor lymphocyte infusions after CD34-selected allogeneic peripheral blood stem cell transplantation. *Bone Marrow Transplant* 2001; **28**: 963–968.
75 Nakamura R, Bahceci E, Read EJ, Leitman SF, Carter CS, Childs R *et al.* Transplant dose of CD34(+) and CD3(+) cells predicts outcome in patients with haematological malignancies undergoing T cell-depleted peripheral blood stem cell transplants with delayed donor lymphocyte add-back. *Br J Haematol* 2001; **115**: 95–104.
76 Schmid C, Schleuning M, Ledderose G, Tischer J, Kolb HJ. Sequential regimen of chemotherapy, reduced-intensity conditioning for allogeneic stem-cell transplantation, and prophylactic donor lymphocyte transfusion in high-risk acute myeloid leukemia and myelodysplastic syndrome. *J Clin Oncol* 2005; **23**: 5675–5687.
77 Schmid C, Schleuning M, Tischer J, Holler E, Haude KH, Braess J *et al.* Early allo-SCT for AML with a complex aberrant karyotype--results from a prospective pilot study. *Bone Marrow Transplant* 2012; **47**: 46–53.
78 Jedlickova Z, Schmid C, Koenecke C, Hertenstein B, Baurmann H, Schwerdtfeger R *et al.* Long-term results of adjuvant donor lymphocyte transfusion in AML after allogeneic stem cell transplantation. *Bone Marrow Transplant* 2015; **51**: 663–667.
79 Liga M, Triantafyllou E, Tiniakou M, Lambropoulou P, Karakantza M, Zoumbos NC *et al.* High alloreactivity of low-dose prophylactic donor lymphocyte infusion in patients with acute leukemia undergoing allogeneic hematopoietic cell transplantation with an alemtuzumab-containing conditioning regimen. *Biol Blood Marrow Transplant* 2013; **19**: 75–81.
80 Wang Y, Liu DH, Xu LP, Liu KY, Chen H, Zhang XH *et al.* Prevention of relapse using granulocyte CSF-primed PBPCs following HLA-mismatched/haploidentical, T-cell-replete hematopoietic SCT in patients with advanced-stage acute leukemia: a retrospective risk-factor analysis. *Bone Marrow Transplant* 2012; **47**: 1099–1104.
81 Huang XJ, Wang Y, Liu DH, Xu LP, Chen H, Chen YH *et al.* Modified donor lymphocyte infusion (DLI) for the prophylaxis of leukemia relapse after hematopoietic stem cell transplantation in patients with advanced leukemia--feasibility and safety study. *J Clin Immunol* 2008; **28**: 390–397.
82 de Lima M, Porter DL, Battiwalla M, Bishop MR, Giralt SA, Hardy NM *et al.* Proceedings from the National Cancer Institute's Second International Workshop on the Biology, Prevention, and Treatment of Relapse After Hematopoietic Stem Cell Transplantation: part III. Prevention and treatment of relapse after allogeneic transplantation. *Biol Blood Marrow Transplant* 2014; **20**: 4–13.
83 Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, Giagounidis A *et al.* Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. *Lancet Oncol* 2009; **10**: 223–232.
84 de Lima M, Giralt S, Thall PF, de Padua Silva L, Jones RB, Komanduri K *et al.* Maintenance therapy with low-dose azacitidine after allogeneic hematopoietic stem cell transplantation for recurrent acute myelogenous leukemia or myelodysplastic syndrome: a dose and schedule finding study. *Cancer* 2010; **116**: 5420–5431.
85 Platzbecker U, Wermke M, Radke J, Oelschlaegel U, Seltmann F, Kiani A *et al.* Azacitidine for treatment of imminent relapse in MDS or AML patients after allogeneic HSCT: results of the RELAZA trial. *Leukemia* 2012; **26**: 381–389.
86 Craddock C, Jilani N, Siddique S, Yap C, Khan J, Nagra S *et al.* Tolerability and clinical activity of post-transplantation azacitidine in patients allografted for acute myeloid leukemia treated on the RICAZA trial. *Biol Blood Marrow Transplant* 2016; **22**: 385–390.
87 Chen YB, Li S, Lane AA, Connolly C, Del Rio C, Valles B *et al.* Phase I trial of maintenance sorafenib after allogeneic hematopoietic stem cell transplantation for fms-like tyrosine kinase 3 internal tandem duplication acute myeloid leukemia. *Biol Blood Marrow Transplant* 2014; **20**: 2042–2048.
88 De Freitas T, Marktel S, Piemontese S, Carrabba MG, Tresoldi C, Messina C *et al.* High rate of hematological responses to sorafenib in FLT3-ITD acute myeloid leukemia relapsed after allogeneic hematopoietic stem cell transplantation. *Eur J Haematol* 2015; **96**: 629–636.
89 Tschan-Plessl A, Halter JP, Heim D, Medinger M, Passweg JR, Gerull S. Synergistic effect of sorafenib and cGvHD in patients with high-risk FLT3-ITD+AML allows long-term disease control after allogeneic transplantation. *Ann Hematol* 2015; **94**: 1899–1905.
90 Sandmaier B, Khaled S, Oran B, Gammon G, Trone D, Frankfurt O. *Results of a Phase 1 Study of Quizartinib (AC220) As Maintenance Therapy in Subjects with Acute Myeloid Leukemia in Remission Following Allogeneic Hematopoietic Cell Transplantation*. American Society of Hematology Annual Meeting: San Francisco, CA, USA, 2014.
91 Hu B, Vikas P, Mohty M, Savani BN. Allogeneic stem cell transplantation and targeted therapy for FLT3/ITD+ acute myeloid leukemia: an update. *Expert Rev Hematol* 2014; **7**: 301–315.
92 Gratwohl A, Stern M, Brand R, Apperley J, Baldomero H, de Witte T *et al.* Risk score for outcome after allogeneic hematopoietic stem cell transplantation: a retrospective analysis. *Cancer* 2009; **115**: 4715–4726.
93 Sorror M, Storer B, Sandmaier BM, Maloney DG, Chauncey TR, Langston A *et al.* Hematopoietic cell transplantation-comorbidity index and Karnofsky performance status are independent predictors of morbidity and mortality after allogeneic nonmyeloablative hematopoietic cell transplantation. *Cancer* 2008; **112**: 1992–2001.