Review Article

Stem Cell INVESTIGATION

Hypomethylating agents after allogeneic blood stem cell transplantation

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Abstract: Allogeneic blood stem cell transplantation (allo-SCT) is a potentially curative treatment for patients with myeloid malignancies such as acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS), but relapse remains the major cause of treatment failure. So far, therapeutic options for patients with AML or MDS who relapse after allo-SCT generally consisted of palliative care, low-dose or intensive chemotherapy as well as cellular therapies such as donor lymphocyte infusions (DLI) and second transplantation in selected cases. Nevertheless, the prognosis of patients with myeloid malignancies relapsing after allo-SCT remains dismal therefore asking for novel treatment strategies. Considering their well-balanced profile of good efficacy and moderate toxicity in the non-transplant setting, the hypomethylating agents (HMA) azacitidine (Aza) and decitabine (DAC) have also been tested either alone or in combination with DLI in the post-transplant period. This review summarizes the current knowledge about the use of these two HMA as pre-emptive, salvage or consolidation therapy mostly retrieved from retrospective studies but also from a few prospective trials. Within this review, we also comment on some practical issues such as optimal dose and schedule, the choice of HMA candidates and the role of additional cellular interventions. Finally, we also give an overview on the assumed mode of actions, ongoing research, clinical studies and potential combination partners aiming to improve this treatment approach.

Keywords: Myelodysplastic syndromes (MDS); acute myeloid leukemia (AML); allogeneic transplantation; relapse; maintenance; decitabine (DAC); azacitidine (Aza)

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Introduction

For many patients with acute myeloid leukemia (AML) allogeneic blood stem cell transplantation (allo-SCT) offers the highest potential for long-term survival, while for patients with myelodysplastic syndromes (MDS) allo-SCT is the only curative treatment option (1). During the last decades, many advances have been made to reduce non-relapse mortality, such as improvements in donor selection, immunosuppression and supportive care. Furthermore, the introduction of reduced toxicity conditioning as well as the use of alternative donor sources have improved outcome and also broadened the access for more, in particular older patients to this treatment option (2).

Despite these advances concerning the pre- and direct transplant phase, relapse still represents the main cause of treatment failure and is associated with a poor prognosis. Treatment options in this challenging situation are limited and have generally consisted of palliative care, low-dose or intensive chemotherapy as well as cellular therapies such as donor lymphocyte infusions (DLI) and second transplantation in selected cases. However, many patients can either not tolerate intensive therapies or are refractory

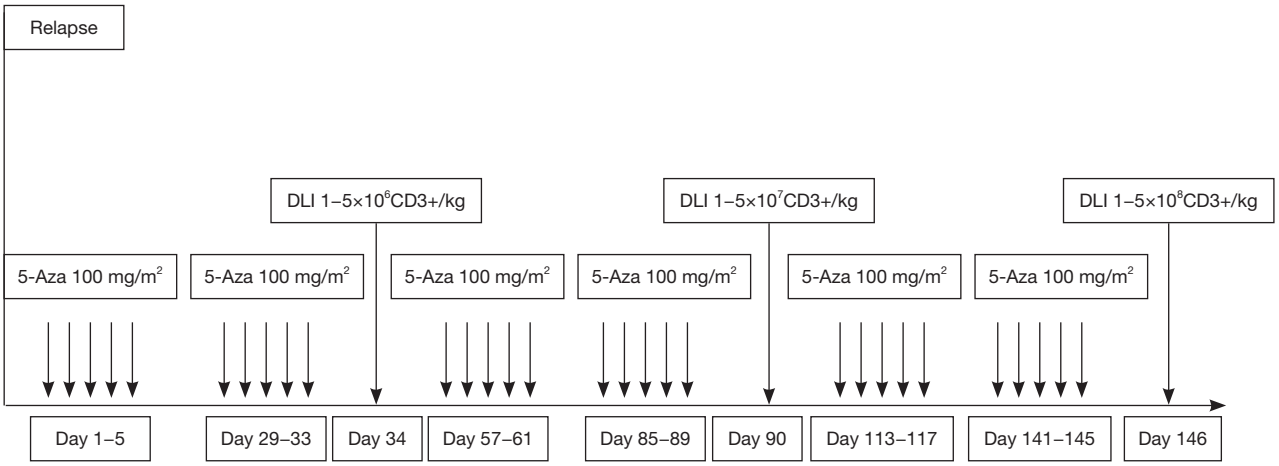

Figure 1 Treatment schedule of the AZARELA-trial (12)

to those conventional interventions (3). Thus, there is a need for novel treatment approaches, which on the one side exert a direct antileukemic effect and ideally direct the donor immune system towards an enhanced graft-versus-leukemia (GvL) reaction. On the other hand, such a therapy should have an acceptable toxicity profile and prevent severe graft-versus-host disease (GvHD).

The two hypomethylating agents (HMA) azacitidine (Aza) and decitabine (DAC) might provide these properties. Both are licensed for the treatment of older patients with AML and/or MDS not eligible for intensive therapies due to their balance between good efficacy and moderate toxicity (4-7). Based on these considerations others and we have tested these substances in the post-transplant period either alone or in combination with DLI.

This review aims to summarize the current knowledge about the use of Aza and DAC to prevent or to treat relapse of AML and MDS after allo-SCT. In addition, we will also give an overview about ongoing research and clinical studies to investigate the use of these two HMA after transplant.

HMA for the treatment of relapse

Aza for the treatment of relapse

Considering the poor outcome after conventional salvage therapies our group treated the first patient with early relapse of an AML evolved from MDS after allo-SCT with Aza and DLI in 2007 (8). Although this was rather due to the lack of realistic treatment alternatives than a decision based on a pathophysiological rationale, we were successful and this woman achieved a complete remission (CR) following this combined pharmacological and cell-based approach. Following this first case, a few small retrospective studies reported on the use of Aza as salvage therapy for relapse of myeloid malignancies after allo-SCT (9-11). These data built the rationale for the first prospective multicenter trial (AZARELA, Eudra-CT 2007-004860-37) (12). In this study, Aza had to be the first intervention for the treatment of relapse and DLI were envisaged in all patients (*Figure 1*).

The majority of patients (92%) included in this trial suffered from AML (15 *de novo* AML, 5 secondary following MDS) and 2 patients had MDS and MDS-MPS, respectively. All patients had hematologic relapse and received a median of 3 courses Aza (range, 1–8 courses) and 22 patients (73%) finally received DLI.

Following this treatment, we observed an encouraging overall response rate of 47%. Seven patients (23%) achieved CR, 2 patients (7%) partial remission (PR), and 5 patients (17%) had stable disease (SD). Of the 7 patients who achieved CR, 5 patients continued in CR for a median of 777 days (range, 461–890 days) without any additional treatment. Interestingly, this approach was especially effective in patients with high-risk cytogenetics as 6 of the 7 patients who achieved CR had a complex karyotype. In contrast, the rate and severity of GvHD as well as toxicities following the treatment with Aza and DLI were rather mild and compared well, if not better with other treatment options. Together with the experience from the early retrospective reports this prospective trial demonstrated that the combination of Aza and DLI was safe and effective in patients with AML or MDS who relapse after allo-SCT.

Overall, as indicated in *Table 1*, a total of 215 patients with AML, MDS and other related myeloid malignancies had been reported in the literature until 2015 (8-21,23,25). Treatment schedules and dosages of Aza varied between these reports. Furthermore, Aza was the first treatment of relapse and combined with DLI in some patients, while other patients had previously received other salvage therapies or did not receive DLI. Probably, due to this heterogeneity CR rates and overall survival (OS, not given in details in a relevant number of many studies) ranged from 14% to 60% and from 12% to 80%, respectively.

In addition, this heterogeneity and limited number of patients in the retrospective studies but even in the prospective trial did not enable to identify predictive factors for response and long-term survival. This prompted us to perform a retrospective analysis of 154 patients with relapse of AML or MDS after allo-HSCT who were treated with Aza and were scheduled for DLI at 12 German transplantation centers (22). The majority of these patients suffered from hematologic relapse (88%), whereas 19 patients (12%) had molecular relapse. In 143 patients (93%), Aza was the first therapy for relapse, while 11 patients (7%) had received antileukemic treatment before Aza therapy. Patients received a median of 4 courses Aza (range, 4–14 courses) and DLI were administered to 105 patients (68%).

The size of this patients group and the quality of data provided by the participating centers allowed us to identify patients who may benefit most from the combination of Aza and DLI. In multivariate analyses, in particular patients with MDS and those with a molecular relapse had a significantly higher probability to achieve CR following Aza and DLI. This also applied to OS, which was predicted to be significantly longer in patients with MDS as well as in those with a low disease burden (molecular relapse or bone marrow blast count <13%) at the time of relapse.

In general, some of the findings were recently confirmed by results from a retrospective EBMT analysis investigating a similar-sized patient group (n=181) (24). Here, Craddock and colleagues identified the diagnosis of MDS instead of AML and transplantation in remission as predictors for response. For OS disease burden defined by the bone marrow blast count at the time of relapse (cut-off 20%) and a longer interval between allo-SCT and relapse (cut-offs 6 and 12 months) turned out to be predictive in multivariate analysis. Based on these variables the authors proposed a so-called AZA Relapse Prognostic Score (ARPS). Although this score could clearly divide their cohort in 3 prognostically different subgroups, this score has not been validated in an independent cohort yet. Another drawback of this study is that only patients with haematological, but not molecular relapse were included. Given the opportunity of continuously optimized molecular methods to monitor minimal residual disease (MRD) to direct MRD-triggered interventions after transplant, a cut-off of 20% BM blasts cast the practicability of this score for real life into doubt.

Despite these controversies, these two large analyses together with the prospective and retrospective studies have univocally shown that the combination of HMA and DLI is of therapeutic value for patients relapsing after allo-SCT, in particular for those with MDS or AML with low disease burden.

DAC for the treatment of relapse

In contrast to Aza, the evidence regarding the use of DAC, the second HMA approved in Europe for the treatment of AML, as salvage therapy for relapse after allo-SCT is limited covering a total of 9 patients so far (*Table 1*). In these three case series 5 patients achieved CR and no excess of toxicity was reported (15,18,26). Although these reports suggest that DAC might also have some efficacy in patients with myeloid malignancies relapsing after allo-SCT, they do not provide sufficient information on survival and response rates due to the limited number of patients. Results from prospective trials investigating DAC as salvage therapy for relapse after allo-SCT have not been published so far and, to the best of our knowledge, will also not be available in the near future.

HMA for the prevention of relapse

Maintenance vs. consolidation therapy with HMA after allo-SCT

Even though HMA can induce long-term remissions in a relevant number of patients with relapse after allo-SCT, it is rather better to avoid than to treat relapse. One potential approach to reduce the risk of AML or MDS relapse following allo-SCT could be prophylactic therapy. Prophylactic approaches can be separated into maintenance or consolidation therapies. While the former means a continuous therapy until disease progression or intolerability, the latter represents a therapy phase defined by a limited time interval and/or number of treatment cycles. Independent from this rather academic distinction, such prophylactic treatment may be either able to directly

Table 1 Retrospective and prospective studies investigating Aza and DAC as treatment of relapse

Author	Year	Type of study	Drug	Schedule	DLI	Patients	Diagnosis	Overall response (CR, PR)	Survival	Acute GvHD	Chronic GvHD	Reference
Graef *et al.*	2007	Case report	Azacitidine	100 mg/m^2 5 days	Yes	1	sAML	CR	Alive, 7 months after start of therapy	No	No	(8)
Kim *et al.*	2010	Case series	Azacitidine	75 mg/m^2 7 days	No	4	MDS	75% (50%, 25%)	2 patients alive, 14 and 35 months after start of therapy	No	Limited 75%	(13)
Jabbour *et al.*	2009	Case series	Azacitidine	16–40 mg/m^2 5 days	No	9	AML	55% (33%, 22%)	2-year overall survival 80%	Not reported	Not reported	(10)
Lübbert *et al.*	2010	Retrospective	Azacitidine	100 mg absolute 3 days	Yes	26	AML, CMML	16% CR, PR not reported	Median survival 136 days, 2-year overall survival 16%	8%	Limited, 4%	(11)
Czibere *et al.*	2010	Retrospective	Azacitidine	100 mg/m^2 5 days	Yes	22	AML, MDS, MPN	41% (23%, 18%)	Median survival 144 days, 2-year overall survival 23%	33%	18%	(9)
Bolanos-Meade *et al.*	2011	Retrospective	Azacitidine	75 mg/m^2 5 to 7 days	Yes	10	AML, MDS	60% (60%, 0%)	Median survival 423 days	0%	10%	(14)
Singh *et al.*	2012	Case report	Decitabine	20 mg/m^2 5 days	No	1	AML	CR	Alive, 26 months after start of therapy	No exacerbatio	No exacerbatio	(15)
Drozd-Sokolowska *et al.*	2016	Case series	Azacitidine	75 mg/m^2 7 days	Yes	9	AML, MDS	0% (0%, 0%)	Median survival 6.8 months	11%	0%	(16)
Steinmann *et al.*	2015	Retrospective	Azacitidine	100 mg absolute 3 days	Yes	72	AML, MDS, CMML	10% CR, PR not reported	Median survival 108 days	10%	4%	(17)
Ganguly *et al.*	2013	Case series	Decitabine	20 mg/m^2 5 days	Yes	8	AML	38% CR, PR not reported	Not reported	75%	Not reported	(18)
Wang *et al.*	2016	Case report	Decitabine	10 mg/m^2 5 days	Yes	1	sAML	CR	Alive, 10 months after start of therapy	yes	Not reported	(19)
Ghobadi *et al.*	2016	Prospective, phase I	Azacitidine	45–75 mg/m^2 d4/6/8/10 post DLI	Yes	8	AML	75% (75%, 0%)	Median survival 12.5 months	62.5%	0%	(20)
Tessoulin *et al.*	2014	Retrospective	Azacitidine	75 mg/m^2 7 days	Yes	31	AML, MDS, MPN	14% (14%, 0%)	Median survival 153 days, 1-year overall survival 14%	Not reported	Not reported	(21)

Table 1 (*continued*)

Table 1 (*continued*)

Author	Year	Type of study	Drug	Schedule	DLI	Patients	Diagnosis	Overall response (CR, PR)	Survival	Acute GvHD	Chronic GvHD	Reference
Schroeder *et al.*	2013	Prospective, phase II	Azacitidine	100 mg/m^2 5 days	Yes	20	AML, MDS	30% (23%, 7%)	2-year overall survival 17%	37%	17%	(12)
Schroeder *et al.*	2015	Retrospective	Azacitidine	50–100 mg/m^2 for 5–7 days	Yes	154	AML, MDS, MPN	33% (27%, 6%)	2-year overall survival 29%	23%	27%	(22)
Inoue *et al.*	2014	Case report	Azacitidine	32–75 mg/m^2 5 days	No	1	tMDS	CR	Alive, 26 months after HSCT	No	No	(23)
Craddock *et al.*	2016	Retrospective	Azacitidine	75 mg/m^2 5–7 days	Yes	181	AML, MDS	29% (15%, 14%)	2-year overall survival 12%	7%	Not reported	(24)
Antar *et al.*	2013	Case series	Azacitidine	32–75 mg/m^2 5–7 days	No	2	AML	1 ongoing CR	One patient alive, 17 month after start of therapy	Not reported	Not reported	(25)

eliminate MRD or to control disease activity until the donor immune system is sufficiently reconstituted to mediate the desired GvL effect.

In accordance with this definition, no maintenance trials but 5 prospective single-arm studies investigating Aza (n=3) or DAC (n=2) as consolidation therapy for patients with AML or MDS after allo-SCT have been published (27-31). The major aims of these early-phase studies covering a total of 130 patients were to demonstrate feasibility and to find an appropriate dose for future trials based on the balance between tolerability and efficacy. As indicated in *Table 2* consolidation therapy was planned to start within the first 2 to 3 months after transplant in order to cope with the fact that most relapses occur within the first year after transplant. However, treatment onset was delayed due to toxicity reasons in most trials. In addition, in the trials of Craddock *et al.* and de Lima *et al.*, 27% and 50% of enrolled patients dropped out prior the first administration of Aza as a consequence of toxicity, patient wish or relapse (27,28). Major adverse events related to the study drugs were hepatotoxicity and infections. Although the doses of Aza and DAC were significantly lower than the approved dosages in the non-transplant setting only a minority of patients could receive the complete number of envisaged treatment cycles. Overall, this indicates, that the study population represents a selected group of patients and highlights the potential risks of post-transplant cytotoxic therapy. Given the limited number of patients and the lack of a control arm, a definitive ranking of outcome results in-terms of relapse incidence, survival and GvHD is impossible so far. However, with regard to the risk of GvHD, there was a hint in the study of de Lima *et al.* that post-transplant therapy with Aza might be associated with a lower probability to develop chronic GvHD.

Finally, the Aza dose identified in the study of Lima *et al.* provided the basis for an ongoing randomized phase III trial investigating Aza for relapse prevention after allo-SCT in patients with myeloid malignancies (NCT00887068) (28). In this trial, 246 patients will be included and receive Aza or placebo for a period of 1 year after transplant. Estimated primary completion date is April 2018 and results from this trial will hopefully elucidate the impact of Aza-based consolidation on relapse risk and GvHD.

Pre-emptive therapy with HMA

Although consolidation therapy may be an important concept, the following concerns must be raised. Generally,

Table 2 Overview of studies investigating Aza and DAC as consolidation therapy

Author	Year	Type of study	Drug	Schedule	Starting time	Duration	Patients	Diagnosis	Overall survival	Relapse-free survival	NRM	CIR	Acute GvHD	Chronic GvHD	Reference
de Lima *et al.*	2010	Prospective, phase I	Azacitidine	8-40 mg/m^2 for 5 days every 30 days	d+30 to d+90	4 cycles	45	AML, MDS	Median 30.8 months	Not reported	9%	Not reported in detail	Not reported in detail	37%	(28)
Craddock *et al.*	2016	Prospective	Azacitidine	36 mg/m^2 for 5 days, every 4 weeks	d+42	12 months	37	AML	81% at 1 year, 49% at 2 years	57% at 1 year, 49% at 2 years	0% at day 100, 8% at 1 year	Not reported in detail	46%	27%	(27)
Pusic *et al.*	2015	Prospective	Decitabine	5-15 mg/m^2 for 5 days every 6 weeks	d+50 to d+100	8 cycles	22	AML	56% at 2 years	48% at 2 years	Not reported in detail	28% at 2 years	41%	63%	(31)
Oshikawa *et al.*	2014	Prospective	Azacitidine	30 mg/m^2 for 7 days, every 4 weeks	d+79	4 cycles	10	AML	70% at 1 year	60% at 1 year	Not reported in detail	Not reported in detail	Not reported in detail	50%	(30)
Han *et al.*	2015	Prospective, phase I	Decitabine	individualized schedules	d+86	12 cycles	16	MDS, sAML	Not reported in detail	Not reported in detail	Not reported in detail	Not reported in detail	Not reported in detail	Not reported in detail	(29)

allo-SCT is a potentially curative therapy and many patients with high-risk myeloid malignancies have a chance to achieve long-term cure without post-transplant cytotoxic therapy. Therefore, it is obvious that for some patients HMA given after transplant may represent over-treatment associated with potentially detrimental side effects in "already cured" patients. This can include cytopenias and infections but also eventually secondary malignancies in the long-term.

To deal with this, MRD-triggered pre-emptive therapy including DLI instead of treatment in remission may be a better strategy. In our large retrospective analysis, we recently found that treatment at the time of molecular instead of haematological relapse was associated with a higher likelihood of remission and survival (22). This idea has also been addressed by Platzbecker and colleagues in a prospective trial, where pre-emptive Aza therapy was guided by donor chimerism in circulating CD34+ cells. Twenty patients with decreasing CD34+ donor chimerism (<80%) who still were in haematological remission received treatment with up to 4 cycles of Aza. Although an improvement of chimerism (>80%) was observed in 50% (10 of 20) of patients, haematological relapse occurred in the majority of patients and continuous remission was only achieved in 3 (30%) of the responders (32). This was probably related to the limited number of Aza cycles, but in particular to the fact that DLI were not part of the protocol. The same group has employed this approach in patients with NPM1-mutated AML including 3 of them with MRD after allo-SCT highlighting this concept to guide post-transplant interventions (33).

The recent discovery of several distinctive gene mutations in patients with myeloid malignancies by genomic high-throughput techniques together with technical advances regarding PCR-based methods will enable a stringent MRD monitoring for the majority of patients. This will help to employ and optimize MRD-based pre-emptive therapies with HMA and other compounds in the close future.

Mode of action

The underlying mechanisms, by which HMA mediate relevant anti-leukemic efficacy accompanied by a relatively low incidence and severity of GvHD, have been addressed in animal models but also in translational analyses of human samples. Besides a primarily cytotoxic effect lower doses of HMA have shown to upregulate several antigens including HLA epitopes, cancer testis antigens and minor

histocompatibility molecules on leukemic cells *in vitro* and *in vivo* (34-37). This re-expression of epigenetically silenced genes by HMA is thought to render malignant cells more immunogenic toward T-cell killing. Along with this, several groups have shown effects of HMA on T cell mediated antitumor activity by increasing tumor-specific CD8 T cell responses, for example against cancer testis antigens (38). Furthermore, treatment with DNA-demethylating agents can induce cell surface expression of formerly unexpressed killer Ig-like receptors (KIRs) in natural killer cells and may hereby contribute to a HMA-mediated GvL effect (39) .

The reproducible observation of relatively infrequent and mostly mild GvHD also supported the idea that HMA might offer immunoregulatory properties. In this context, two groups demonstrated that HMA convert conventional T cells to Tregs and hereby prevent GVHD after allogeneic transplant or DLI in mice with no effect on GVL. HMA after allo-SCT and DLI reduce GvHD in these animals by a direct T cell suppression and by conversion of alloreactive donor T cells into Tregs (CD4+, CD25+, FOXP3+) through enhancement of FOXP3 expression (40,41). Expansion of Tregs has also been observed in humans who received Aza either as maintenance or salvage therapy for relapsed AML and MDS (42,43). Taken together, these results suggest that Aza might target different immunological pathways and may hereby separate GvHD and GvL to a certain extent. Still, many of the underlying mechanisms need to be deciphered to gain a better understanding of the molecular and immunologic events associated with the use of HMA after allo-SCT.

Open questions

Overall, HMA and in particular Aza have proven to be a valuable treatment alternative for relapse. Still, most of the evidence is based on retrospective reports, but not on prospective randomized trial covering a large number of patients. As a consequence, several questions regarding the practical use of HMA as salvage therapy after transplant have not been answered sufficiently yet. Here, we comment on some of these issues based on the current knowledge:

What is the optimal dose and number of cycles of HMA for treatment of relapse?

With regard to this question no definitive answer can be given. As indicated in *Table 1* different schedules with daily dosages ranging from 16 to 100 mg/m^2 for 3 to 7 days have been used. Responses have been observed with all dosages and there is no clear relationship between dosage and response. One could assume that a potentially higher anti-leukemic effect mediated by a higher dose might be relevant for patients with high leukemic burden or rapid relapse kinetics but should be balanced against potential side effects, in particular cytopenias and cytopenia related complications.

We currently start with the approved Aza dosage of 75 mg/m^2 for 7 days in accordance to the protocol of our prospective trial (12) and adapt dosages during the following cycles in case of hematotoxicity.

Median time and number of cycles to response also varied between the reports. This suggests, that whenever feasible at least 4 cycles should be administered before a definitive evaluation of response can be made. In addition, also duration of treatment is not defined by any evidence from the literature, whether to administer a definitive number of cycle or to continue until progression or intolerability as recommended in the non-transplant setting. Again, we try to follow the scheme of our prospective trial and administer 6 to 8 cycles of Aza if feasible. In addition, we aim to infuse DLI after every second Aza cycle with increasing T cell dosages until GvHD occurs. Based on our personal experience, we administer at least 1 cycle of Aza after the last DLI, since we have observed some cases of severe GvHD in cases where DLI was the last intervention.

Aza or DAC—which one to choose for relapse after allo-SCT?

No randomized trial has addressed this question so far. As described above, both HMA can to induce remissions in patients relapsing after allo-SCT. However, currently the literature definitively covers more patients treated with Aza than with DAC in this situation, including 2 prospective trials. Furthermore, in Europe DAC is only licensed for patients with AML but not MDS. For this reason, we generally use Aza in this setting and only consider DAC in patients with contraindications against Aza or in case of Aza failure. Furthermore, DAC might also an alternative for AML patients with high blast counts or rapid disease kinetics at relapse.

Are DLI and/or second transplant needed in addition to HMA?

Again, this relevant question, whether a combination with donor cells is required for response and long-term survival, cannot be answered on the basis of prospective

randomized trials. The results of the studies published so far were heterogeneous regarding the use of DLI and second transplant (*Table 1*).

In the recent retrospective EBMT analysis, the administration of DLI had no impact on either the probability of achieving a major response or on 2-year OS. However, in this analysis only 38% of patients (n=69) received DLI, and only those 39 of whom received DLI within two months of commencing AZA salvage and in the absence of a clinical response were included in multivariate analysis (24). Besides this methodological limitation, these results are in strict contrast to results from another retrospective EBMT analysis. Here, Schmid and colleagues clearly demonstrated, that reinduction of CR by pharmacological compounds including Aza alone is not sufficient for long-term survival, but donor-cell-based consolidation is required (44). The results from our retrospective analysis are in further support of this idea, as in 78% of patients CR was obtained after the first DLI, suggesting a pronounced cell-induced immune reaction. Remissions induced by Aza and DLI in our analysis were ongoing for a median period of 20 months in 66% of patients and lasted for a median of 13 months in those who finally relapsed again (22). In contrast, in the study of Platzbecker *et al.*, even though it was given pre-emptively, Aza alone could prevent hematologic relapse only in a minority of patients, probably related to the fact that DLI were not part of the protocol (32).

Therefore, to us it seems reasonable to follow the general principle that relapse after allo-SCT needs treatment with a cellular approach either alone or in combination with cytotoxic therapy.

Prophylactic or preemptive therapy with HMA?

As discussed above, prophylactic treatment might represent over-treatment associated with relevant side effects in a relevant proportion of patients. In the absence of randomized trials demonstrating a benefit of a prophylactic approach with HMA so far, no recommendation for its use can be made and patients should be treated in clinical trials.

In the future, to tailor post-transplant treatment a special attention has to be paid to patient selection based on relapse risk. One approach for a better risk stratification could be the knowledge about the adverse prognosis of karyotype alterations, gene mutations and their combination. For example, some studies have shown that TP53 mutations indicate a dismal prognosis for MDS patients after allo-SCT and may be able to subdivide patients with complex karyotypes with regard to their prognosis (45,46). The goal is to identify a patient population with an extraordinary high relapse risk for further studies to test innovative post-transplant strategies applied in remission. In patients with an intermediate risk for relapse after allo-SCT over-treatment should be avoided. Therefore, MRD-triggered, pre-emptive therapy including DLI instead of treatment in remission may be a better strategy as recent studies have shown that it can achieve excellent outcomes.

Potential combination partners

In the non-transplant setting several compounds such as HDAC inhibitors, tyrosine kinase inhibitors (TKI) or the immunomodulator lenalidomide have been tested in combination with HMA. Based on initial favourable results from these trials (47) some combinations are currently also under investigation for treatment of relapse after allo-SCT. For example, there are currently 2 trials investigating the potentially additive effect of lenalidomide (VIOLA trial and NCT02472691). In the ongoing trials others and we hope to exploit a potential stimulation of the donor immune system by lenalidomide in order to enhance the Aza-mediated GvL effect.

Another interesting class of drugs are TKI, which have inhibitory effects on internal tandem duplications (ITD) in the gene encoding for the Fms-like tyrosine-3 (FLT3) kinase receptor. One of these candidates is sorafenib, a multikinase inhibitor with activity against FLT3 kinase. Sorafenib with or without DLI has demonstrated antileukemic activity in this situation and may induce complete molecular remissions in some patients (48,49). Furthermore, given the promising results of a recent phase-II trial combining Sorafenib and Aza in relapsed or refractory FLT3–ITD mutated AML (50), this combination may also be worth testing after transplant in patients with FLT3–ITD+ AML.

Finally, immune checkpoint blockade is also entering the field of haematological malignancies. There are early preliminary signals from *in-vitro* and *in-vivo* systems suggesting that a combination of HMA and PD1-blocking agents may have a pathophysiological rationale in AML and MDS (51,52). Clinical trials investigating such approaches have just started in the non-transplant setting and, if successful, might be expanded to the field of relapse after allo-SCT.

Conclusions

HMA and in particular Aza have proven to be a valuable treatment for MDS and AML patients relapsing after allo-SCT. A better understanding of the underlying mechanisms and identification of target patient populations will hopefully help to further optimize this approach. In addition, the arrival of new pharmacological compounds together with the upcoming improvements of specialized cellular products and antibodies may also help to further improve prognosis of relapse after allo-SCT.

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Footnote

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