5-azacitidine prolongs overall survival in patients with myelodysplastic syndrome - a systematic review and meta-analysis

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ABSTRACT

Hypomethylating agents have recently been shown to improve the outcome of patients with myelodysplastic syndrome. A meta-analysis and systematic review was carried out of randomized controlled trials comparing treatment with hypomethylating agents to conventional care, i.e., best supportive care or chemotherapy, in patients with myelodysplastic syndrome. The outcomes assessed were overall survival, time to transformation or death, overall response rate and toxicity. Hazard ratios with 95% confidence intervals were estimated and pooled for time-to-event data. For dichotomous data, relative risks were estimated and pooled. Four trials including 952 patients examined the effect of 5-azacitidine and decitabine. Treatment with hypomethylating agents significantly improved overall survival (hazard ratio 0.72, 95% confidence interval 0.60-0.85, three trials) and time to transformation or death (hazard ratio 0.69, 95% confidence interval 0.58- 0.82, four trials). In a subgroup analysis per type of drug, these benefits could be shown for 5-azacitidine but not for decitabine. Both agents favorably influenced response rates. A higher rate of grade 3/4 adverse events was observed with their use. Since 5-azacitidine prolongs overall survival and time to transformation or death it should be highly considered in the treatment of patients with high-risk myelodysplastic syndrome. Further studies are needed to establish the exact role of decitabine compared to 5-azacitidine in these patients.

Key words: myelodysplastic syndrome, hypomethylating agents, 5-azacitidine, decitabine.

Citation: Gurion R, Vidal L, Gafter-Gvili A, Belnik Y, Yeshurun M, Raanani P, and Shpilberg O. 5-azacitidine prolongs overall survival in patients with myelodysplastic syndrome - a systematic review and meta-analysis. Haematologica. 2010; 95:303-310. doi:10.3324/haematol.2009.010611

Introduction

Patients with intermediate-2 and high-risk myelodysplastic syndrome (MDS) have a survival rate of 0.4 to 1.2 years as well as a high risk of their disease progressing to acute myeloid leukemia (AML).[1] The only treatment with a curative potential is allogeneic stem cell transplantation. However, in the majority of patients, this treatment is not applicable, mainly due to the age of the recipients and co-morbid conditions. *Best supportive care* consisting of blood product transfusions and antibiotics has been the most frequently administered treatment for MDS patients until recently.[2]

Lately, several new treatments including immunomodulatory agents, histone deacetylase inhibitors and DNA methyltransferase inhibitors (hypomethylating agents) have emerged as options for the treatment of patients with MDS.[2] Hypomethylating agents, 5-azacitidine and 5-aza-2'-deoxycitidine (decitabine) are nucleoside analogs that covalently bind to the DNA methyltransferases, irreversibly inhibiting their function, leading to the progressive loss of methylation and reversal of gene silencing. This results in gene expression and in differentiation of myeloid cells.[3] In addition to their differentiation-inducing activity, these agents also have direct cytotoxic effects.[4]

In a number of phase 2 trials 5-azacitidine and decitabine given to patients with MDS resulted in an overall response rate of 50%.[5-8] This led to the initiation of phase 3 trials comparing 5-azacitidine or decitabine to best supportive care. Although all trials showed complete and partial response rates of about 15-20%, results regarding time to leukemic transformation or death and overall survival were not consistent.[9,10]

We, therefore, conducted a systematic review and meta-analysis in order to assess the role of hypomethylating agents in patients with MDS and specifically to elucidate whether these agents offer a survival advantage over conventional care.

Design and Methods

Data sources

We searched PubMed (January 1966 to March 2009), the Cochrane

**The authors Ronit Gurion and Liat Vidal contributed equally to this manuscript.*
Manuscript received April 27, 2009; revised version arrived July 4, 2009; manuscript accepted August 5, 2009.
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Library (issue 3/2008), LILACS (up to March 2009) and the following conference proceedings for trials in hematology (2002-2008): Annual Meetings of the American Society of Hematology, European Group for Bone and Marrow Transplantation, Annual Meetings of the European Hematology Association, Annual Meetings of the Society for Hematology and Stem Cells and the Annual Meetings of the American Society of Clinical Oncology. In addition we searched databases of ongoing and unpublished trials: *http://www.controlled-trials.com*, *http://www.clinicaltrials.gov/ct* and *http://clinicaltrials.nci.nih.gov*.

We used the following search terms: (myelodysplastic OR MDS) AND (decitabine OR azacitidine OR deoxycytidine OR hypomethylating OR dacogen OR 5 azacytidine OR 5-aza 2' deoxycytidine OR vidaza OR DNA methyltransferase inhibitors). For PubMed, we added the Cochrane highly sensitive search term for identification of clinical trials.[11] We scanned the references of all included studies and reviews identified for additional trials that did not come up in our search.

Study selection

We included all randomized, controlled trials comparing hypomethylating agents (5-azacitidine, decitabine) to conventional care in patients with MDS. We included trials regardless of publication status, date of publication and language. Two reviewers (*RG, LV*) screened all references identified through our search strategy and applied inclusion criteria. For possibly relevant articles or in the event of disagreement between the two reviewers, we obtained and independently inspected the full text article.

Data extraction and quality assessment

Two reviewers (*RG, LV*) independently extracted data from the included trials. In the event of disagreement between the two reviewers, a third reviewer (*AG*) extracted the data and results were attained by consensus. We contacted the authors of trials for missing data when necessary. We assessed allocation concealment, allocation generation and blinding and graded allocation concealment and generation as adequate, unclear, inadequate or not used according to the criteria specified in the Cochrane Handbook.[11]

Definition of outcomes

The primary outcome was overall survival at the end of the study period. Secondary outcomes included early mortality at 3 months, treatment-related mortality, time to transformation to AML or death, hematologic response (overall, complete, partial, improved) as defined by International Working Group response criteria,[12] freedom from red blood cell (RBC) transfusions and adverse events.

Data synthesis and analysis

For each trial, results were expressed as relative risks (RR) with 95% confidence intervals (CI) for dichotomous data; a RR greater than 1 favors the control. Time-to-event outcomes were analyzed as hazard ratios (HR) and their variances as described by Parmar *et al.*[13] and pooled according to Peto's method [Review Manager (RevMan), version 4.2 for Windows; the Cochrane Collaboration, Oxford, UK].

We assessed heterogeneity in the results of the trials using a χ^2-squared test of heterogeneity and the I^2 measure of inconsistency. We conducted meta-analysis using a fixed-effect model. In case of significant heterogeneity ($P<0.10$ or $I^2>50\%$), for dichotomous data meta-analysis, we used a random-effects model (the DerSimonian and Laird method).

We performed sensitivity analyses to assess the effect of individual methodological quality measures on effect estimates, including allocation generation, concealment and blinding. We assessed the effect of the type of drug (5-azacitidine and decitabine) on the overall effect through mixed effect meta-regression (Comprehensive Meta-Analysis 2.2).

Results

The computerized search strategy identified 271 trials, 20 of which were considered relevant for this review. In addition one abstract from a conference proceeding was also relevant. Sixteen of the identified trials were excluded for various reasons (Figure 1).[5,8,14-27] Of the five publications considered relevant for the meta-analysis, two reported different outcomes on the same trial, of which only one was relevant. Thus, four trials including 952 patients and performed between the years 2002 and 2008 fulfilled the inclusion criteria (Table 1).[9-10,28,29]

Two trials examined the effect of 5-azacitidine[10,29] and two evaluated decitabine.[9,28] The control arm consisted of best supportive care in three trials[9-10,28] and of either best supportive care, low dose cytarabine or intensive chemotherapy in one trial.[29]

The medians of the patients' age ranged between 67 and 70 years. In three trials most patients (>70%) were considered to have high-risk MDS according to the International Prognostic Scoring system (IPSS). In one trial the IPSS was calculated for less than 50% of the patients, half of whom were considered high-risk patients.[10] Table 2 presents the definitions of response criteria for each trial.

Primary outcome

Data from three trials including 782 patients were available for the analysis of overall survival.[10,28,29] Treatment with hypomethylating agents significantly improved overall survival (HR 0.72, 95% CI 0.60 to 0.85) (Figure 2). One trial could not be included in the analysis of overall survival since it did not report sufficient data. This trial reported that median survival was not significantly different between the two arms.[9]

When overall survival was analyzed per type of drug, there was an advantage for 5-azacitidine (HR 0.67, 95% CI 0.54 to 0.83; two trials, 549 patients). This survival benefit could not be shown for decitabine (HR 0.88, 95% CI 0.66 to 1.17; one trial, 233 patients). Hypomethylating agents were advantageous over both best supportive care and low dose cytarabine in terms of overall survival (HR 0.77, 95% CI 0.64 to 0.92; three trials, 646 patients and HR 0.38, 95% CI 0.22 to 0.66; one trial, 94 patients), respectively. However, when compared to intensive chemotherapy, there was no difference in overall survival (HR 0.76, 95% CI 0.34 to 1.71; one trial, 42 patients) (Figure 2).

Quality of allocation concealment (adequate and unclear) did not have a significant impact on the results for overall survival. Trials with adequate allocation concealment had a HR of 0.72 (95% CI 0.58 to 0.88; two trials).[9,28,29]

Secondary outcomes

There was no difference in early mortality, at 3 months, between patients treated with hypomethylating agents and those managed with conventional care (RR 0.99, 95% CI 0.72 to 1.37; four trials). However, treatment-related mortality was significantly higher among the patients treated with hypomethylating agents than among those treated with conventional care (RR 7.27, 95% CI 1.67-31.64; three trials).

Hypomethylating agents prolonged time to AML transformation or death compared to conventional care (HR 0.69, 95% CI 0.58 to 0.82; four trials). When time to AML or death was analyzed per type of drug, there was an advantage for 5-azacitidine (HR 0.54 95% CI 0.42 to 0.70; two trials). This advantage could not be shown for decitabine (HR 0.85 95% CI 0.66 to 1.07; two trials) (Figure 3).

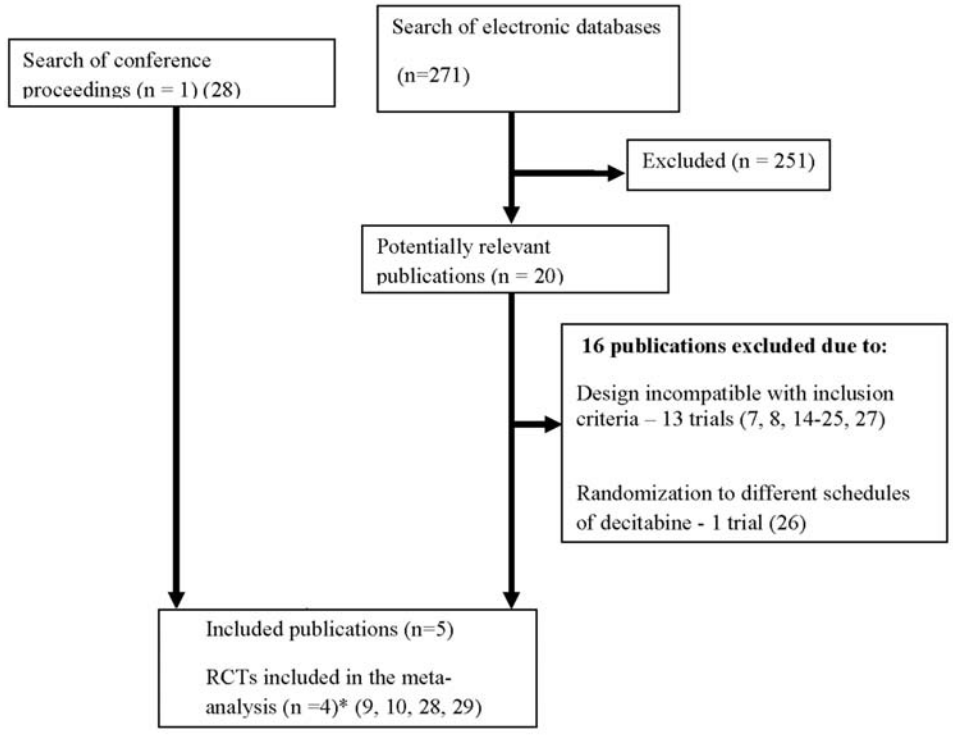
Hypomethylating agents improved the rates of complete response (RR 7.63, 95% CI 1.41 to 41.17; four trials, random effects model), partial response (RR 6.01, 95% CI 2.93 to 12.32; four trials), hematologic improvement (RR 3.06, 95% CI 1.09 to 8.6; four trials, random effects model) and overall response (including all the above) (RR 5.72, 95% CI 1.60 to 20.39; four trials, random effects model) (Figure 4). Response rates were not affected by the type of drug.

There was no difference in freedom from RBC transfusions between the patients treated with hypomethylating agents and those managed with conventional care (RR 10.65, 95% CI 0.29 to 388.82; two trials, random effects model). Treatment with hypomethylating agents was associated with a significantly higher rate of grade 3/4 adverse events (RR 1.21, 95% CI 1.10 to 1.33; three trials), most of which were hematologic effects – mainly neutropenia and thrombocytopenia. The type of drug did not affect the risk of grade 3/4 adverse events. The rate of febrile neutropenia was also significantly higher among the patients treated with hypomethylating agents (RR 8.93, 95% CI 1.29 to 62.07; two trials, random effects model).

Discussion

Our systematic review demonstrates that, compared to conventional care, treatment with hypomethylating agents and specifically 5-azacitidine, prolongs overall survival and time to AML transformation or death, despite increased treatment-related mortality and lack of difference in early mortality. Treatment with both 5-azacitidine and decitabine improves the rate of complete response, partial response, hematologic improvement and overall response. Nevertheless, it is associated with a significantly higher rate of adverse events.

Current guidelines recommend the use of hypomethylating agents (e.g., 5-azacitidine or decitabine) in patients with MDS who are classified as high-risk according to the IPSS and who are not candidates for intensive chemotherapy.[30] The improvement in overall survival and time to AML transformation or death with 5-azacitidine shown in



*Two publications reported different outcomes on the same trial, only one of which was relevant for the meta-analysis

Figure 1. Flow diagram: publications identified for study and exclusions.

our review support these recommendations. A recent meta-analysis published as an abstract also supports our results.[31]

The overall survival advantage with 5-azacitidine was achieved despite the higher toxicity profile of this drug and treatment-related mortality. It might be that with further improvement in supportive care techniques during treatment, the substantial survival advantage will improve even further. Despite the higher treatment-related mortality, early mortality was not affected. This might be because the detrimental treatment-related mortality during the treatment period is counterbalanced by the favorable effect on disease-related mortality. It should be noted that the effect of an improvement in overall survival seen with 5-azacitidine was not established for decitabine. This might be explained, at least partially, by the limited number of trials and patients treated with decitabine. The effect of decitabine on overall survival must, therefore, be interpreted with caution.

A subgroup analysis according to the type of drug showed a difference between the two agents regarding time to AML transformation or death in favor of 5-azacitidine. This might have contributed to the difference in their impact on overall survival. Other factors could account for the difference in outcomes between the two agents. Both drugs are chemically similar, decitabine being the deoxy derivative of 5-azacitidine, and 10-fold more potent than the latter.[3] However, our analysis showed an inferiority of decitabine; this might be related to the different durations of treatment with the two agents. Studies conducted in cell lines showed that re-methylation occurs shortly after withdrawal of hypomethylating agents, making it reasonable to believe that this treatment should be administered continuously for longer periods.[32] Decitabine was administered for a median of only three to four cycles as compared to nine cycles for 5-azacitidine. Furthermore,

Table 1. Characteristics of included trials.

Study year, [ref]	Intervention (Type of HA, dose, schedule)	N. of pts.	Age (years.) median (range)	N. of pts. according to FAB classification	N. (%) of pts. according to IPSS risk groups	Allocation generation, concealment
Silverman 2002[10]						
	s.c. 5-azacitidine 75 mg/m²/d for 7 d. Cycles begin on d1, 29, 57, 85	92	69 (31-92)	RA=17, RARS=5, RAEB=32, RAEB-t=27, CMML=7, OTHER=11	low risk=23 (59%) high risk=16 (41%) not reported=53	B, B
	Best supportive care	99	67 (35-88)	RA=20, RARS=3, RAEB=34, RAEB-t=18, CMML=7, OTHER=10	low risk=21 (50%) high risk=21(50%) not reported=57	
Kantarjian 2006[9]						
	IV decitabine 15 mg/m²/d for 3 hours every 8 hours for 3 d (135mg/m² per course) every 6 wk	89	70 (65-76)	RA=12, RARS=7, RAEB=47, RAEB-t=17, CMML=6	low risk=28 (31.5%) high risk=61 (68.5%)	B, A
	Best supportive care	81	70 (62-74)	RA=12, RARS=4, RAEB=43, RAEB-t=14, CMML=8	low risk=24 (30%) high risk=57 (70%)	
Fenaux 2009[29]						
	s.c 5-azacidine 75mg/m2/d for 7 days every 28 d for at least 6 cycles	179	≤64 yrs: 57 pts; >64 yrs: 122 pts	RAEB=104, RAEB-t=61, CMML=6 AML=1	low risk=5 (3%) high risk=158 (97%) not reported-16	A, A
	conventional care: Best supportive care S.C low dose ARA-C 20 mg/m²/d for 14d every 28 d at least for 4 cycles Intensive chemotherapy (induction "7+3")	179	≤64 yrs: 43pts >64 yrs: 136 pts	RAEB=103, RAEB-t=62, CMML=5 AML=1	low risk=13 (8%) high risk=155 (92%) not reported-11	
WijerMans 2008[28]						
	IV decitabine 15 mg/m² over 4 hr every 8 hr for 3d every 6 wk for max. of 8 cycles	119	70 (60-90)	RAEB-t=75	Low risk-16 (7%) high risk=217 (93%)	B, A
	Best supportive care	114				

HA: hypomethylating agents, pts- patients, IPSS- international prognosis scoring system, low risk MDS-low and intermediate 1, high risk MDS-intermediate 2+ high; RA: refractory anemia, RARS: refractory anemia with ringed sideroblasts; RAEB: refractory anemia with excess of blasts; RAEB-t: refractory anemia with excess of blasts in transformation; CMML: chronic myelomonocytic leukemia; yrs: years; wk: weeks; d: days; hr:hours; pts: patients; max: maximum; ARA-C: cytarabine.

about 40-50% of patients received only two or fewer cycles of decitabine, usually because of the toxicity of the drug.[9,28] We could not demonstrate a difference between the two agents when grade 3/4 toxicity was compared for the full treatment. Unfortunately, a subgroup analysis for toxicity per cycle could not be conducted due to lack of data. The inferiority of decitabine might, therefore, stem from its shorter duration of administration leading to reduced efficacy.

Our analysis demonstrated a significantly prolonged time to AML or death with the use of hypomethylating agents. Although this finding was shown only in two trials, the effect was amplified by the meta-analysis, reaching statistical significance. The molecular basis for this outcome is unclear as the exact gene targets for the drugs have not been identified yet. Potential target genes are those of the *p53* family, affecting cell differentiation and apoptosis, or the *p21* and *p18* genes affecting the behavior of stem cells.[33] It is also unknown whether the drugs exert their effect by restoring gene expression and blast cell differentiation or by induction of apoptosis.[2] Interestingly, a recent study showed that decitabine induces expression of p53-inducible ribonucleotide reductase, an effect that is independent of its hypomethylating activity.[34] Moreover, in a trial published by Fenaux *et al.*, the overall survival benefit observed with 5-azacitidine was not dependent on baseline methylation status.[35] Thus, the effects of these agents are not necessarily mediated by their hypomethylating features but might be exerted through non-hypomethylating pathways as well.

Despite the improvement of response rate in favor of patients treated with the hypomethylating agents shown in our systematic review, there was no difference in freedom from RBC transfusions between patients receiving these treatments and patients treated with conventional care. This could be explained by limited data, since only two trials reported this outcome. Indeed, the main limitation of our review is the small number of trials and the diversity of definitions and treatments between them.

The trials differed in the distribution of risk groups as defined by the IPSS, in their definitions of response and in the type of treatment administered to the control group. The clinical heterogeneity between the trials with respect to these issues might account for the statistical heterogeneity in some of the analyses. While at least 70% of the patients were at high risk in three of the trials (Table 1),

Table 2. Definitions of response criteria for each trial.

Study year, [ref] (years in which study was conducted)	Complete response	Definition of hematologic response Partial response	Hematologic improvement
Silverman, 2002[10] (1994-1996)			
	Normal BM or <5% blasts in BM and some dyshematopoietic features In PB - Complete normalization of count: Hb≥13.3gr/dL for male, Hb≥11.7gr/dL for female; WBC≥4.4×10⁹/L; ANC≥1.8×10⁹/L; PLT≥140×10⁹/L No blasts in PB No transfusions	In BM ≤50% of initial marrow blasts In PB trilineage response No blasts in PB No transfusions For patients with RA/RARS, PB criteria alone were used	In PB monolineage or bilineage response or ≥50% decrease from baseline in transfusion requirements
Kantarjian, 2006[9] (2001-2004)			
	According to the response criteria for MDS: In BM < 5% blasts and without dysplasia In PB: Hb>11 g/dL, ANC≥1.5× 10⁹/L, PLT>100×10⁹/L No blasts in PB No transfusions or GF Minimum duration of response 8 weeks	According to the IWG response criteria for MDS: In BM ≤50% decrease in blasts Other response criteria the same as CR or downgrade in FAB category	Was described as: Magnitude of response (major or minor) Individual responsive cell lines
Fenaux, 2009[29] (2004-2006)			
	According to the IWG response criteria for MDS	According to the IWG response criteria for MDS	Was described as: Magnitude of response (major or minor) Individual responsive cell lines
WijerMans, 2008[28] (2002-2007)			
	Not reported	Not reported	Not reported

BM: bone marrow; PB: peripheral blood; CBC: complete blood count; Hb: hemoglobin; PLT: platelets; WBC: white blood cell count; ANC: absolute neutrophil count; GF: growth factors; IWG response criteria for MDS: International Working Group response criteria for Myelodysplatic Syndrome (Cheson, Blood 2000;96:3671).

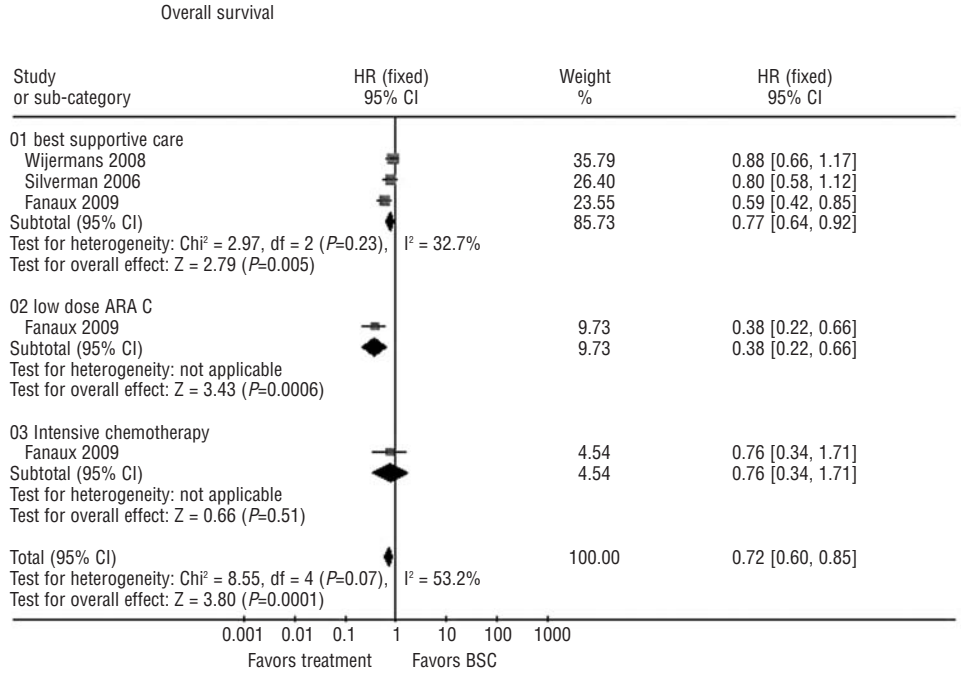


Figure 2. Overall survival in patients treated with hypomethylating agents as compared to best supportive care, low dose cytarabine (ARA C) or intensive chemotherapy.

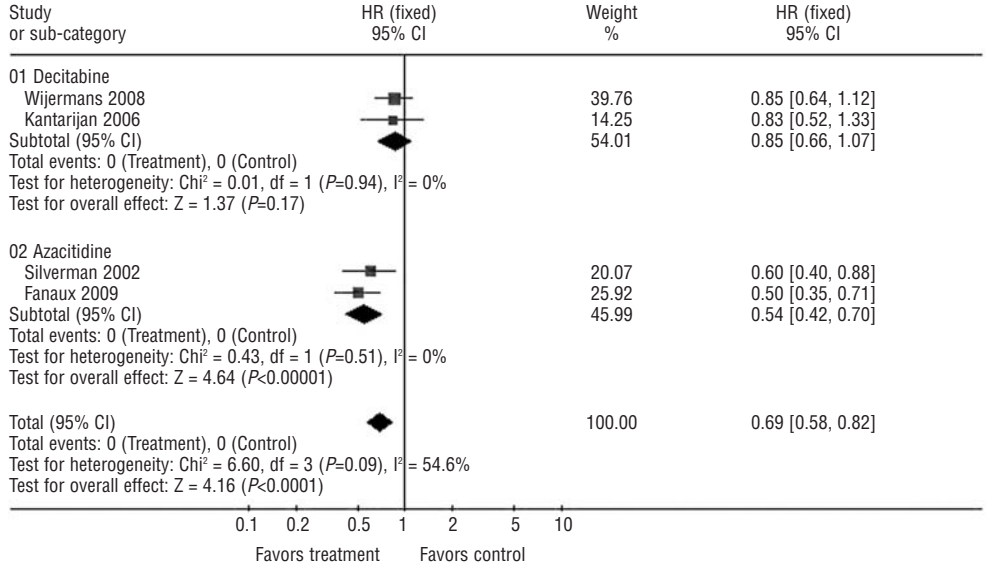


Figure 3. Time to AML or death in patients treated with hypomethylating agents as compared to conventional care.

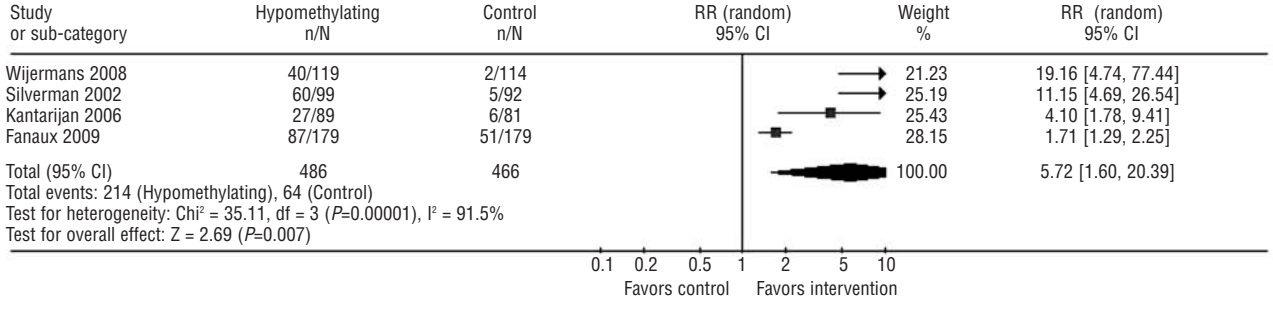


Figure 4. Overall response including complete response, partial response and haematologic improvement in patients treated with hypomethylating agents as compared to conventional care.

IPSS criteria could be applied to merely half of the patients in the trial published earlier by Silverman *et al.*, namely those for whom cytogenetic data available.[10] Of note, about 50% of the patients in this trial were at higher risk (intermediate-2 or high-risk) according to the IPSS criteria. Ideally we would have conducted an analysis excluding the low-risk MDS patients, since hypomethylating agents appear to have a role mainly in high-risk patients. Unfortunately, the data for such an analysis could not be retrieved from the original trials.

Although most of the trials used the International Working Group criteria for the definitions of response,[12] Silverman *et al.* used more flexible criteria allowing for some degree of dyshematopoiesis in patients achieving complete response and not entailing a minimal response duration. The variability in treatments in the control arm should also be mentioned. While in three of the trials the control arm included patients treated by *best supportive care only*[9,10,28] patients were treated with either supportive care, low-dose cytarabine or intensive chemotherapy in one trial.[29] Since there is no established standard of care for high-risk MDS patients, we, as well as others previously, included all three older therapeutic options used in common practice in the control arm of our meta-analysis.

In terms of overall survival, results were in favor of hypomethylating agents, especially 5-azacitidine, as compared to best supportive care. Conversely, when compared to intensive chemotherapy, there was no difference in overall survival. However, the ease of administration of hypomethylating agents and the fewer adverse events associated with their use, especially in the elderly, make these drugs more attractive than intensive chemotherapy in many respects.

In conclusion, our review shows that, according to current evidence, hypomethylating agents, especially 5-azacitidine, have a major role to play in the treatment of patients with MDS. Our conclusions apply mainly to high-risk patients, as defined by the IPSS, since most of the patients included in the trials belonged to high-risk groups. Future trials should address further issues including comparisons between the two agents and different doses, the role of these agents compared to intensive chemotherapy, their place in the treatment of low-risk MDS patients, the number of cycles required for treatment and their use as maintenance therapy for MDS patients.

Authorship and Disclosures

RG: conception and design of the study, protocol development, searching for trials, acquisition, analysis and interpretation of data, drafting the article; LV, AG-G, YB, MY and PR: conception and design of the study, acquisition, analysis and interpretation of data, drafting the article; OS: conception and design of the study, protocol development, acquisition, analysis and interpretation of data, drafting the article. All authors approved the final version of the article to be published.

The authors reported no potential conflicts of interest.

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