Invited review

Minimizing risk of hypomethylating agent failure in patients with higher-risk MDS and practical management recommendations

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ABSTRACT

In Europe, azacitidine is the only hypomethylating agent approved for the treatment of patients with int-2-/high-risk myelodysplastic syndromes, offering significantly improved survival compared with conventional care. However, not all patients treated with azacitidine respond to treatment, and the vast majority of responders subsequently relapse. Currently, no standard care regimens have been established for patients after failure of azacitidine. Here, we discuss treatment options after loss of response or progression on azacitidine. In addition, we briefly consider optimization of first-line treatment along with potential biomarkers for identifying and monitoring response during treatment with azacitidine.

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1. Introduction

Myelodysplastic syndromes (MDS) are clonal hematopoietic disorders characterized by ineffective hematopoiesis, cytological dysplasia, cytopenias and an increased risk of progression to acute myeloid leukemia (AML) [1]. MDS usually affect the elderly and are often associated with diminished quality of life (QoL) and poor prognosis, especially in higher-risk cases [2]. Prognosis among patients with MDS is estimated using the International Prognostic Scoring System (IPSS) [3], which was recently revised (IPSS-R), and now considers bone marrow (BM) blast percentage, the number and severity of cytopenias and additional cytogenetic risk categories [4,5]. Without disease-modifying therapy patients with IPSS high-risk MDS generally have a life expectancy of <6 months following diagnosis [3]. Until some years ago, active treatment options for patients with high-risk MDS were limited to hematopoietic stem cell transplantation (HSCT) and chemotherapy. While HSCT remains the only potentially curative treatment in eligible candidates, it is rarely applicable to the majority (mainly due to advanced age and/or comorbidities) [6], and chemotherapy has shown limited efficacy [7,8] and should be reserved for patients with a normal karyotype. However, alternative non-aggressive treatment options that can improve outcomes in a broader range of patients are now available or are under development.

In recent years, new therapeutic strategies have been developed with the hypomethylating agents (HMAs), decitabine and azacitidine. These nucleoside analogs are both approved by the US Food and Drug Administration (FDA) for the treatment of MDS. In the confirmatory AZA-001 trial, azacitidine was shown to significantly prolong overall survival (OS) compared with conventional care regimens (CCR; 24.5 months vs. 15.0 months, respectively; $p = 0.0001$) in patients with intermediate (int)-2-/high-risk MDS or AML with 20–30% BM blasts (World Health Organization-defined AML [WHO-AML]) who achieved any kind of response, including hematologic improvement (HI) [2,9]. These results led to its approval by the European Medicines Agency in these patient subsets and in patients with chronic myelomonocytic leukemia (CMML) with >10% BM blasts [10]. As decitabine was not able to demonstrate significant survival benefits in MDS patients in Phase 3 trials, and for that reason has not received European authorization for use in MDS, azacitidine is the only approved therapy for IPSS int-2-/high-risk MDS in Europe.

Despite the efficacy and reported activity of HMAs in large numbers of patients, these agents are not curative; principal clinical studies and emerging patient registry data indicate that as many as 40% of patients do not respond to treatment or subsequently relapse (Table 1). In the AZA-001 trial which assessed patients with IPSS higher-risk MDS, 29% of patients treated with azacitidine (75 mg/m^2/day for 7 continuous days during every 28-day cycle) failed to achieve a response (complete response [CR], partial response [PR] or stable disease [SD]) and most patients who responded experienced disease progression within 2 years

[2]. Similarly, in the CALGB-8421 and -9221 studies which also assessed azacitidine (at the same dose and schedule as in AZA-001), 40–56% of patients failed to achieve a response (CR or PR or HI) [11,12]. Recently published data from the Groupe Francophone des Myélodysplasies (GFM) MDS patient registry have indicated that 40% of patients treated with azacitidine in a 'real-world' setting failed to respond (22% of patients achieved SD without HI; 18% progressed) [13]. Mechanisms of resistance to azacitidine have not been fully elucidated but may be due to intrinsic biological characteristics of the disease such as presence of specific gene mutations, e.g. *TP53*, upregulation of BCL2 family proteins, e.g. BCL2L10 [14], DNA methylation density, and differential expression of the enzyme UCK1 involved in nucleoside metabolism [15].

Once patients lose their response or progress on azacitidine, their prognosis is poor [17,18]. In a recent analysis of 435 patients with high-risk MDS and primary (no response) or secondary (loss of response) failure to azacitidine, median OS after azacitidine failure was 5.6 months and 2-year survival probability was 15% [18]. Similarly, in an analysis of 59 patients who had relapsed on or were refractory to azacitidine, median OS was 252 days and the estimated 12-month survival rate was only 20% [19]. At present, the absence of a standard salvage treatment following azacitidine failure [18] constitutes a major unmet medical need in patients with higher-risk MDS.

Given the poor outcomes in patients following failure of azacitidine, considerable efforts have been made to ensure optimal response rates with first-line therapy. Recommendations have been published elsewhere, and have highlighted, for example, the need to treat patients for at least 6 cycles or until disease progression with the approved dose and schedule (75 mg/m^2/day for 7 continuous days during every 28-day cycle) [20,21]. There has also been much research into the possibility of improving response rates or the depth and durability of response with azacitidine, by combining it with other epigenetic modifying agents such as histone deacetylase (HDAC) inhibitors, lenalidomide, *All* trans retinoic acid and other agents [22–29]. However, despite some early promising data, no combination regimens to-date have been validated in randomized clinical trials, some of which are still ongoing. At the moment, therefore, it is imperative that viable post-azacitidine salvage options are developed.

In order to gain an up-to-date perspective on the management of patients relapsed or refractory to azacitidine, a panel of international experts was convened to discuss; (1) the best current treatment approaches for patients failing azacitidine and (2) the most promising investigational agents currently under development in these patients. Given the poor prognosis of relapsed/refractory patients, the panel also discussed; (3) how best to monitor treatment to facilitate early recognition of non-responders and signs of relapse during first-line treatment with azacitidine; and (4) recent developments in biomarkers that may be able to predict and monitor response.

Table 1
Principal clinical studies of azacitidine and real-world data in patients with IPSS higher-risk MDS (azacitidine-treated patients only).

Study	Baseline characteristics	Treatment	Response	Progression/relapse	Survival
Fenaux et al. (2009) **AZA-001** [2]	• Patients, $n = 179$	• Azacitidine 75 mg/m^2/day for 7 days every 28 days (SC)	• CR: 17% • PR: 12% • SD: 42% • HI: 20%	• Time to AML progression: 17.8 months • Time to disease progression, relapse after CR or PR, and death: 14.1 months • Duration of CR, PR or HI: 13.6 months • Duration of CR + PR: 3.0 months	• OS: 24.5 months
Silverman et al. (2002) **CALGB-9221** [11]	• Patients, $n = 99$	• Azacitidine 75 mg/m^2/day for 7 days every 28 days (SC)	• CR: 7% • PR: 16% • HI: 37% • No response: 40%	• Time to AML progression or death: 21 months • Time to treatment failure: 9.1 months	• OS: 20 months
Silverman et al. (2006) **CALGB-8921** [16]	• Patients, $n = 70$	• Azacitidine 75 mg/m^2/day for 7 days every 28 days (SC)	• CR: 17% • PR: 0% • HI: 23% • No response: 60%	• NR	• NR
Silverman et al. (2006) **CALGB-8421** [16]	• Patients, $n = 48$	• Azacitidine 75 mg/m^2/day for 7 days every 28 days (IV)	• CR: 15% • PR: 2% • HI: 27% • No response: 56%	• NR	• NR
Patient registries Itzykson et al. (2011) **GFM registry** [13]	• Patients, $n = 282$	• Azacitidine 75 mg/m^2/day for 7 days every 28 days: 72% • Azacitidine <75 mg/day for 7 days every 28 days: 6% • Azacitidine 75 mg/day for 5 days every 28 days: 19% • Azacitidine <75 mg/day for 5 days every 28 days: 3%	• CR: 14% • PR: 3% • mCR: 11% • SD with HI: 15% • SD without HI: 22% PD: 18%	• Duration of CR: 10.4 months • Duration of PR: 9.8 months • Duration of mCR: 8.0 months • Duration of SD with HI: 7.9 months • Median duration of any response: 9.5 months	• OS: 13.5 months

CR, complete response; HI, hematologic improvement; IV, intravenous; mCR, marrow CR; NR, not reported; ORR, overall response rate; PD, progressive disease; PR, partial response; SC, subcutaneous; SD, stable disease; TTP, time to progression.

2. Panel recommendations for the treatment of patients with higher-risk MDS following azacitidine failure

2.1. What is the best current treatment approach in patients after azacitidine?

2.1.1. Background

Currently, there are few data regarding the treatment and outcomes of patients post-azacitidine with only one large analysis published to-date [18]. In this study, clinical outcomes were compared in 270 patients with higher-risk MDS who received HSCT ($n = 37$), intensive chemotherapy ($n = 35$), low-dose chemotherapy ($n = 32$), investigational agents ($n = 44$) or BSC ($n = 122$) following azacitidine failure. HSCT was associated with the best clinical outcomes with an overall response rate (ORR) of 68%. Median OS in these patients was 19.5 months, which was significantly greater than that observed with either best supportive care (BSC: 4.1 months; $p < 0.001$) or intensive chemotherapy (8.9 months; $p = 0.008$). Among patients who received investigational therapies (including amongst others, kinase inhibitors, epigenetic agents, lenalidomide or thalidomide) median OS was 13.2 months, which was not significantly different from HSCT results, but was significantly longer compared with all other treatment options. Response rate in patients who received intensive chemotherapy was poor at 14%, with a median OS of 8.9 months. Patients who received low-dose chemotherapy had a median OS of 7.3 months with no objective responses. Based on these data, only HSCT seems to offer the best potential for longer term disease control following azacitidine failure. Overall however, treatment outcomes post-azacitidine

remain disappointing. Although investigational therapies appear to extend survival, these results should be interpreted with caution, given the inherent selection bias introduced by entry criteria for clinical trials.

In the first-line setting, intensive chemotherapy is generally considered of limited utility in higher-risk MDS patients due to poor tolerability, limited efficacy and high risk of relapse. However, some smaller studies have assessed chemotherapy in the post-azacitidine setting. In one study, CLAG-M (cladribine, cytarabine, G-CSF, mitoxantrone; $n = 25$) and standard 3 + 7 (anthracycline and cytarabine, $n = 24$) regimens were assessed in non-randomized patients with secondary AML, arising from MDS, after failure of HMAs (azacitidine or decitabine) [30]. Median OS was significantly better in patients treated with CLAG-M compared with those who received 3 + 7 chemotherapy (202 days vs. 86 days, $p = 0.025$). Moreover, more patients in the CLAG-M cohort were able to proceed to allogeneic HSCT (28% vs. 4%; $p = 0.024$). In another retrospective study, Bello, et al. assessed intensive chemotherapy in 61 patients with secondary AML post-MDS of whom 45% had previously been treated with HMAs (azacitidine or decitabine) or lenalidomide. Among these patients, 32% achieved a CR or marrow CR (mCR) with low platelets, and median OS was only 3.7 months [31]. Thus, the role of intensive chemotherapy in the salvage setting appears to remain uncertain and requires further investigation.

2.1.2. Views and recommendations of the panel

The panel agreed that HSCT seems to offer encouraging outcomes following azacitidine therapy, with seemingly reduced impact caused by the presence of active disease at the time of

transplant. Furthermore, it was suggested that eligibility for transplantation should be assessed as early as possible before and during treatment with azacitidine. Accumulating evidence indicate that azacitidine can facilitate HSCT in some patients who were previously ineligible [32,33]. However, it was noted that only limited numbers of patients who received azacitidine are likely to be eligible for transplantation, or become fit to receive pre-transplant conditioning therapy in this setting.

There were conflicting views within the panel regarding the role of intensive chemotherapy following azacitidine failure. Some panel members reported that, in their experience, marrow regeneration is poor in patients who undergo intensive chemotherapy post-azacitidine. Furthermore, it was noted that relapsed/refractory MDS patients with a complex karyotype are unlikely to respond, as is the case in the front-line setting [34]. Therefore, some panel members recommended that intensive chemotherapy is only undertaken with a view to preparing patients for subsequent HSCT. However, sporadic good responses were reported by other members of the panel. The panel concluded that more data needs to be collected in patients who have received intensive chemotherapy as a salvage treatment. Indeed, at present, there is a lack of prospective clinical data and only limited case series reports.

2.2. What are the key investigational strategies in the post-azacitidine setting?

2.2.1. Background

Several promising investigational strategies are currently being investigated in the post-azacitidine setting. Results from early clinical trials are outlined in Table 2, and discussed briefly here.

2.2.1.1. Nucleoside analogs.
Certain novel nucleoside analogs have shown anti-leukemic activity in MDS and AML in the post-azacitidine setting. For example, clofarabine in both oral and intravenous formulations has been assessed in patients with higher-risk relapsed/refractory MDS. At doses of 15 and 30 mg/m^2 IV for 5 days, patients previously treated with HMAs ($n = 22$ and $n = 13$, respectively) achieved response rates of 15–19% [35]. Additionally, in a study of 20 patients with HMA-refractory/relapsed MDS, oral clofarabine led to a 33% response rate and appeared to be better tolerated than the IV formulation [38]. However, tolerability concerns with this agent, such as prolonged myelosuppression, and hepatic and renal toxicities remain an important barrier when considering clofarabine in this setting [35–37].

Sapacitabine, an oral deoxycytidine nucleoside analog, has also demonstrated promising activity in patients with MDS who had been previously treated with HMAs. Interim results from a Phase 2 trial in patients who were refractory to azacitidine or decitabine indicated a post-treatment ORR of 14–19%, median OS of approximately 8 months and acceptable tolerability when treated with oral sapacitabine (200 mg bid/300 mg qd for 7 days every 4 weeks or 100 mg qd for 5 days a week × 2 weeks) [39]. Based on these data, further prospective trials of sapacitabine in this setting are ongoing.

2.2.1.2. HMAs.
The HMAs azacitidine and decitabine have been assessed in several clinical trials for MDS and AML. In the first-line setting, azacitidine is the only treatment to have demonstrated a survival advantage in patients with higher-risk MDS ineligible for transplantation. However, no prospective, randomized trials of the two agents have been conducted. Currently, the only available comparisons are retrospective, observational analyses [51,52], which should be interpreted with caution given the inherent selection bias of such studies. Importantly however, although azacitidine and decitabine are both DNMT inhibitors, they have different

mechanisms of action which could facilitate sequential treatment. A key difference is that azacitidine is predominantly incorporated into RNA (65–90%) with the remainder becoming incorporated into DNA [53,54]. Decitabine, on the other hand is only incorporated into DNA [53]. Therefore, it is an oversimplification to refer to azacitidine as a DNA HMA because this description overlooks additional mechanisms of activity which affect RNA and protein metabolism. Indeed, azacitidine and decitabine appear to have different effects on cell viability, protein synthesis, the cell cycle and gene and protein expression [53,55]. Based on these differences, it was hypothesized that patients may not be cross-resistant to both agents [17]. Some very limited data from 2 small studies suggest that decitabine may have activity in some patients who have failed treatment with azacitidine. Response rates of 28% and 33%, respectively, were achieved in these studies of 14 patients each, while OS remained limited ranging from 6 to 12 months [40,42]. In addition, in a recently reported retrospective analysis, sequential therapy with decitabine after azacitidine elicited a median OS of 48 months in 10 patients with predominantly lower-risk disease [41]. Based on these results, further investigation of decitabine in this setting is warranted.

Recently, a second-generation HMA (SGI-110, a dinucleotide of decitabine and deoxyguanosine), has been developed that potentially offers improved clinical activity over decitabine based on its improved resistance to degradation by cytidine deaminase and favorable pharmacokinetic profile. In an ongoing Phase 1/2 trial of 78 patients with relapsed or refractory AML/MDS, SGI-110 yielded some responses in this heavily pre-treated population (AML: 6%; MDS: 14%) and was well tolerated at doses higher than the biologically effective dose [56]. Further clinical studies of SGI-110 in the relapsed/refractory setting are therefore warranted.

2.2.1.3. Tyrosine kinase inhibitors.
A small number of tyrosine kinase inhibitors (TKIs) are currently under investigation as post-azacitidine salvage therapy. Notably, in a recent Phase 1/2 trial of rigosertib, a dual PLK1/PI3K inhibitor, 50% of evaluable patients demonstrated either a BM or peripheral blood response following HMA failure. Median OS was approximately 10 months [44]. As a result, a Phase 3 trial comparing intravenous rigosertib with BSC in patients with higher-risk MDS refractory, relapsed or intolerant to HMAs and with excess blasts has recently been undertaken (NCT01241500) [57]. Although the primary endpoint of this trial (median OS for the whole cohort) was not met, early subanalyses indicate that a survival benefit can be seen in patients who had progressed or failed on prior HMAs who had been treated with rigosertib + BSC compared with patients who received BSC alone (8.5 months vs. 4.7 months, $p = 0.02$) [58]. However, the same improvement in OS was not seen in patients who had relapsed after initially responding to treatment with HMAs [58]. In addition to this study, a recent Phase 1 study of oral rigosertib in 37 patients with low- and high-risk MDS indicated that the drug was bioavailable, well tolerated and clinically active in patients who had received prior therapy, including HMAs [45]. Further investigation of this oral formulation is warranted.

Other agents include the epidermal growth factor receptor (EGFR) TKI erlotinib, which has demonstrated some efficacy following first-line treatment with either azacitidine or decitabine [46]. As preclinical data have indicated that the spectrum of activity of erlotinib extends to JAK2 and other kinases [59], it may synergize with HMAs. As such, it could be considered as a possible add-on therapy in patients who are losing response to azacitidine.

Tosedostat is an orally available aminopeptidase inhibitor that, similarly to TKIs, is thought to selectively block proliferation in tumor cells. Having shown some activity in a Phase 1/2 trial in AML [60], a Phase 3 randomized study is now planned in patients with higher-risk MDS and AML.

Table 2

Trials of investigational agents in the post-azacitidine setting.

Agent	Treatment schedule	Prior treatment	Patients	Key findings	Reference
Nucleoside analogs					
Clofarabine	15–30 mg/m^2 for 5 days every 4–8 weeks (IV)	Azacitidine or decitabine	35 patients with high-risk MDS or sAML (interim analysis)	• 6/35 patients (17%) achieved a response • Significant myelosupression and toxicity	Faderl et al. (2012) [35]
Clofarabine	5–10 mg/m^2 for 5 days every 4–8 weeks (IV)	Azacitidine ± decitabine or lenalidomide	9 evaluable patients with MDS	• CR/PR: 22% • HI: 22% • Severe and prolonged pancytopenia • Median duration of any response: 12 months	Lim et al. (2010) [36]
Clofarabine	5–10 mg/m^2 for 5 days every 4–8 weeks (IV)	Azacitidine	19 patients with higher-risk MDS or AML	• CR/PR: 11% • mCR: 16% • HI-any: 5 • 7 patients hospitalised due to fever or bleeding	Braun et al. (2011) [37]
Clofarabine	20–40 mg/m^2 for 5 days every 4–8 weeks (oral)	Azacitidine or decitabine	20 evaluable patients with higher-risk MDS, AML or CMML	• CR: 10% • HI: 10% • Myelosuppression was common	Faderl et al. (2010) [38]
Sapacitabine	100 mg/day every 5 days for 2 weeks or 300–400 mg/day every 7 days for 2 weeks	Azacitidine or decitabine	61 patients with int-2 or high-risk MDS (interim analysis)	• CR/CRp: 7% • HI: 7% • SD: 10% • Well tolerated	Garcia-Manero et al. (2012) [39]
Hypomethylating agents					
Decitabine	20 mg/m^2 for 5 days every 28-day cycle	Azacitidine	14 patients with MDS (interim analysis)	• CR: 21% • HI-P: 7% • SD: 36% • Non-hematologic grade 3–4 AEs: 29% • Febrile neutropenia: 33%	Borthakur et al. (2008) [40]
Decitabine	NR	Azacitidine	21 evaluable patients with MDS	• mCR: 10% • Median OS from diagnosis: 48 months	Komrokji et al. (2013) [41]
Decitabine	NR	Azacitidine	10 evaluable patients with high-risk MDS or AML	• 0/10 patients achieved a response • Median OS: 11.8 months	Prebet et al. (2011) [18]
Decitabine ± gemtuzumab ozogamicin (GO)	Decitabine: 20 mg/m^2 for 5 days every 28-day cycle GO: 3 mg/m^2 on days 5 and 9 of every 28-day cycle	Azacitidine	9 patients with MDS, CMML or AML	• CR + PR + HI: 33% • Median OS in refractory patients: 2 months • Median OS in relapsed patients: 4 months • Two patients hospitalised due to sepsis	Sanna et al. (2011) [42]
SGI-110	Phase I dose escalation study (SC administration)	Azacitidine or decitabine	15 evaluable patients with MDS	• mCR: 13% • HI-any: 27% • Generally well tolerated	Kantarjian et al. (2012) [43]

Table 2 (*Continued*)

Agent	Treatment schedule	Prior treatment	Patients	Key findings	Reference
Tyrosine kinase inhibitors					
Rigosertib	1375 mg/m^2/day IV infusion for 72 h every 2 weeks	Azacitidine or decitabine	18 evaluable patients with MDS, AML or CMML	• mCR: 22% • HI: 11% • SD: 17% • Median OS in responders: 10.1 months • Median OS in non-responders: 2 months	Navada et al. (2012) [44]
BSC ± rigosertib	Rigosertib: 1800 mg/m^2/day IV infusion for 72 h every 2 weeks (1st 8 cycles), every 4 weeks thereafter BSC: schedule not specified	Azacitidine or decitabine	299 patients with higher-risk MDS (5–30% BM blasts)	• Primary endpoint not met • Median OS for rigosertib + BSC vs. BSC alone: 8.2 months vs. 5.8 months (p = 0.27) • Median OS for rigosertib + BSC vs. BSC alone in primary non-responders to HMAs: 8.5 months vs. 4.7 months (p = 0.02) • Generally well tolerated	NCT01241500 [48] Onoconova press release, February 2014 [49]
Rigosertib	Phase I dose escalation study (PO administration)	Azacitidine, decitabine, lenalidomide or ESA	37 patients with MDS	• mCR: 5% • TTP in IPSS higher-risk patients: 16 weeks	Komrokji et al. (2013) [45]
Erlotinib	150 mg/day for 16 weeks	Azacitidine or decitabine	35 evaluable patients with MDS	• mCR: 9% • HI: 6% • SD: 31% • Median OS: 6.8 months • Manageable toxicity	Komrokji et al. (2011) [46]
HDAC inhibitors					
Vorinostat + LDAC	Vorinostat: 400 mg/day for 7, 10 or 14 days LDAC: 10–20 mg/m^2/day for 14 days	Azacitidine or decitabine	35 evaluable patients with int-2 or high-risk MDS	• CRi/mCR: 11% • HI: 1% • Median duration of response: 3 months (range 2–6) • Median OS: 9.2 months	Prebet et al. (2012) [47]
Panobinostat	30 mg three times weekly	Azacitidine or decitabine	10 patients with MDS (interim analysis)	• SD: 70% • Three patients experienced SAEs • Well tolerated	Flinn et al. (2010) [48]
Immunomodulatory agents					
Lenalidomide	5–10 mg/day for 21 days every 28-day cycle	Azacitidine	10 patients with MDS or AML	• CR: 30% • HI-E: 10% • Median duration of response: 6 months • Median OS post-AZA: 19.5 months	Prebet et al. (2012) [49]
Lenalidomide + azacitidine	10 mg/day for 21 days every 28-day cycle	Azacitidine	3 patients with higher-risk MDS	• All 3 patients achieved a CR • Duration of CR: 5–7+ months • Well tolerated	Sekeres et al. (2011) [50]

AEs, adverse events; AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; CR, complete response; HI, hematologic improvement; HI-E, hematologic improvement-erythroid; HI-P, hematologic improvement-platelet; IV, intravenous; LDAC, low dose cytarabine; mCR, marrow complete response MDS, myelodysplastic syndromes; NR, not reported; OS, overall survival; PR, partial response; SAE, serious adverse event; sAML, secondary AML; SD, stable disease; TTP, time to progression.

2.2.1.4. HDAC inhibitors. As well as first-line combination regimens mentioned previously, some recent studies have assessed the activity of HDAC inhibitors in the salvage setting. For example, a Phase 2 trial has indicated a possible role for vorinostat combined with low-dose chemotherapy in MDS patients who have failed on azacitidine [47]. Panobinostat may also be a useful combination agent in the salvage setting, but is yet to demonstrate a significant clinical benefit in first-line treatment [48].

2.2.1.5. Immunomodulatory agents. It was recently hypothesized that lenalidomide may have activity in the post-azacitidine setting given the two agents' different mechanisms of action. Although the precise molecular rationale for lenalidomide in this setting is yet to be elucidated, there is empirical evidence that it has clinical activity post-azacitidine [49,50]. In a retrospective analysis of 10 patients (low-risk and high-risk MDS) who received at least 1 cycle of lenalidomide (5 or 10 mg/day [both *n* = 5] for 21 days every 28 days) post-azacitidine, an ORR of 40% and median OS of 19.5 months was reported [49]. One may speculate that the antiproliferative effects of azacitidine may be complemented by the immunomodulatory effects of lenalidomide on the BM microenvironment [27,50].

2.2.2. Views and recommendations of the panel

Overall, the panel agreed that despite intensive and ongoing evaluation of investigational agents in the post-azacitidine setting, no convincing data currently exist to inform treatment decisions. Furthermore, in the absence of prospective comparative trials, panel members stated that it is currently difficult to compare the relative merits of investigative salvage treatments. Therefore, there is a clear and urgent need for additional clinical trials that assess novel salvage therapies as well as potential add-on strategies to first-line azacitidine. Consequently, all panel members recommended that patients who have failed azacitidine therapy (and are not eligible for HSCT) should be enrolled in a clinical trial. Indeed, as shown previously, treatment with experimental agents may offer the prospect for better survival than conventional chemotherapy or BSC [18].

Panel members are personally engaged in a number of clinical trials of investigational agents and regimens including rigosertib, erlotinib and clofarabine. Some panel members have also used lenalidomide in patients who have failed on azacitidine therapy. Discussing lenalidomide in a salvage setting, the panel agreed that prospective studies in post-azacitidine patients were warranted, particularly in patients who have a chromosome 5q deletion, either as an isolated aberration or as part of a complex karyotype, but that at present it cannot be recommended for routine use. Finally, the panel agreed that HDAC inhibitors, decitabine and potentially SGI-110 warrant further investigation in the post-azacitidine setting.

3. Panel recommendations for monitoring patients during treatment with azacitidine

Despite ongoing clinical investigations, no clear salvage strategies in patients with relapsed/refractory MDS who have failed treatment with azacitidine are currently available. Consequently, appropriate selection of patients for azacitidine in first-line therapy is of paramount importance. To this end, the French Prognostic Score (FPS) has been developed, based on performance status, presence or absence of circulating blasts and red blood cell (RBC) transfusion requirement, that categorizes prognosis in patients treated with azacitidine [13]. As well as ensuring that patients most likely to benefit from azacitidine receive it, it is important that patients are monitored for early signs of relapse, particularly in those patients who are candidates for stem cell transplant, given

the limited salvage options. Discussing appropriate monitoring of patients, the panel considered the following questions.

3.1. When monitoring patients on azacitidine, how do you distinguish between peripheral blood (PB) fluctuations (during response) and a true loss of response, and when should the BM be assessed?

3.1.1. Background

Exacerbation of cytopenias and myelosuppression-related complications are not unexpected in patients treated with azacitidine and may lead initially to deterioration of the clinical condition of patients, especially during early treatment cycles. In the AZA-001 trial, anemia, neutropenia and thrombocytopenia were observed in 51.4%, 65.7% and 69.7% of patients treated with azacitidine, respectively [21]. Proportionately more patients experienced hematologic adverse events (AEs) during the first 2 cycles of azacitidine compared with later cycles [21]. Therefore, reductions in PB counts should not necessarily be considered as evidence of primary or secondary treatment failure, particularly when noted during early treatment cycles.

3.1.2. Views and recommendations of the panel

In terms of monitoring patients for response, the panel agreed that the most important parameter for assessing patients during the first 6 cycles was regular assessment of PB counts rather than BM assessment, given the correlation of PB counts with patients' prognosis, QoL and overall wellbeing. BM assessment should be undertaken, only after prior analysis of the PB, and after at least 6 cycles of azacitidine. In addition, the panel recommended that BM analysis be performed in patients who appear to be primary non-responders in the PB in order to confirm that there is no existing or imminent response, and in secondary non-responders (i.e. patients who had relapsed on azacitidine), to determine the cause of cytopenia, e.g. infection, loss of cellularity, drug-related toxicity.

Regarding fluctuations in PB counts, rapid changes, especially in early cycles, may be due to transient toxic effects of the drug rather than disease progression. It is important therefore that drug-related cytopenias are managed during treatment, without dose reductions/delays if at all possible so as to maximize the chances of response to azacitidine [21]. In contrast, loss of hematologic response to azacitidine tends to be gradual and occur during later cycles after a response has been achieved. As such, reappearance of RBC-transfusion dependence (RBC-TD) in a patient who had previously achieved RBC-transfusion independence (RBC-TI) is a likely sign of impending relapse. To identify loss of response, all lineages should be assessed over time to gauge an overall impression of trends. Consequently, the panel agreed that the most important indicators of loss of response to azacitidine and/or progression to AML were deteriorating cytopenias (which may take several successive measurements to confirm) [61], an increase or recurrence in transfusion requirements or emergence of circulating blasts.

Continuing their discussion the panel suggested that the following observations could indicate relapse: a progressive decline in PB counts over time that do not return to baseline levels; unexpected changes in established, stable blood counts (after a hematologic response is achieved); and a gradual decrease in platelet count with or without an accompanying increase in PB blast count. Platelet levels were considered a particularly sensitive parameter to monitor, given their shorter circulation time compared with RBCs. Consequently, reduced platelet counts are likely to be one of the earliest indicators of pending disease relapse.

Finally, when assessing response and improvement of response in patients treated with azacitidine in clinical trials, some panel members highlighted that it is important to remember that most of the principal Phases 2 and 3 trials were conducted using

Table 3
Emerging predictive factors of response to azacitidine.

Clinical factors	
Positive	Negative
Doubling of platelet count after first cycle [70]	Grade 3 BM fibrosis [72]
	ECOG PS > 2 [13]
Reduction in leukemic stem cells during treatment [71]	Circulating blasts [13]
	BM blasts >15% [13]
	Transfusion dependence [13]
	Previous therapy [13]
Molecular/cytogenetic factors	
Positive	Negative
Mutations in TET2 [65,73]	Mutations in TP53 [74]
Mutations in EZH2 [73,75]	Abnormal/complex karyotype [13]
Decreased methylation of the promoter region of PI-PLCbeta1 [69]	Expression levels of BCL2L10 [14]

BM, bone marrow; ECOG PS, Eastern Cooperative Oncology Group performance status.

standardized International Working Group (IWG) 2000 criteria to assess response [62]. More recently however, some trials and patient registries are now using the updated IWG 2006 criteria [63]. Changes between the two sets of criteria, including modified definitions of HI and mCR, need to be taken into account when comparing studies.

3.2. Are there any biomarkers available to monitor response, or predict relapse, to azacitidine?

3.2.1. Background

Further research is required to identify potential markers that can effectively predict response and loss of response to azacitidine. Current promising baseline markers include; mutations of *EZH2* [64], *TET2* [65], *DNMT3A* and *IDH1/IDH2* [66], *BCL2L10* gene or protein expression levels [14], methylation status of *CDKN2B* [67], serum ferritin level [68], levels of expression of UCK1 enzyme [15]. Other promising biomarkers for the monitoring of response to azacitidine during treatment include methylation status of phosphoinositide-phospholipase C beta 1 [69], increase in platelet levels after 1–2 cycles of therapy [18,70], and assessment of leukemic stem cell numbers using flow cytometry as a means of predicting relapse (Table 3) [71].

3.2.2. Views and recommendations of the panel

The panel noted that specific biomarkers that might predict response/relapse to azacitidine are yet to be validated in a prospective study, and as a result, none can be recommended at this time. Promising biomarkers should be tested prospectively in large clinical trials.

3.3. Are there any factors that may contribute to relapse that might be prevented?

3.3.1. Background

Several preventable factors have been postulated to increase the likelihood of relapse in patients treated with azacitidine. These include early treatment withdrawal and potentially the concomitant use of agents such as hydroxyurea.

Although treatment with azacitidine should be discontinued if there is evidence of relapse, it is possible that long-term clinical outcomes may be compromised in patients who discontinue azacitidine prematurely. For example, in a recent case series of 13 patients with MDS who discontinued treatment with azacitidine

while in hematologic remission, 77% relapsed within 6 months [76]. Moreover, recent multivariate analysis of the AZA-001 trial has demonstrated that patients who achieve HI with azacitidine, but do not achieve CR or PR, are also afforded a survival advantage compared with patients with progressive disease [77]. Notably however, prolongation of OS in patients who achieved SD was seen in both treatment arms [77].

These observations emphasize the importance of routinely treating patients until overt disease progression, and AEs should be managed aggressively whenever possible. Furthermore, it is noteworthy that there are no data indicating transformation potential of prolonged exposure to azacitidine, nor reports of secondary tumors in the post-marketing evaluation.

Preclinical studies have demonstrated that hydroxyurea blocks the conversion of azacitidine to deoxycytidine to inhibit DNA methylation [78]. This relates to the fact that hydroxyurea is a ribonucleotide reductase inhibitor that is necessary for azacitidine to influence genomic methylation. As a result, in addition to its antiproliferative effects, hydroxyurea could be antagonistic to azacitidine. However, as the activity of azacitidine is also attributable to its incorporation into RNA as well as DNA [53], its activity may only be partially inhibited by interaction with hydroxyurea. Nevertheless, in the absence of any clear clinical demonstration of compatibility, hydroxyurea and azacitidine should be used sequentially rather than concomitantly.

It should also be noted that, to-date, survival benefits with azacitidine have only been demonstrated with the approved dose and schedule of 75 mg/m^2 subcutaneous injection for 7 continuous days every 28 days. Therefore, if possible, this schedule should constitute the preferred treatment for patients with MDS. However, due to impracticalities of this schedule (i.e. weekends are problematic in most healthcare systems), there is an ongoing need for collecting efficacy data from alternative treatment schedules (e.g. 100 mg/m^2 for 5 continuous days, 75 mg/m^2 on a 5–2–2 schedule, 75 mg/m^2 on 5 consecutive days, or intravenous vs. subcutaneous administration).

3.3.2. Views and recommendations of the panel

There was consensus among the panel that in order to minimize risk of relapse, azacitidine should not be discontinued, if at all possible, until overt disease progression. Some panel members reported that in their experience, treatment discontinuation in responding patients could lead to rapid loss of response and disease progression. Moreover, they highlighted that patients who prematurely discontinue azacitidine treatment are unlikely to respond if treatment is resumed.

The panel agreed that the most common reason for discontinuation of azacitidine during the first few cycles of treatment is myelosuppression, particularly neutropenia. Although most panel members routinely manage neutropenia with myeloid growth factors, they did not think that this was always the case with referring hematologists. Although some panel members restrict the use of growth factor to patients with febrile neutropenia, the consensus was that growth factors should be used in all cases of prolonged severe neutropenia (ANC < 500 cells/μL). This is despite early data that suggest an increased risk of clonal evolution in patients with monosomy 7 treated with myeloid growth factors [79]. In addition, the panel suggested growth factors could be used in patients at greatest risk for infection, for example those with diabetes, old age or chronic pulmonary disease. Furthermore, it is important that growth factor administration is sequenced separately from azacitidine to avoid enhanced uptake of the drug as a result of S phase recruitment. Given the lack of any formal demonstration of efficacy, there was no consensus amongst the panel regarding the use of prophylactic antibiotics and antifungals in severely neutropenic patients. Some panel members routinely use antibiotic prophylaxis

in patients with MDS as well as AML. However, emerging data indicate that patients with unfavorable cytogenetics and low platelet counts are at high risk of infection and prophylaxis may only be particularly appropriate in such cases [80].

The panel also discussed patient-related factors that may result in premature discontinuation of treatment. Understandably, a frequent cause of cycle delays and treatment discontinuation is the inconvenience of repeated injections over 7 days every month in an outpatient setting. To address this, development of at-home nursing strategies, combined with a possible oral formulation of azacitidine will be of particular use for the long-term management of patients with MDS. It is also important that physicians make their patients fully aware of the likely consequences of treatment termination.

Finally, the panel debated the role of treatment delays in the management of AEs. They highlighted that there remains a lack of consensus among practicing hematologists regarding whether treatment delay or dose reduction is the best initial approach to manage AEs. In general, physicians seem to rely on personal experience and preference [20,21]. Members of the panel were uncomfortable with reducing dose, owing to a fear of losing response (although this is not based on prospective clinical data); therefore, as a first option, they prefer extending the interval between cycles, but only to a maximum of 6 weeks.

4. Conclusions

There is a clear unmet clinical need for effective treatments for patients who have failed azacitidine therapy. Although allogeneic HSCT seems to be associated with encouraging outcomes, few post-azacitidine patients are eligible. Treatment with azacitidine may facilitate allogeneic HSCT in patients who were previously ineligible. While intensive chemotherapy may benefit a small minority of patients (e.g. patients undergoing subsequent HSCT), more data are required to assess its role in a salvage setting. There is considerable ongoing research with investigational agents following azacitidine failure, but, at present, there are no final and convincing data to guide treatment decisions. Therefore, the panel strongly recommended that patients who have failed azacitidine therapy (and are not eligible for HSCT and/or intensive chemotherapy) should be enrolled in a clinical trial.

According to the panel recommendations, when azacitidine is prescribed, responding patients should be closely monitored through evaluation of PB counts. The most important indicators of imminent loss of response to azacitidine and/or progression to AML are a clear trend toward deteriorating cytopenias and/or reappearance of RBC-TD in patients who were previously RBC-TI. Of note, deteriorating cytopenias may take successive measurements over several cycles to confirm due to fluctuations in PB counts commonly observed during treatment with azacitidine. Finally, early discontinuation of azacitidine (e.g. due to myelosuppression, other AEs or patient request) should be avoided if at all possible due to the increased probability of relapse. Treatment delays and supportive therapies (e.g. myeloid growth factors) are recommended in order to manage AEs, and avoid dose reductions or treatment discontinuation.

In summary, until effective post-azacitidine treatment options are identified and supported by prospective clinical data, current recommendations should be considered when treating with azacitidine. This should ensure maximum clinical benefit is obtained for patients, who face a poor prognosis should they fail to respond or lose response to azacitidine.

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