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PROGRESS IN HEMATOLOGY

New immunotherapy-based approach in allogeneic hematopoietic stem cell transplantation

Hypomethylating agents for treatment and prevention of relapse after allogeneic blood stem cell transplantation

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Abstract
Despite the curative potential of allogeneic stem cell transplantation (allo-SCT) in patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS), many patients will relapse. Until recently therapeutic options mainly consisted of palliative care, chemotherapy, donor lymphocyte infusions and second transplantation in selected cases. Still many patients either do not tolerate intensive therapies or do not achieve durable remissions and will finally succumb. Given this unmet medical need the hypomethylating agents (HMA), Azacitidine (Aza) and Decitabine (DAC) have been tested as salvage therapy in patients with myeloid malignancies relapsing after allo-SCT. Furthermore, they have also been incorporated into prophylactic and pre-emptive approaches to avoid haematological relapse. In this review, we summarize the evidence from retrospective studies but also from a few prospective trials regarding the use of HMA after transplant. To aid clinicians in their daily clinical practice, we also comment on some practical aspects such as dosing and schedule, the choice of HMA and the use of complementary cellular therapies. Finally, this review also gives an overview on potential mechanisms mediating the efficacy of HMA after transplant as well as ongoing preclinical research and clinical activities aiming to further improve this treatment approach.

Keywords Myelodysplastic syndromes · Acute myeloid leukemia · Allogeneic transplantation · Relapse · Maintenance · Decitabine · Azacitidine

Introduction

Allogeneic blood stem cell transplantation (allo-SCT) is a potentially curative treatment option for many patients with acute myeloid leukemia (AML) and represents the only chance for long-term survival in patients with myelodysplastic syndromes (MDS) [1]. In the past, several improvements including donor selection, immunosuppression and supportive care have been made to reduce non-relapse mortality. In addition, the introduction of reduced toxicity conditioning has broadened the access for more, in particular older patients to this treatment option [2].

In contrast to this, relapse still represents the main cause of treatment failure and is associated with a poor prognosis. The principles of treatment in this challenging situation are to reduce the disease burden on the one hand and to induce an allogeneic immune reaction on the other hand to achieve long-term disease control. Traditionally, treatment options were limited and have generally consisted of palliative care, low-dose or intensive chemotherapy as well as cellular therapies such as donor lymphocyte infusions [3] and second transplantation in selected cases. Still, the fact that many patients can either not tolerate intensive therapies or are refractory to these conventional interventions indicates the relevant need for novel treatment approaches [4]. Ideally, such a therapy mediates direct antileukemic effects and strengthens the graft-versus-leukemia (GvL) reaction, while on the other hand is not associated with an extensive risk for severe graft-versus-host disease (GvHD) and offers an acceptable toxicity profile.

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Given their balance between efficacy and moderate toxicity, the two hypomethylating agents (HMA) Azacitidine (Aza) and Decitabine (DAC) might meet many of these demands. Both are licensed and usually employed for the treatment of older patients with AML and MDS not eligible for intensive therapies [5–8]. Taking this into account, these two substances have also been tested in the post-transplant period.

In this review, we aim to summarize the current literature reporting on the use of Aza and DAC to prevent or to treat relapse of myeloid malignancies after allo-SCT. Besides an overview about ongoing research and clinical studies in this field, we also address practical issues regarding the use of these two HMA after transplant.

Treatment of relapse with HMA

Azacitidine for the treatment of relapse

In the absence of realistic treatment alternatives, we treated the first patient with early relapse of an AML evolved from MDS after allo-SCT with Aza and DLI in 2007 [9]. Following this combined pharmacological and cell-based approach, this woman achieved a complete remission (CR) and our observation presented the starting point for several retrospective studies reporting on the use of Aza as salvage therapy for relapse of myeloid malignancies after allo-SCT in a limited number of patients [10–12]. These data built the rationale for the first prospective multicenter trial (AZA-RELA, Eudra-CT 2007-004860-37) [13], where Aza was administered as first intervention for relapse and DLI were scheduled after every second Aza cycle.

All 30 patients included in this trial had hematologic relapse of AML ($n = 28$, 92%) or MDS and MDS-MPS ($n = 2$, 8%) in median 175 days after transplantation. They received a median of 3 courses of Aza (range 1–8) and 22 patients (73%) finally received at least one DLI. This treatment resulted in an overall response rate of 30% including 7 patients (23%) achieving CR and 2 patients (7%) partial remission (PR). These remissions were durable in 5 of 7 patients lasting for a median of 777 days (range 461–890). One of these patients remains in ongoing remission without any further antileukemic treatment for 56 months until now. The finding that this therapy was in particular effective in patients with high-risk cytogenetics such as complex karyotype is in accordance with its primary indication in the non-transplant setting and gave already an early hint, which patients might benefit from this approach. This efficacy was not counterbalanced by an excess of toxicity and compared well if not better with other treatment options. Indeed, the rate and severity of GvHD as well as toxicities following the treatment with Aza and DLI were rather low and mild. Altogether, this prospective study confirmed the observation from the retrospective reports that the combination of Aza and DLI could by a safe and effective treatment alternative for patients with myeloid malignancies who relapse after allo-SCT.

Table 1 summarizes the publications regarding the use of Aza as salvage treatment for relapse after allo-SCT: until now a total of 601 patients with AML, MDS and other related myeloid malignancies have been published with varying schedules and dosages of Aza. These included 3 prospective, non-randomized trials and the majority of patients reported retrospectively [9–25]. Furthermore, Aza was the first treatment of relapse and combined with DLI in some of these patients, while other patients had previously received other salvage therapies or did not receive DLI. This heterogeneity of treatment strategies explains CR rates and overall survival (OS, not given in details in a relevant number of many studies) ranging from 14 to 75% and from 12 to 80% after treatment with Aza.

Furthermore, as a consequence of this heterogeneity and limited number of patients in most of these analyses, the reproducible identification of factors predictive for response and long-term survival was not possible. For this purpose, two larger retrospective surveys were performed. In the first one, we analysed the outcome of 154 patients with hematologic (88%) or molecular (12%) relapse of AML or MDS after allo-HSCT. All were treated with Aza (median 4 courses; range 4–14) and DLI (administered to 105 patients, 68%) either as first (93%) or later (7%) salvage approach at 12 transplant centres participating in the german cooperative transplant study group [22]. The size of this patient group and the quality of data provided by the participating centres enabled us to identify patients who may benefit most from the combination of Aza and DLI. Multivariate analyses carved out that the diagnosis of MDS and detection of relapse at a molecular stage were significantly predictive for the likelihood to achieve CR. In congruency with this, a low disease burden (molecular relapse or bone marrow blast count < 13%) at the time of relapse and the diagnosis of MDS were also significant predictors for a longer overall survival [22].

These issues were also addressed in another retrospective analysis of a similar-sized patient group ($n = 181$) by Craddock et al. within the EBMT [16]. They also identified the diagnosis of MDS instead of AML and in addition transplantation in remission as predictors for response. Confirming our results with regard to overall survival disease burden defined by the bone marrow blast count (cut-off 20%) at the time of relapse turned out to be predictive in multivariate analysis. Furthermore, a longer interval between allo-SCT and relapse (cut-offs 6 and 12 months) was also associated with a better outcome. These variables were included into a so-called AZA Relapse Prognostic Score (ARPS), which

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

Author	Year	Type of study	Drug	Schedule	DLI	Patients (*n*)	Diagnosis	Overall response (CR, PR)	Survival	Acute GvHD	Chronic GvHD	References
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	[9]
Kim et al.	2010	Case series	Azacitidine	75 mg/m^2 7 days	No	4	MDS	75% (50, 25%)	2 pts alive, 14 and 35 months after start of therapy	No	Limited 75%	[21]
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	[11]
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%	[12]
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%	[10]
Bolanos-Meade et al.	2011	Retrospective	Azacitidine	75 mg/m^2 5–7 days	Yes	10	AML, MDS	60% (60, 0%)	Median survival 423 days	0%	10%	[15]
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 exacerbation	No exacerbation	[36]
Drozd-Sokolowska	2016	Case series	Azacitidine	75 mg/m^2 7 days	Yes	9	AML, MDS	0% (0, 0%)	Median survival 6.8 months	11%	0%	[17]
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%	[23]
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	[34]
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	[38]

Table 1 (continued)

Author	Year	Type of study	Drug	Schedule	DLI	Patients (*n*)	Diagnosis	Overall response (CR, PR)	Survival	Acute GvHD	Chronic GvHD	References
Ghobadi et al.	2016	Prospective, phase I	Azacitidine	45-75 mg/m^2 day 4/6/8/10 post DLI	Yes	8	AML	75% (75, 0%)	Median survival 12.5 months	62.5%	0%	[18]
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	[24]
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%	[13]
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	[19]
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	[16]
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	[14]
Ishikawa et al.	2017	Case report	Azacitidine	75 mg/m^2 5–7 days	Yes	1	tMDS	CR for 15 months	Dead, after relapse #2 and second transplant	Yes	Yes	[20]
Liu et al.	2017	Case report	Decitabine	Data not available	No	1	AML	CR	Not reported	No	No	[37]
Woo et al.	2017	Prospective, phase II	Azacitidine	75 mg/m^2 7 days	Allowed	39	AML, MDS	31% (8, 23%)	2-year overall survival 25%	8%	Not reported	[25]

could clearly divide their cohort in 3 prognostically different subgroups. Still, this score has not been validated in an independent cohort yet and has only been tested in patients with haematological, but not molecular relapse due to the inclusion criteria of this registry-based analysis. In the light of the continuously optimized molecular methods to monitor minimal residual disease (MRD) and to guide MRD-triggered interventions after transplant, a cut-off of 20% BM blasts cast the practicability of this score for real life into doubt.

During the last years, several molecular alterations, mostly mutations have been unraveled in patients with MDS and AML [26, 27]. These mutations have substantially improved our pathophysiological understanding and can augment the outcome prediction after conventional therapy as well as after allo-SCT. As many of the identified mutations affect the so-called "epigenetic machinery", it was assumed that such mutations could potentially serve as predictors for response and survival after HMA treatment. Unfortunately, this has not proven to be the case in elderly patients treated with HMA, but not undergoing allo-SCT [28–30]. In the context of HMA as salvage therapy after allo-SCT, one group has investigated this aspect so far. Woo and colleagues recently reported their results in 21 Aza-treated patients using a targeted 54 NGS gene panel. In their analysis, TP53 mutations were significantly associated with poor responsiveness to Aza and inferior survival, while the opposite applies by trend to TET2 mutations [31]. Nevertheless, the number of patients is too small to draw any conclusions asking for validation in larger patient groups.

Despite these controversies and the ongoing research including the search for biomarkers, the two large analyses together with the prospective and retrospective studies have univocally shown that the combination of Aza and DLI is of therapeutic value for patients relapsing after allo-SCT. Therefore, this approach has been incorporated as a treatment option for these patients in the current recommendations of the EBMT and the European Leukemia Net [32, 33].

Decitabine for the treatment of relapse

DAC is the second HMA, which is approved in Europe for the treatment of elderly patients with AML. Furthermore, in the USA, it is also available for the treatment of patients with MDS. In the post-transplant setting, the literature reporting on the use of DAC as salvage therapy for relapse after allo-SCT was restricted to a total of 11 patients reported so far (Table 1). With 6 of the treated patients achieving a complete remission, these case series suggested that DAC might also have some efficacy in patients with myeloid malignancies relapsing after allo-SCT [34–38]. 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. Again, this prompted us to perform a retrospective survey on the use of DAC as salvage within the German Cooperative Transplant Study group. Hereby, we were able to analyse data of 36 patients with haematological ($n = 35$) or molecular relapse ($n = 1$), who received DAC as first salvage therapy (44%) or after 1–5 previous lines of salvage therapy. As a result, CR rate was 23% ($n = 6$, 17%) including 3 patients within the first-line group. Of particular interest, 3 patients receiving DAC as second-line treatment after Aza (2 patients with failure, 1 patient with intolerability) also achieved CR. The 2-year OS rate was 11 ± 6% without any difference between first-line and pretreated patients [39].

Together with an acceptable toxicity profile, these data suggest that also the second HMA DAC exerts clinical efficacy and can induce durable remissions in individual patients. Prospective trials are warranted to confirm that DAC may be an alternative to Aza or even a second choice after Aza failure.

HMA for the prevention of relapse

Prophylactic versus pre-emptive therapy with HMA after allo-SCT

As mentioned above, HMA can induce long-term remissions in a relevant number of patients with relapse after allo-SCT. Nevertheless, the results of our analysis and the results from Craddock et al. showed that the success of HMA after transplant directly correlates with disease burden. Thus, it is rather better to avoid than to treat relapse or if not possible to start therapy at the lowest level of measurable disease. Approaches to reduce the risk of frank AML or MDS relapse following allo-SCT can be separated into prophylactic and pre-emptive strategies. Prophylactic approaches can be further subdivided 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 (Fig. 1).

Prophylactic treatment strategies aim to directly eliminate residual malignant cells, which cannot be detected with the currently available monitoring techniques. In addition, even if prophylactic treatment on its own fails to eradicate these undetectable, but present malignant cells, it may help to control disease activity until the donor immune system is sufficiently reconstituted to mediate the desired GvL effect. For this reason, prophylaxis concepts after allo-SCT not only incorporating HMA but also other compounds such as tyrosine kinase inhibitors are currently tested. However, in this context some concerns should be taken into account. Generally, some patients with high-risk myeloid malignancies will not require post-transplant cytotoxic therapy to achieve

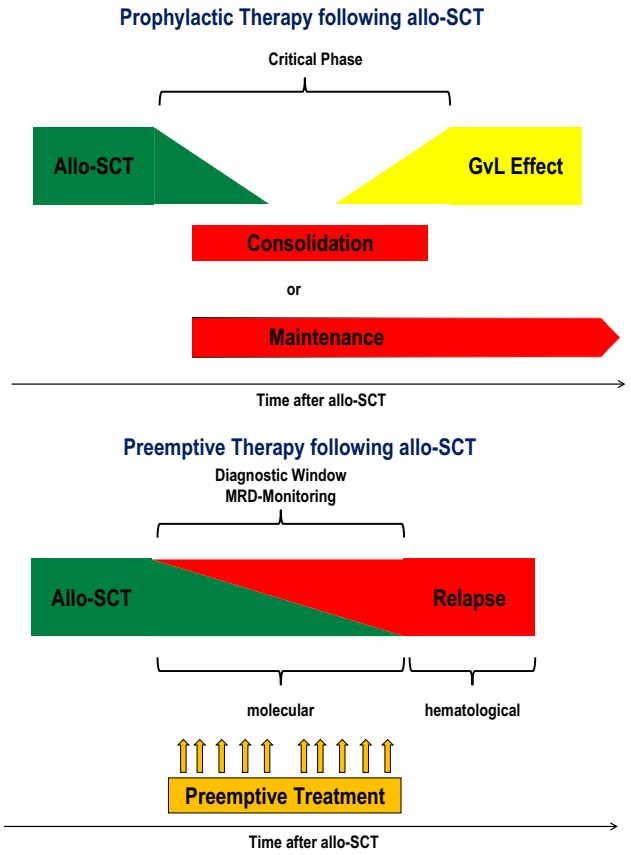

Fig. 1 Scheme of prophylactic and pre-emptive therapy

long-term cure since allo-SCT can be a potentially curative therapy already on its own. Therefore, for some patients, HMA given after transplant may represent over-treatment associated with potentially detrimental side effects such as cytopenias and infections as well as eventually even secondary malignancies in "already cured" patients.

Taking this into account, pre-emptive therapy instead of prophylactic treatment in remission may be a better strategy. Such pre-emptive approaches are initiated as soon as there is any evidence of relapse at a submicroscopical level to avoid conversion to frank haematological relapse.

Adhering to these definitions, no prospective maintenance trials have been published so far. Only one retrospective case series recently reported on a maintenance approach in 18 patients, who were envisaged to receive Aza continuously until progression or intolerability [40]. In contrast, 5 prospective single-arm studies have been performed so far investigating consolidation therapy with either Aza ($n = 3$) or DAC ($n = 2$) for patients with AML or MDS after allo-SCT [41–45]. These early-phase studies covered 130 patients and demonstrated feasibility. In addition, one aim of these studies was to identify the optimal dosage and schedule for future trials based. As most relapses occur within the first year after transplant, consolidation therapy was planned to start within the first 2–3 months after transplant in these studies (Table 2). Nevertheless, toxicity can be a relevant problem in this early phase after transplant and contributed to a treatment onset later than per-protocol in most trials. Furthermore, de Lima et al. and Craddock et al. reported a drop-of rate of 50 and 27% of enrolled patients prior to the first administration of Aza due to toxicity, patient wish or relapse [41, 42]. This indicates that the study population represents a selected group of patients. As expected from the known toxicity profile of these substances, hematotoxicity and infections were the most common adverse events related to the study drugs. Consequently, despite the use of dosages that were significantly lower than the approved dosages, only a minor fraction of patients could receive all of the envisaged treatment cycles. Taken together, these trials highlighted the potential risks of post-transplant cytotoxic therapy and the limited size of patients and the absence of a control arm do not allow a definitive ranking of efficacy and safety so far. Still, the study of de Lima et al. suggested a lower likelihood to develop chronic GvHD in patients receiving Aza maintenance therapy [42].

Finally, the Aza dose identified in the study of Lima et al. provided the basis for an ongoing randomized phase III trial investigating Azacitidine for relapse prevention after allo-SCT in patients with myeloid malignancies (NCT00887068) [42]. This trial is currently recruiting patients and 246 patients will be randomized to receive Aza or placebo for 12 months after allo-SCT. Results from this trial will hopefully elucidate the impact of Aza-based consolidation on relapse risk and GvHD.

With regard to pre-emptive therapy, the use of Aza has been tested in a prospective trial reported by Platzbecker and colleagues. Here, pre-emptive Aza therapy in 20 patients with MDS and AML was triggered by falling donor chimerism in circulating CD34+ cells. Up to 4 cycles of Aza were started as soon as the CD34+ donor chimerism dropped below a threshold of 80%, while patients were still in haematological remission. Despite an improvement of chimerism (> 80%) in half of the patients, this early intervention was able to induce durable remissions only in 3 (30%) of the responders and did not avoid progression towards haematological relapse in the majority of patients [46]. 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. Given the opportunity of sensitive methods to detect NPM1, the same group has applied this concept of pre-emptive Aza treatment also to this molecularly defined AML subgroup including 3 patients of them with MRD after allo-SCT [47].

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

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

Author	Year	Type of study	Drug	Schedule	Starting time	Duration	Patients (*n*)	Diagnosis	Overall Survival	Relapse-free survival	NRM	CIR	Acute GvHD	Chronic GvHD	References
de Lima et al.	2010	Prospective, phase I	Azacitidine	8–40 mg/m^2 for 5 days every 30 days	Day + 30 to day + 90	4 cycles	45	AML, MDS	Median 30.8 months	Not reported	9%	Not reported in detail	Not reported in detail	37%	[42]
Craddock et al.	2016	Prospective	Azacitidine	36 mg/m^2 for 5 days, every 4 weeks	Day + 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%	[41]
Pusic et al.	2015	Prospective	Decitabine	5–15 mg/m^2 for 5 days every 6 weeks	Day + 50 to day + 100	8 cycles	22	AML	56% at 2 years	48% at 2 years	Not reported in detail	28% at 2 years	41%	63%	[45]
Oshikawa et al.	2014	Prospective	Azacitidine	30 mg/m^2 for 7 days, every 4 weeks	Day + 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%	[44]
Han et al.	2015	Prospective, phase I	Decitabine	Individualised schedules	Day + 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	[43]
El-Cheikh et al.	2017	Retrospective	Azacitidine	32 mg/m^2 for 5 days, every 4 weeks	Median: day + 60	Median: 16 cycles	18	AML, MDS	70% at 1 year	63% at 1 year	Not reported in detail	Not reported in detail	11%	Not reported in detail	[40]

employ and optimize MRD-based pre-emptive therapies with HMA and other compounds in the close future.

Mode of action

To understand mechanisms by which HMA mediates its efficacy after allo-SCT animal models but also translational investigations of human samples have been exerted. In these analyses, HMA have shown to upregulate several antigens on leukemic cells in vitro and in vivo that were previously epigenetically silenced. This includes HLA epitopes, cancer testis antigens and minor histocompatibility molecules thought to render malignant cells more immunogenic toward T cell killing [48–51]. In further support of this, HMA seem to enhance T-cell mediated antitumor activity by increasing tumor-specific CD8 T cell responses against these upregulated molecules such as cancer testis antigens [52]. Another interesting mechanism seems to be the activation of endogenous retroviral elements (ERVs) through demethylation, which has been recently demonstrated in ovarian and colon cancer [53, 54]. By this 'viral mimicry', an interferon response in the tumor cells is induced resulting in an immune-mediated cancer cell killing.

Finally, cell surface expression of formerly unexpressed KIRs (killer Ig-like receptors) in natural killer (NK) cells is also modulated by HMA suggesting that interference with NK cell activity may also contribute to a HMA-mediated GvL effect [55].

The relatively infrequent and mostly mild GvHD observed in the majority of reports also supports the idea that HMA might offer immunoregulatory properties. In line with this, it was shown in mice that HMA convert conventional T cells to Tregs thereby preventing GVHD after allogeneic transplant or DLI without attenuating GVL. In these animals, HMA diminished GvHD severity and rate after allo-SCT and DLI resulted from direct suppression of T cell functionality and from conversion of allo-reactive donor T cells into Tregs (CD4+CD25+FOXP3+) through enhancement of FOXP3 expression [56, 57]. Correlating with this, Tregs seem to expand also in patients with AML and MDS during maintenance or salvage therapy after transplant [58, 59]. Taken together, these results suggest that HMA might target different immunological pathways and may hereby separate GvHD and GvL to a certain extent.

Besides these diverse immunomodulatory effects, another interesting mode of action could be related to the effects of HMA on the bone marrow microenvironment. We have recently demonstrated that mesenchymal stromal cells from patients with MDS and AML are aberrantly methylated [60–62]. Along with this, Verma et al. showed that Aza might mediate at least some of its efficacy via demethylation of the BM stroma [3].

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.

Practical issues

Results from the retrospective reports and the limited number of prospective trials have established HMA and in particular Aza as a valuable treatment alternative for patients who relapse after allo-SCT [32, 33]. Still, several questions regarding the practical use of HMA as salvage therapy after transplant have not been answered sufficiently yet. To aid clinicians in their daily practice, we here comment on some of these issues based on the current knowledge and our own experience:

Choice of HMA (Aza vs. DAC) for relapse after allo-SCT

This question has not been addressed in randomized trials so far and the literature currently covers more patients treated with Aza than with DAC. Furthermore, DAC is only licensed for patients with AML but not MDS in Europe. For these reasons, we generally consider Aza as first choice in this setting and only use DAC in patients with contraindications against Aza or in case of Aza failure. However, both HMAs can induce remissions in patients relapsing after allo-SCT and the evidence regarding the use of DAC for relapse after allo-SCT is just growing. DAC might also be an alternative for AML patients with high blast counts or rapid disease kinetics at relapse. In addition, it was recently shown that patients exhibiting a TP53 mutation had an extraordinary high response rate to DAC [63]. Although this observation needs to be confirmed prospectively, it might be worth to consider DAC in this molecularly defined patient group.

Dosing and schedule of HMA for treatment of relapse

Table 1 depicts that different schedules with daily dosages ranging from 16 to 100 mg/m^2 for 3–7 days have been used and were able to induce durable remissions. Indeed, no clear correlation between dosage and response has been found. Therefore, a definitive recommendation regarding dosage and schedule cannot be made. For patients with high leukemic burden or rapid relapse kinetics, one could assume that a higher dose might mediate a potentially stronger antileukemic effect. However, this 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 and adapt dosages during the following cycles in case of hematotoxicity. Similar to the situation in the non-transplant setting, we try to administer at least 4 cycles before a definitive evaluation of response can be made. In addition, duration of treatment is also not defined by any evidence from the literature. It remains unclear so far whether it is better to administer a definitive number of cycles or to continue until progression or intolerability as recommended in the non-transplant setting. Again, we follow the scheme of our prospective trial and administer 6–8 cycles of Aza if feasible and aim to infuse repetitive DLI until GvHD occurs. Since some of our patients experienced severe GvHD if DLI was the last intervention, we administer at least 1 cycle of Aza after the last DLI to take advantage of its assumed immunomodulatory effects.

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

In case of relapse, after allo-SCT, it is a general principle to reduce disease burden by cytotoxic therapy and to combine this with a cellular approach to reinforce an allo-immune reaction. Amongst others, this principle has been exemplified by a large retrospective EBMT analysis. Here, Schmid and colleagues clearly demonstrated that re-induction of CR by pharmacological compounds alone is not sufficient for long-term survival, but donor-cell-based consolidation is required [64].

With regard to the use of DLI and second transplant HMA, the reports published so far were heterogeneous (Table 1) and give no answer whether a combination with donor cells is required for response and long-term survival. In the recent retrospective EBMT analysis, only those 39 patients who received DLI within 2 months of commencing AZA salvage and in the absence of a clinical response were included in multivariate analysis [16]. Probably as a result of this methodological limitation, the administration of DLI had no impact on either response or on 2-year overall survival in this register-based analysis. In contrast, in our retrospective analysis, 78% of the CR were obtained after the first DLI suggesting a pronounced cell-induced immune reaction. In further support of this idea, remissions induced by Aza and DLI in our analysis were durable in 66% of patients for a median time of 20 months and lasted for a median of 13 months even in those who finally relapsed again [22]. Finally, in the study of Platzbecker et al., DLI were not part of the protocol and probably relating to this pre-emptive Aza therapy could not avoid progression to frank hematologic relapse in the majority of patients [46].

Based on these considerations, we envisage combining Aza with DLI in all patients with relapsed AML and MDS. In those patients who achieve CR after this approach, we generally do not consolidate CR with a second transplant. We consider a second transplant only in those patients where no DLI are available or in patients who fail to Aza and DLI.

Prophylactic or pre-emptive therapy with HMA?

Results from randomized trials demonstrating a benefit of a prophylactic approach with HMA either as consolidation or maintenance are lacking so far. To us, prophylactic treatment might represent over-treatment associated with relevant side effects in a relevant proportion of patients. Thus, no recommendation for a prophylactic approach can be made and patients should be treated in clinical trials.

In the future, a relevant challenge will probably be risk stratification and to tailor post-transplant treatment based on the individual risk for relapse risk. Besides known risk factors such as remission state or karyotype knowledge about specific somatic mutations will also be incorporated into such stratification algorithms. Potential candidates for this seem to be TP53 mutations, as several analyses indicated a dismal prognosis for MDS patients after allo-SCT [65–68]. The goal is to identify a patient population with an extraordinary high relapse risk for further studies to test innovative prophylactic strategies after transplant. In patients with an intermediate risk for relapse, MRD-triggered pre-emptive therapy including DLI instead of treatment in remission may be a better strategy.

Potential combination partners

Several compounds such as HDAC- and tyrosine kinase inhibitors (TKI) or the immunomodulator Lenalidomide have been tested in combination with HMA in the non-transplant setting. Unfortunately, all of them have failed to improve response rate and survival when compared to monotherapy with HMA [69]. Nevertheless, based on early positive signal from one of these trials [70] some combinations are currently also under investigation for treatment of relapse after allo-SCT. Currently, the potentially additive effect of Lenalidomide is under investigation in 2 prospective trials (VIOLA trial and NCT02472691). The rationale for this combination is a potential stimulation of the donor immune system by Lenalidomide to potentiate the Aza-mediated GvL effect.

Several TKI with inhibitory effects on internal tandem duplications (ITD) in the gene encoding for the Fms-like tyrosine-3 (FLT3) kinase receptor are currently tested in clinical trials. Midostaurin will be the first one to be approved for conventional AML therapy [71]. For this reasons, TKI with FLT3-inhibiting activity have also been tested after allo-SCT. For example, therapy with Sorafenib, a multikinase inhibitor with activity against FLT3 kinase, has demonstrated antileukemic activity with or without DLI in this situation and

can induce complete molecular remissions in some patients [72, 73]. Given the promising results of a recent phase-II trial combining Sorafenib and Aza in relapsed or refractory FLT3-ITD-mutated AML [74], we tested this combination based on an individual decision in 8 patients with relapsed FLT3+ AML after transplant. Following this combination, 4 patients achieved a complete remission (50%) with two of them remaining in remission > 1 year now without any anti-leukemic treatment [75].

The IDH2 inhibitor Enasidenib appears to be another very interesting compound for the post-transplant period. This small molecule mediates its effect rather by differentiation than by a direct cytotoxic effect against myeloid blasts and induces a promising response rate of 40% [76, 77]. Enasidenib has just recently been approved in the USA for the treatment of patients with IDH2-mutated relapsed/refractory AML. Based on its efficacy and its low toxicity, it seems reasonable to test Enasidenib as mono- or combination with HMA also in patients relapsing after allo-SCT.

Finally, there are early preliminary signals from in vitro and in vivo analyses suggesting that a combination of HMA and PD1-blocking agents may have a pathophysiological rationale in AML and MDS [78, 79]. Along with this, first reports suggest that immune checkpoint blockade may also be efficient in case of relapse after allo-SCT [80, 81].

Conclusions

HMA and in particular Aza have proven to be a valuable treatment for MDS and AML patients relapsing after allo-SCT and have consequently been integrated into clinical guidelines. To further optimize this approach, a better understanding of the underlying mechanisms and identification of target patient populations are required. Together with new pharmacological compounds, specialized cellular products and antibodies, this will hopefully help to further improve the prognosis of relapse after allo-SCT.

Compliance with ethical standards

Financial disclosure statement T S. had a consulting role for Celgene Corporation, Germany and received financial travel support and lecture fees from Celgene Corporation, Germany. T. S. also received financial travel support and lecture fees from Janssen-Cilag GmbH, Germany. C. R. received financial travel support from Celgene Corporation, Germany. R. H. and U. G. have nothing to declare. G. K. received financial travel support, research funding and lecture fees from Celgene Corporation, Germany.

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