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ORIGINAL ARTICLE

# Decitabine improves progression-free survival in older high-risk MDS patients with multiple autosomal monosomies: results of a subgroup analysis of the randomized phase III study 06011 of the EORTC Leukemia Cooperative Group and German MDS Study Group

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**Abstract** In a study of elderly AML patients treated with the hypomethylating agent decitabine (DAC), we noted a surprisingly favorable outcome in the (usually very unfavorable) subgroup with two or more autosomal monosomies (MK2+) within a complex karyotype (Lübbert et al., Haematologica 97:393-401, 2012). We now analyzed 206 myelodysplastic syndrome (MDS) patients (88 % of 233 patients randomized in the EORTC/GMDSSG phase III trial 06011, 61 of them

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with RAEBt, i.e. AML by WHO) with cytogenetics informative for MK status.. Endpoints are the following: complete/partial (CR/PR) and overall response rate (ORR) and progression-free (PFS) and overall survival (OS). Cytogenetic subgroups are the following: 63 cytogenetically normal (CN) patients, 143 with cytogenetic abnormalities, 73 of them MK-negative (MK−), and 70 MK-positive (MK+). These MK+ patients could be divided into 17 with a single autosomal monosomy (MK1) and 53 with at least two monosomies (MK2+). ORR with DAC in CN patients: 36.1 %, in MK− patients: 16.7 %, in MK+ patients: 43.6 % (MK1: 44.4 %, MK2+ 43.3 %). PFS was prolonged by DAC compared to best supportive care (BSC) in the CN (hazard ratio (HR) 0.55, 99 % confidence interval (CI), 0.26; 1.15, $p=0.03$) and MK2+ (HR 0.50; 99 % CI, 0.23; 1.06, $p=0.016$) but not in the MK−, MK+, and MK1 subgroups. OS was not improved by DAC in any subgroup. In conclusion, we demonstrate for the first time in a randomized phase III trial that high-risk MDS patients with complex karyotypes harboring two or more autosomal monosomies attain encouraging responses and have improved PFS with DAC treatment compared to BSC.

**Keywords** Monosomal karyotype · Adverse cytogenetics · Hypomethylating agents · Azacytidine · Epigenetic therapy · Elderly patients

## Introduction

The treatment of elderly myelodysplastic syndrome (MDS)/AML patients with a complex karyotype still poses a highly unmet clinical need, given the low complete response rate and high relapse rate even after standard induction/consolidation chemotherapy or allogeneic transplantation. Regarding non-curative AML treatment of older patients in the randomized MRC trial AML14, comparing low-dose cytarabine to best supportive care (BSC), patients with adverse cytogenetics (the majority with complex karyotypes) had an equally dismal outcome in both treatment arms, with a median survival of less than 2 months and a 1-year survival below 5 % [1]. Not surprisingly, a complex karyotype thus scores in the very high-risk cytogenetic groups of AML and MDS [2–5]. Notably, it recently became apparent that clofarabine and the DNA hypomethylating agents azacytidine and decitabine (DAC) have marked activity in complex-karyotype AML [6–9] and MDS [10–16]. However, it is unclear why these drugs differ from cytarabine and other cytotoxic agents in that regard. Equally notable was the recurrent observation of activity of DAC [13, 15–18] and azacytidine [10, 19] in MDS/AML patients with sole monosomy 7—also a robust clinical result but as yet lacking a mechanistic explanation. A monosomy might be only one of several abnormalities and may not exhibit prognostic impact itself [20, 21].

We therefore recently hypothesized that AML patients with complex karyotype (CK+) including one or more autosomal monosomies (MK+, most frequently monosomy 7) might show a response to hypomethylating agent treatment that is absent in complex-karyotype patients without autosomal monosomies (MK−/CK+). Since the large phase II DAC trial 00331 had recruited 54 CK+ patients, their outcome was compared with regard to the presence (MK+/CK+, $n=37$) or absence (MK−/CK+, $n=17$) of the monosomal karyotype [8]. Intriguingly, the response rate was superior in the MK+/CK+ AML patients compared to MK−/CK+ patients (complete plus partial remissions 37 vs. 12 %). Seemingly paradoxical was the result in the group of 22 patients with at least two monosomies (MK2+): these patients showed a 45 % complete remission (CR)/partial remission (PR) rate vs. 25 % in the 15 patients with a single monosomy (MK1) [8].

To extend this observation (collected from a single AML trial) also to higher-risk MDS, we now conducted identical cytogenetic subgroup analyses on 206 higher-risk MDS patients (FAB classification) with informative cytogenetics included in the randomized phase III trial 06011 of the EORTC Leukemia Group and the German MDS Study Group [11] (constituting, after the recent report of a subgroup analysis of the RAEBt patients [22], the second subgroup analysis from this study; for a listing of the Study Consortium see Supplementary Information). We also provide supportive evidence from 118 additional higher-risk MDS patients from two phase II DAC trials [13].

Key findings are a comparable rate of overall and objective responses to DAC in the normal karyotype and MK+ patients, and prolonged progression-free (though not overall) survival in patients with complex karyotype and two or more autosomal monosomies treated with DAC compared to best supportive care. The in vivo mechanism of action of DAC in these patients may be distinct from standard chemotherapy such as low-dose AraC.

## Patients and methods

Patients were eligible for the EORTC Leukemia Cooperative Group phase III trial 06011 [11] if aged ≥60 years and diagnosed with primary or treatment-related MDS or chronic

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myelomonocytic leukemia (CMMoL) irrespective of white blood counts; IPSS intermediate-1, IPSS intermediate-2, or high; bone marrow blasts 11–30 %; or ≤10 % but with poor cytogenetics (IPSS) and ECOG performance status 0–2. Severe cardiovascular disease was an exclusion criterion, whereas previous treatment of MDS was not (except aggressive chemotherapy or treatment with a hypomethylating agent). The study was performed in accordance with the Declaration of Helsinki, all patients provided written informed consent, and the study was approved by the local ethics committees at all participating trial sites. The definition of monosomal karyotype was as by Breems et al. [23], i.e., at least two autosomal monosomies or one single autosomal monosomy in combination with at least one structural abnormality.

## Statistical analysis

Responses were assessed according to the International Working Group criteria [24, 25], with sequential bone marrow studies planned after every other course of decitabine and at weeks 24 and 48 for patients on the BSC arm, earlier in both arms in case of suspected progression. Before and during the study, evaluations of bone marrow aspirates were performed locally by the investigators.

Progression-free survival (PFS) was defined as time from random assignment to progression, relapse after attainment of complete remission (CR) or partial response (PR), or death, whichever occurred first. Overall survival (OS) was defined as time from randomization until death (whatever the cause) or the last follow-up (censored observation). The Kaplan-Meier method was used to estimate PFS and OS. The two-sided, log-rank test was used for comparisons of treatment outcome. The Cox proportional hazards model was used to obtain hazard ratio (HR) estimates and corresponding confidence intervals (CIs). Subgroup analyses were performed using forest plot techniques. For efficacy analyses, the intent-to-treat principle was followed.

## Results

### EORTC/GMDSSG randomized phase III trial 06011: frequent recruitment of MDS patients with monosomal karyotype

Of 233 patients randomized to either DAC or BSC, 206 had cytogenetic analyses allowing evaluation for the absence or presence of a monosomal karyotype (MK; CONSORT information: Table 1, clinical characteristics: Table 2). For the remaining 27 patients, cytogenetic scoring for MK status was not feasible. As shown in Table 3, 63 of the 206 patients were cytogenetically normal (CN), and 143 had cytogenetic abnormalities (CA). These CA patients can be subdivided into those without (MK−, $n$=73) and with MK (MK+, $n$=70). Of the MK− patients, 54 had one or two cytogenetic abnormalities (i.e., "non-complex," MK−/CK−), and 18 had a complex karyotype (MK−/CK+). Among the MK+ patients, four scored as CK−, the remaining 66 patients as CK+. Overall, this patient cohort has a strong representation of very high-risk cytogenetic patients.

**Table 1** CONSORT diagram (06011 phase III trial, 206 patients with informative karyotypes)

| | $n$=206 patients | |
|---|---|---|
| | Treatment arm: supportive care ($N$=101) $n$ (%) | Treatment arm: decitabine ($N$=105) $n$ (%) |
| Reason off-protocol | | |
| Normal completion | 18 (17.8) | 26 (24.8) |
| PD | 49 (48.5) | 36 (34.3) |
| Toxicity | 0 (0.0) | 19 (18.1) |
| Hypoplasia | 0 (0.0) | 5 (4.8) |
| Death | 15 (14.9) | 10 (9.5) |
| Refusal | 12 (11.9) | 3 (2.9) |
| Protocol violation | 4 (4.0) | 3 (2.9) |
| Ineligibility | 1 (1.0) | 1 (1.0) |
| Other | 2 (2.0) | 2 (1.9) |

### DAC induces objective responses in all cytogenetic subgroups including complex karyotypes with multiple monosomies

Among all 206 patients with cytogenetics informative for MK, the overall response rate (ORR, complete and partial remissions, hematological improvement) was 33.3 % in the 105 patients on the DAC arm vs. 2 % on the BSC arm (two patients had hematological improvement), making comparisons between the two treatment arms not very meaningful (Table 3). We therefore focused on the ORR in the different cytogenetic subgroups of the DAC arm. The 36 CN patients had a 36.1 % ORR, and the 69 CA patients had a 31.9 % ORR. When next looking to the CA patients without MK (MK−, $n$=30), an ORR of 16.7 % was seen, in the CA patients with MK (MK+, $n$=39) an ORR of 43.6 %. Notably, this response rate was comparable between patients with one (MK1, $n$=9, 44.4 %) and two or more monosomies (MK2+, $n$=30, 43.3 %), the latter generally being considered the cytogenetic subgroup with the most adverse prognosis.

**Table 2** Patient baseline characteristics (all patients, $n$=233; patients informative for monosomal karyotype, $n$=206)

| Baseline characteristics | Patients total ($n$=233) | | Patients informative for monosomal karyotype ($n$=206) | |
|---|---|---|---|---|
| | DAC $n$=119 | BSC $n$=114 | DAC $n$=105 | BSC $n$=101 |
| Age, median (range) | 69 (60; 90) | 70 (60; 86) | 69 (60; 90) | 70 (60; 86) |
| >=75 years, $n$ (%) | 33 (27.7) | 34 (29.8) | 28 (26.7) | 31 (30.7) |
| Sex, $n$ (%) | | | | |
| Male | 76 (63.9) | 73 (64) | 65 (61.9) | 62 (61.4) |
| FAB, $n$ (%) | | | | |
| RA/RARS | 8 (6.7) | 10 (8.8) | 9 (8.5) | 10 (9.9) |
| RAEB | 61 (51.3) | 64 (56.1) | 52 (49.5) | 59 (58.4) |
| RAEBt | 40 (33.6) | 35 (30.7) | 34 (32.4) | 27 (26.7) |
| AML | 1 (0.8) | 1 (0.9) | 1 (1.0) | 1 (1.0) |
| CMMoL | 10 (8.4) | 4 (3.5) | 9 (8.6) | 4 (4.0) |
| IPSS, $n$ (%) | | | | |
| Low | – | – | – | – |
| Int 1 | 8 (6.7) | 8 (7.0) | 5 (4.8) | 8 (7.9) |
| Int 2 | 64 (53.8) | 63 (55.3) | 55 (52.4) | 52 (51.5) |
| High | 46 (38.7) | 42 (36.8) | 45 (42.9) | 41 (40.6) |

Considering that these results might be skewed by a lower rate of objective responses (CR, PR) and a higher rate of hematological improvement in the cytogenetically less favorable compared to the cytogenetically more favorable subgroups, we next looked to the CR/PR rate. Here in the CN group, three and four patients had a CR or PR, respectively (19.4 %); there were four CRs in the MK− group (13.3 %) and seven CRs and two PRs in the MK+ group (23.0 %). When dividing the MK+ group into MK1 and MK2+, the respective CR/PR rates were 44.4 and 16.7 %.

Further support for the ability of DAC to induce objective responses also in MK+ patients is provided by a retrospective cytogenetic analysis we now performed of two phase II trials of DAC in MDS [13]. One hundred eighteen of the 143 patients were informative for MK status (Supplementary Table 1A). Here, the ORR was 48.3 % in the entire cohort, 46.7 % in the CN group, 46.6 % in the MK− group, and 60.0 % in the MK+ group (MK1, 66.7 %; MK2+, 58.3 %, see Supplementary Table 1B).

## Outcome with DAC vs. best supportive care in MDS patients in different cytogenetic subgroups

To next address possible interactions between treatment and cytogenetic subgroups, we looked to PFS and OS. For the entire study cohort of the trial (233 patients) [11], for the 206 patients informative for MK status (Table 3), and for the 27 patients with unknown MK status (Supplementary Figure 1), PFS but not overall survival (OS) was sensitive enough to detect a significant effect of DAC treatment compared to BSC.

When looking to the different cytogenetic groups, the CN patients treated with DAC had a prolonged PFS (HR 0.55 [0.26; 1.15], $p$=0.03) but not OS compared to patients receiving sole BSC (Table 3, Figs. 1 and 2a, b). Among the 143 patients with any cytogenetic abnormalities, this difference in PFS was not so impressive and did not reach statistical significance (HR 0.76 [0.49; 1.18], $p$=0.11). There was no difference in OS.

When dividing this group into MK− ($n$=73) and MK+ ($n$=70), we first looked to the MK− patients: their median PFS was 0.36 years with DAC treatment vs. 0.26 years with BSC (HR 0.81 [0.43; 1.55], $p$=0.41). Median OS was 0.63 years in MK− patients receiving DAC vs. 0.68 years in those receiving BSC (HR 0.98 [0.49; 1.97], $p$=0.93), as shown in Table 3 and Fig. 1b.

When dividing this MK− group into patients without or with complex karyotype (CK), in the 54 MK−/CK− patients, there seemed to be a trend towards longer PFS with DAC treatment compared to BSC (HR 0.70 [0.33; 1.48], $p$=0.22) that was not apparent in the 18 MK−/CK+ patients (HR 0.95 [0.26; 3.49], $p$=0.92). However, it should be stressed that such trend may also be due to the increasingly smaller patient group sizes when conducting such comparisons. In both subgroups, OS was similar between treatment arms (Table 3).

Among the 70 MK+ patients, by trend, DAC also seemed to have a positive effect on PFS (median 0.47 years) when compared to BSC (median 0.21 years; HR 0.73 [0.38; 1.38], $p$=0.20). Here also, the OS was similar between the two treatment modalities (HR 0.93 [0.49; 1.75], $p$=0.76).

**Table 3** Patient distribution and comparison decitabine vs. best supportive care regarding response rate, PFS, and OS of 206 MDS patients with cytogenetics informative for MK status

| Group | Patient distribution (*n*) | | | CR/PR/HI (*n*) (% ORR) | | PFS | | | | OS | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | All | DAC | BSC | DAC | BSC | DAC median (years) | BSC Median (years) | HR* [CI] | *p* value** | DAC median (years) | BSC median (years) | HR* [CI] | *p* value** |
| All | 206 | 105 | 101 | 14/6/15 (33.3) | 0/0/2 (2.0) | 0.50 | 0.25 | 0.72 [0.54; 0.96] | 0.02 | 0.77 | 0.74 | 0.93 [0.69; 1.26] | 0.65 |
| CN | 63 | 36 | 27 | 3/4/6 (36.1) | 0/0/0 (0) | 0.86 | 0.38 | 0.55 [0.26; 1.15] | 0.03 | 1.3 | 0.95 | 0.85 [0.40; 1.79] | 0.57 |
| CA | 143 | 69 | 74 | 11/2/9 (31.9) | 0/0/2 (2.7) | 0.45 | 0.25 | 0.76 [0.49; 1.18] | 0.11 | 0.65 | 0.59 | 1.03 [0.65; 1.63] | 0.88 |
| MK− | 73 | 30 | 43[a] | 4/0/1 (16.7) | 0/0/1 (2.3) | 0.36 | 0.26 | 0.81 [0.43; 1.55] | 0.41 | 0.63 | 0.68 | 0.98 [0.49; 1.97] | 0.93 |
| MK−/CK- | 54 | 21 | 33 | 3/0/1 (19.0) | 0/0/1 (3.0) | 0.52 | 0.26 | 0.70 [0.33; 1.48] | 0.22 | 0.7 | 0.78 | 0.98 [0.43; 2.25] | 0.95 |
| MK−/CK+ | 18 | 9 | 9 | 1/0/0 (11.1) | 0/0/0 (0) | 0.30 | 0.23 | 0.95 [0.26; 3.49] | 0.92 | 0.62 | 0.44 | 0.74 [0.19; 2.84] | 0.56 |
| MK+ | 70 | 39 | 31 | 7/2/8 (43.6) | 0/0/1 (3.2) | 0.47 | 0.21 | 0.73 [0.38; 1.38] | 0.20 | 0.67 | 0.5 | 0.93 [0.49; 1.75] | 0.76 |
| MK1 | 17 | 9 | 8 | 4/0/0 (44.4) | 0/0/0 (0) | 0.71 | 0.74 | 0.88 [0.24; 3.24] | 0.81 | 1.02 | 0.96 | 0.98 [0.43; 2.25] | 0.38 |
| MK2+ | 53 | 30 | 23 | 3/2/8 (43.3) | 0/0/1 (4.3) | 0.45 | 0.18 | 0.50 [0.23; 1.06] | 0.016 | 0.63 | 0.45 | 0.99 [0.47; 2.12] | 0.98 |

*CI* confidence interval (95 %, for all patients, or 99 %, for subgroups), *DAC* decitabine, *BSC* best supportive care, *PFS* progression-free survival, *OS* overall survival, *CN* cytogenetically normal, *CA* cytogenetically abnormal, *MK* monosomal karyotype, *CK* complex karyotype, *HR* hazard ratio, *CR* complete remission, *PR* partial remission, *HI* hematological improvement, *ORR* overall response rate

*HR <1 indicates a better outcome of DAC vs BSC (Cox regression); **$p$ value given by the Wald test

[a] One patient was not informative for CK

### MDS patients with a complex karyotype harboring two or more autosomal monosomies have longer progression-free but not overall survival with DAC compared to best supportive care

It is generally accepted that patients with AML/MDS and more than a single autosomal monosomy have an inferior outcome than those with MK1 when treated with standard chemotherapy. We first subdivided the 70 MK+ into those with one and two more monosomies, i.e., MK1 ($n$=17) and MK2+ ($n$=53), respectively. As shown in Table 3, PFS in the MK1 subgroup ($n$=17, four of them not fulfilling CK criteria) was similar in both treatment arms (HR 0.88 [0.24; 3.24], $p$= 0.81), as was overall survival. Interestingly, median PFS of the 30 MK2+ patients treated with DAC was 0.45 years; compared to 0.18 years in the 23 MK2+ patients treated with BSC (Table 3, Fig. 2c), the resultant HR was 0.50 (0.23; 1.05], $p$=0.016). OS was not different between the two treatment groups (Fig. 2d; HR 0.99 [0.47; 2.92], $p$=0.98, Table 3; see also Fig. 2d).

## Discussion

Already in 1997, Wijermans et al. [26] noted the ability of DAC to induce remissions in MDS patients with complex karyotype and other adverse cytogenetics. This—at first counterintuitive—clinical finding was confirmed in MDS [27, 28] and AML [7, 8]. While these trials were ongoing, Breems et al. [23] first described the novel cytogenetic poor-risk category of MK in AML. This genotype is clinically distinct: the presence of one or more autosomal monosomies imbedded in a complex karyotype was associated with a worse prognosis than a complex karyotype *without* a monosomy. The presence of two or more autosomal monosomies increases the negative prognostic compared to a single monosomy impact, as shown in several large AML and MDS patient series (standard chemotherapy, allografting).

In order to confirm the intriguing response rates and overall survival of MK AML patients observed in the 00331 trial, we now analyzed MDS patients of the 06011 EORTC trial. Asking whether overall (ORR: CR/PR/HI) and objective response rates (CR/PR) differed between these groups, we found that for both, the MK+ patients had response rates comparable to the CN group (cytogenetically low risk by IPSS). This encouraging finding is supported by a retrospective analysis of 118 higher-risk MDS patients for response by MK status treated on two previous phase II DAC trials. We also had the chance to cytogenetically analyze a large cohort of MDS patients (mostly IPSS int-2/high) treated within a Dutch named-patient azacytidine program (for patient characteristics see Supplementary Table 2A). While it is difficult to compare response rates between a randomized trial and a treatment

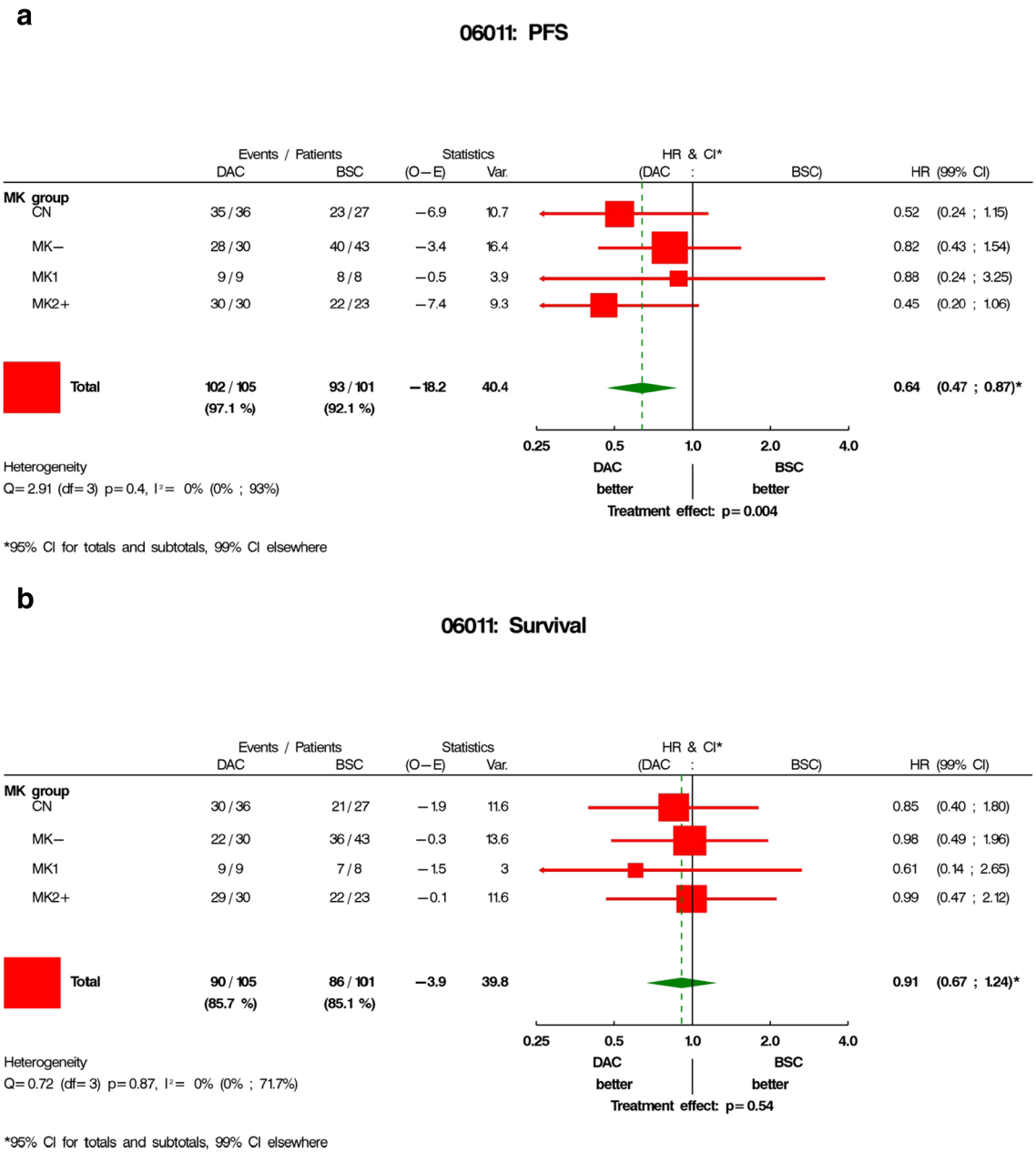


**Fig. 1** Forest plots depicting **a** progression-free and **b** overall survival of the different cytogenetic subgroups in the 06011 phase III trial by random assignment. Patients with informative karyotype with normal cytogenetics (*CN*) and abnormal cytogenetics without monosomal karyotype (*MK−*) and with one (MK1) or two or more (MK2+) monosomies were analyzed. *DAC* decitabine, *BSC* best supportive care, *O* observed, *E* expected, *Var* variance, *CI* confidence interval, *HR* hazard ratio 99 % CIs. Parentheses with *asterisk* indicate 95 % CIs for totals and subtotals

program, when looking to ORR and CR/PR, some of the 26 MK+ patients treated with azacytidine also responded to this hypomethylating agent, even in the MK2+ subgroup ($n$=17, 29.4 % ORR, 17.6 % CR/PR, Supplementary Table 2B). A summarical tabulation of the outcome of all 319 DAC-treated patients from the three data sets analyzed is provided in Supplementary Table 3.

When analyzing the 06011 cohort for progression-free and overall survival in the different cytogenetic subgroups according to treatment arm, only the CN patients and those with MK2+ (the two "extremes" within the prognostic spectrum) treated with DAC had prolonged PFS compared to those on the BSC arm; for the other cytogenetic subgroups, median PFS appeared similar. Overall survival was not prolonged by DAC treatment, and possible reasons have been discussed [11]. Briefly, four major factors could be identified: (i) suboptimal DAC dose and schedule (3-day dosing repeated every 6 weeks); (ii) DAC treatment

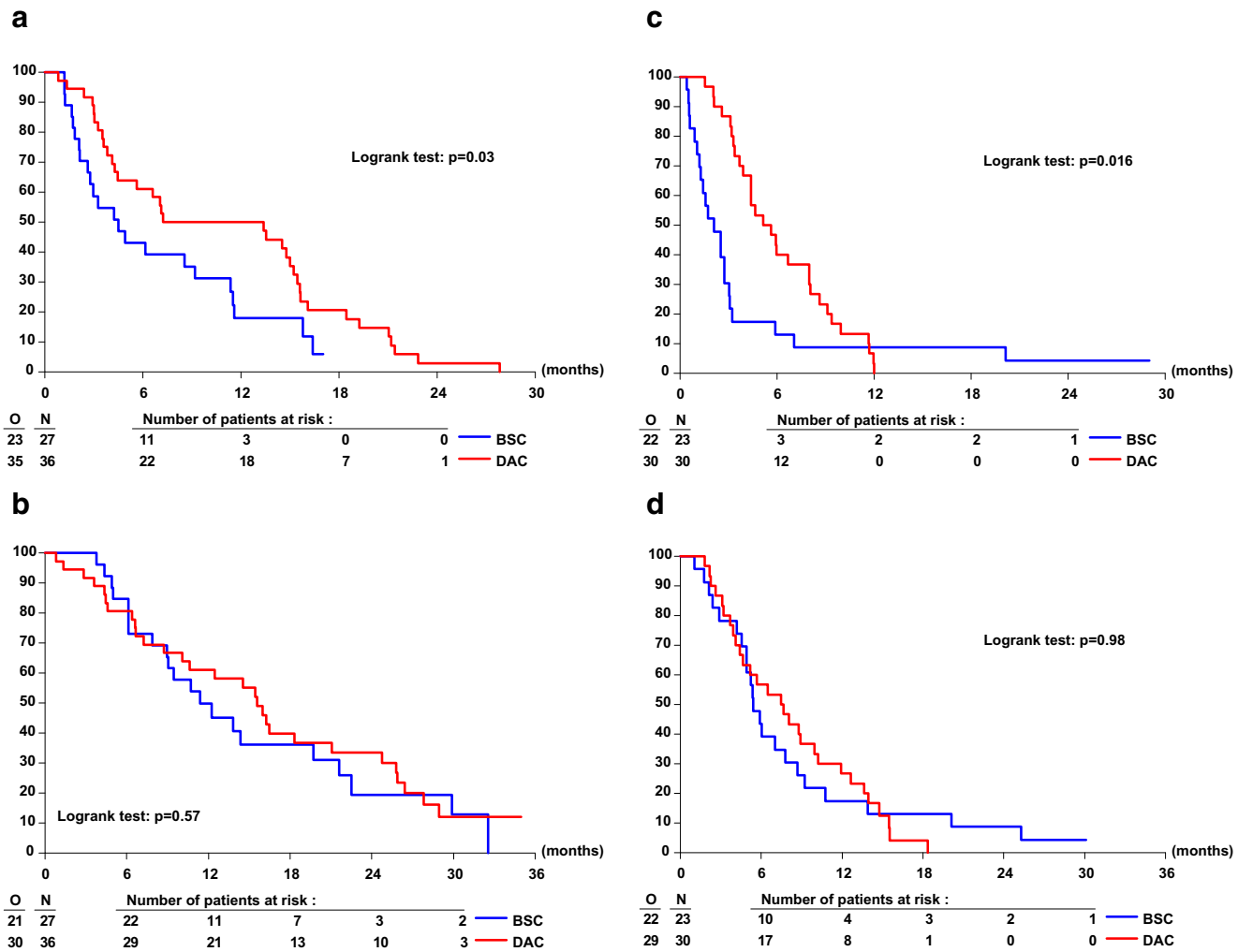


**Fig. 2** Progression-free (**a**, **c**) and overall survival (**b**, **d**) of patients of the 06011 phase III trial with normal karyotype (**a**, **b**) and MK2+ karyotype (**c**, **d**) depicted by Kaplan-Meier plot according to treatment arm. Note that two MK2+ patients receiving sole best supportive care (**c**) also lived without progression of their MDS beyond 18 months. *O* observed events, *N* patients, *BSC* treatment arm best supportive care, *DAC* treatment arm DAC. **a** Progression-free survival in cytogenetically normal patients. **b** Overall survival in cytogenetically normal patients. **c** Progression-free survival in MK2+ patients. **d** Overall survival in MK2+ patients

duration limited to 8 cycles (10 in CR patients); (iii) patient disposition, with a median duration from MDS diagnosis to randomization of only 3 months; and (iv) post-progression treatment as a confounder. It would be of interest to apply the specific scoring system recently described by Oosterveld et al. for AML/MDS patients treated with standard chemotherapy, in which poor cytogenetics predicted inferior survival [29].

Thus far, only very few studies have specifically addressed the role of MK in hypomethylating agent (HMA) treatment. The largest azacytidine studies describe either a similar overall survival of MDS patients with CK+/MK+ or CK+/MK− [30] or a worse outcome of AML patients with CK+/MK+ compared to CK+/MK− [31], albeit with limited patient numbers. Our post hoc analysis constitutes the first analysis of a phase III randomized HMA trial in MDS/AML, but it has similar limitations: when comparing cytogenetic subgroups of DAC-treated patients, the numbers (particularly in the CK+/MK− group, $n=9$) became too small to allow meaningful comparisons of outcomes. In that regard, the test for interaction indicated in Fig. 1a was not significant. However, the power of such a test is very low given the limited number of patients (and events) analyzed. Therefore, even if an interaction between MK status and the treatment difference is observed, it cannot reach statistical significance. If, however, several studies report the same trend, it will become significant in a meta-analysis.

Collectively, our results provide further evidence that DAC has encouraging activity in MDS/AML patients with a complex karyotype including multiple monosomies. It is tempting to speculate that this is mechanistically linked to the DNA hypomethylating and gene-derepressive activity of this agent. In future MDS/AML trials with HMAs, outcome by MK status should be prospectively captured.

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**Compliance with ethical standards**

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## References

1. Burnett AK, Milligan D, Prentice AG, Goldstone AH, McMullin MF, Hills RK, Wheatley K (2007) A comparison of low-dose cytarabine and hydroxyurea with or without all-trans retinoic acid for acute myeloid leukemia and high-risk myelodysplastic syndrome in patients not considered fit for intensive treatment. Cancer 109(6):1114–1124. doi:10.1002/cncr.22496
2. Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Sole F, Bennett JM, Bowen D, Fenaux P, Dreyfus F, Kantarjian H, Kuendgen A, Levis A, Malcovati L, Cazzola M, Cermak J, Fonatsch C, Le Beau MM, Slovak ML, Krieger O, Lubbert M, Maciejewski J, Magalhaes SMM, Miyazaki Y, Pfeilstöcker M, Sekeres M, Sperr WR, Stauder R, Tauro S, Valent P, Vallespi T, van de Loosdrecht AA, Germing U, Haase D (2012) Revised international prognostic scoring system for myelodysplastic syndromes. Blood 120(12):2454–2465. doi:10.1182/blood-2012-03-420489
3. Haase D, Germing U, Schanz J, Pfeilstöcker M, Nösslinger T, Hildebrandt B, Kündgen A, Lübbert M, Kunzmann R, Giagounidis AAN, Aul C, Trümper L, Krieger O, Stauder R, Müller TH, Wimazal F, Valent P, Fonatsch C, Steidl C (2007) New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2124 patients. Blood 110(13):4385–4395. doi:10.1182/blood-2007-03-082404
4. Kayser S, Zucknick M, Döhner K, Krauter J, Köhne CH, Horst HA, Held G, von Lilienfeld-Toal M, Wilhelm S, Rummel M, Germing U, Götze K, Nachbaur D, Schlegelberger B, Göhring G, Späth D, Morlok C, Teleanu V, Ganser A, Döhner H, Schlenk RF (2011) Monosomal karyotype in adult acute myeloid leukemia: prognostic impact and outcome after different treatment strategies. Blood 119(2):551–558. doi:10.1182/blood-2011-07-367508
5. Döhner H, Estey EH, Amadori S, Appelbaum FR, Büchner T, Burnett AK, Dombret H, Fenaux P, Grimwade D, Larson RA, Lo-Coco F, Naoe T, Niederwieser D, Ossenkoppele GJ, Sanz MA, Sierra J, Tallman MS, Löwenberg B, Bloomfield CD (2009) Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European leukemiaNet. Blood 115(3):453–474. doi:10.1182/blood-2009-07-235358
6. Burnett AK, Russell NH, Kell J, Dennis M, Milligan D, Paolini S, Yin J, Culligan D, Johnston P, Murphy J, McMullin MF, Hunter A, Das-Gupta E, Clark R, Carr R, Hills RK (2010) European development of clofarabine as treatment for older patients with acute myeloid leukemia considered unsuitable for intensive chemotherapy. J Clin Oncol 28(14):2389–2395. doi:10.1200/jco.2009.26.4242
7. Cashen AF, Schiller GJ, O'Donnell MR, DiPersio JF (2009) Multicenter, phase II study of decitabine for the first-line treatment of older patients with acute myeloid leukemia. J Clin Oncol 28(4):556–561. doi:10.1200/jco.2009.23.9178
8. Lübbert M, Rüter BH, Claus R, Schmoor C, Schmid M, Germing U, Kuendgen A, Rethwisch V, Ganser A, Platzbecker U, Galm O, Brugger W, Heil G, Hackanson B, Deschler B, Döhner K, Hagemeijer A, Wijermans PW, Döhner H (2012) A multicenter phase II trial of decitabine as first-line treatment for older patients with acute myeloid leukemia judged unfit for induction chemotherapy. Haematologica 97(3):393–401. doi:10.3324/haematol.2011.048231
9. Dombret H, Seymour JF, Butrym A, Wierzbowska A, Selleslag D, Jang JH, Kumar R, Cavenagh J, Schuh AC, Candoni A, Récher C, Sandhu I, Bernal Del Castillo T, Al-Ali HK, Martinelli G, Falantes J, Noppeney R, Stone RM, Minden MD, McIntyre H, Songer S, Lucy LM, Beach CL, Döhner H (2015) International phase 3 study of azacitidine vs conventional care regimens in older patients with newly diagnosed AML with >30% blasts. Blood 126(3):291–299. doi:10.1182/blood-2015-01-621664
10. Fenaux P, Mufti GJ, Hellström-Lindberg E, Santini V, Finelli C, Giagounidis A, Schoch R, Gattermann N, Sanz G, List A, Gore SD, Seymour JF, Bennett JM, Byrd J, Backstrom J, Zimmerman L, McKenzie D, Beach CL, Silverman LR (2009) Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. Lancet Oncol 10(3):223–232. doi:10.1016/s1470-2045(09)70003-8
11. Lübbert M, Suciu S, Baila L, Rüter BH, Platzbecker U, Giagounidis A, Selleslag D, Labar B, Germing U, Salih HR, Beeldens F, Muus P, Pflüger KH, Coens C, Hagemeijer A, Schaefer HE, Ganser A, Aul C, de Witte T, Wijermans PW (2011) Low-dose decitabine versus best supportive care in elderly patients with Intermediate- or high-risk myelodysplastic syndrome (MDS) ineligible for intensive chemotherapy: final results of the randomized phase III study of the European organisation for research and treatment of cancer leukemia group and the German MDS study group. J Clin Oncol 29(15):1987–1996. doi:10.1200/jco.2010.30.9245
12. van der Helm LH, Alhan C, Wijermans PW, van Marwijk KM, Schaafsma R, Biemond BJ, Beeker A, Hoogendoorn M, van Rees BP, de Weerdt O, Wegman J, Libourel WJ, Luykx-de Bakker SA, Minnema MC, Brouwer RE, Croon-de Boer F, Eefting M, Jie K-SG, van de Loosdrecht AA, Koedam J, Veeger NJGM, Vellenga E, Huls G (2011) Platelet doubling after the first azacitidine cycle is a promising predictor for response in myelodysplastic syndromes (MDS), chronic myelomonocytic leukaemia (CMML) and acute myeloid leukaemia (AML) patients in the Dutch azacitidine

compassionate named patient programme. Br J Haematol 155(5): 599–606. doi:10.1111/j.1365-2141.2011.08893.x

13. Wijermans PW, Lübbert M, Verhoef G, Klimek V, Bosly A (2005) An epigenetic approach to the treatment of advanced MDS; the experience with the DNA demethylating agent 5-aza-2′-deoxycytidine (decitabine) in 177 patients. Ann Hematol 84(S1): 9–17. doi:10.1007/s00277-005-0012-1
14. van der Helm LH, Veeger NJGM, Kooy MM, Beeker A, de Weerdt O, de Groot M, Alhan C, Hoogendoorn M, Laterveer L, van de Loosdrecht AA, Koedam J, Vellenga E, Huls G (2013) Azacitidine results in comparable outcome in newly diagnosed AML patients with more or less than 30% bone marrow blasts. Leuk Res 37(8):877–882. doi:10.1016/j.leukres.2013.03.022
15. Jabbour E, Garcia-Manero G, Ravandi F, Faderl S, O'Brien S, Fullmer A, Cortes JE, Wierda W, Kantarjian H (2013) Prognostic factors associated with disease progression and overall survival in patients with myelodysplastic syndromes treated with decitabine. Clin Lymphoma Myeloma Leuk 13(2):131–138. doi:10.1016/j.clml.2012.11.001
16. Estey EH (2013) Epigenetics in clinical practice: the examples of azacitidine and decitabine in myelodysplasia and acute myeloid leukemia. Leukemia 27(9):1803–1812. doi:10.1038/leu.2013.173
17. Lübbert M, Wijermans P, Kunzmann R, Verhoef G, Bosly A, Ravoet C, Andre M, Ferrant A (2001) Cytogenetic responses in high-risk myelodysplastic syndrome following low-dose treatment with the DNA methylation inhibitor 5-aza-2′-deoxycytidine. Br J Haematol 114(2):349–357. doi:10.1111/j.1365-2141.2001.02933.x
18. Rüter B, Wijermans P, Claus R, Kunzmann R, Lübbert M (2007) Preferential cytogenetic response to continuous intravenous low-dose decitabine (DAC) administration in myelodysplastic syndrome with monosomy 7. Blood 110(3):1080–1082. doi:10.1182/blood-2007-03-080630
19. Raj K, John A, Ho A, Chronis C, Khan S, Samuel J, Pomplun S, Thomas NSB, Mufti GJ (2007) CDKN2B methylation status and isolated chromosome 7 abnormalities predict responses to treatment with 5-azacytidine. Leukemia 21(9):1937–1944. doi:10.1038/sj.leu.2404796
20. Schanz J, Tüchler H, Solé F, Mallo M, Luño E, Cervera J, Grau J, Hildebrandt B, Slovak ML, Ohyashiki K, Steidl C, Fonatsch C, Pfeilstöcker M, Nösslinger T, Valent P, Giagounidis A, Aul C, Lübbert M, Stauder R, Krieger O, Le Beau MM, Bennett JM, Greenberg P, Germing U, Haase D (2013) Monosomal karyotype in MDS: explaining the poor prognosis? Leukemia 27(10):1988–1995. doi:10.1038/leu.2013.187
21. Valcarcel D, Adema V, Sole F, Ortega M, Nomdedeu B, Sanz G, Luno E, Canizo C, de la Serna J, Ardanaz M, Marco V, Collado R, Grau J, Montoro J, Mallo M, Vallespi T (2013) Complex, not monosomal, karyotype is the cytogenetic marker of poorest prognosis in patients with primary myelodysplastic syndrome. J Clin Oncol 31(7):916–922. doi:10.1200/jco.2012.41.6073
22. Becker H, Suciu S, Rüter BH, Platzbecker U, Giagounidis A, Selleslag D, Labar B, Germing U, Salih HR, Muus P, Pflüger KH, Hagemeijer A, Schaefer HE, Fiaccadori V, Baron F, Ganser A, Aul C, de Witte T, Wijermans PW, Lübbert M (2015) Decitabine versus best supportive care in older patients with refractory anemia with excess blasts in transformation (RAEBt)—results of a subgroup analysis of the randomized phase III study 06011 of the EORTC Leukemia Cooperative Group and German MDS Study Group (GMDSSG). Ann Hematol 94(12):2003–2013. doi:10.1007/s00277-015-2489-6
23. Breems DA, Van Putten WLJ, De Greef GE, Van Zelderen-Bhola SL, Gerssen-Schoorl KBJ, Mellink CHM, Nieuwint A, Jotterand M, Hagemeijer A, Beverloo HB, Löwenberg B (2008) Monosomal karyotype in acute myeloid leukemia: a better indicator of poor prognosis than a complex karyotype. J Clin Oncol 26(29):4791–4797. doi:10.1200/jco.2008.16.0259
24. Cheson BD, Greenberg PL, Bennett JM, Lowenberg B, Wijermans PW, Nimer SD, Pinto A, Beran M, de Witte TM, Stone RM, Mittelman M, Sanz GF, Gore SD, Schiffer CA, Kantarjian H (2006) Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood 108(2):419–425. doi:10.1182/blood-2005-10-4149
25. Cheson BD, Bennett JM, Kantarjian H, Pinto A, Schiffer CA, Nimer SD, Löwenberg B, Beran M, de Witte TM, Stone RM, Mittelman M, Sanz GF, Wijermans PW, Gore S, Greenberg PL, World Health Organization (WHO) international working group (2000) Report of an international working group to standardize response criteria for myelodysplastic syndromes. Blood 96(12): 3671–3674
26. Wijermans PW, Krulder JWM, Huijgens PC, Neve P (1997) Continuous infusion of low-dose 5-aza-2′-deoxycytidine in elderly patients with high-risk myelodysplastic syndrome. Leukemia 11(1):1–5. doi:10.1038/sj.leu.2400526
27. Wijermans P, Lübbert M, Verhoef G, Bosly A, Ravoet C, Andre M, Ferrant A (2000) Low-dose 5-aza-2′-deoxycytidine, a DNA hypomethylating agent, for the treatment of high-risk myelodysplastic syndrome: a multicenter phase II study in elderly patients. J Clin Oncol Off J Am Soc Clin Oncol 18(5):956–962
28. Kantarjian H, Issa J-PJ, Rosenfeld CS, Bennett JM, Albitar M, DiPersio J, Klimek V, Slack J, de Castro C, Ravandi F, Helmer R, Shen L, Nimer SD, Leavitt R, Raza A, Saba H (2006) Decitabine improves patient outcomes in myelodysplastic syndromes. Cancer 106(8):1794–1803. doi:10.1002/cncr.21792
29. Oosterveld M, Suciu S, Muus P, Germing U, Delforge M, Belhabri A, Aul C, Selleslag D, Ferrant A, Marie JP, Amadori S, Jehn U, Mandelli F, Hess U, Hellström-Lindberg E, Cakmak-Wollgast S, Vignetti M, Labar B, Willemze R, de Witte T (2015) Specific scoring systems to predict survival of patients with high-risk myelodysplastic syndrome (MDS) and de novo acute myeloid leukemia (AML) after intensive antileukemic treatment based on results of the EORTC-GIMEMA AML-10 and intergroup CRIANT studies. Ann Hematol 94(1):23–34. doi:10.1007/s00277-014-2177-y
30. Hwang KL, Song MK, Shin HJ, Na HJ, Shin DH, Kim JK, Moon JH, Ahn JS, Song IC, Hong J, Lee GW, Chung JS (2014) Monosomal and complex karyotypes as prognostic parameters in patients with international prognostic scoring system higher risk myelodysplastic syndrome treated with azacitidine. Blood Res 49(4):234–240. doi:10.5045/br.2014.49.4.234
31. Pleyer L, Burgstaller S, Girschikofsky M, Linkesch W, Stauder R, Pfeilstocker M, Schreder M, Tinchon C, Sliwa T, Lang A, Sperr WR, Krippl P, Geissler D, Voskova D, Schlick K, Thaler J, Machherndl-Spandl S, Theiler G, Eckmüllner O, Greil R (2014) Azacitidine in 302 patients with WHO-defined acute myeloid leukemia: results from the Austrian azacitidine registry of the AGMT-study group. Ann Hematol 93(11):1825–1838. doi:10.1007/s00277-014-2126-9