Dear Editor,

Myelodysplastic syndromes (MDS) emerge as a disorder of stem cell differentiation and maturation, resulting in peripheral cytopenias and eventual progression to acute myeloid leukemia (AML). Numerous clonal genetic abnormalities together with varying degrees of cytopenias and myeloblast (MB) accumulation are the basis for the revised International Prognostic Scoring System (IPSS-R) [1]. Gradual disease progression worsens survival and is an indication for starting treatment with hypomethylating agents (HMAs) such as 5-azacytidine (AZA) or decitabine, in some cases as a bridge to transplantation or as continuous therapy until failure for patients who are not transplant candidates. Compared to conventional chemotherapy, AZA treatment prolongs survival in both higher-risk MDS and oligoblastic (20–30%) MDS/AML (24.5 vs 16 months) [2–4]. AZA induces more sustained hematologic responses, but does not lead to durable remissions and most patients eventually progress and fail therapy. To improve efficacy, new agents such as Venetoclax [5, 6], Pevonedistat [7] or Panobinostat [8] have been tested in combination with the standard AZA regimen, while others (Sabatolimab, Magrolimab, IDH1/IDH2 inhibitors) are being tested. G-CSF (granulocyte colony stimulating factor) activates myeloid gene transcription in stem cells if added prior to HMA [9, 10]. G-CSF administration was verified using the CD64 biomarker [14] on granulocytes (SM10) and by measuring plasma G-CSF levels (SM11) after the first cycle of therapy. OS and therapeutic response between the arms were assessed at multiple time points using longitudinal multivariate data analysis and a Joint model including time-constant (sex, input DNA variants, NGS analysis described in SM12) and time-varying (laboratory data) parameters. The Cox proportional hazards model containing time-varying covariates together with the ordinal multilevel logistic mixed model provide a plausible statistical framework for the aforementioned evaluation (Table 1). Although the Kaplan–Meier plot is crossed between the arms at the end of follow-up in terms of OS (Fig. 1), this view involves only univariate empirical analysis. For the designed arms, the median OS times are 443 days (14.8 months) in the GA arm and 402 days (13.4 months) in the A arm (95% CI: [362,737] and [147,580] days, respectively) (p = 0.300, Cochran-Mantel-Haenszel logrank test). However, there are confounding effects of G-CSF injections in particular, which stem from the fact that patients in both arms could receive G-CSF for ethical reasons in the event of febrile neutropenia (SM13). Thus, there are also patients in arm A who received G-CSF (N = 6, 19%). In addition, there is a trend towards more frequent use of G-CSF upon HMA failure in Year 2, and the difference between arms in terms of the number of G-CSF injections equalizes from a 4:1 to a 2:1 ratio. Thus, patients in the GA arm have a lower risk of death and GA treatment significantly prolongs OS (p = 0.0297). In contrast, for arm A, the risk of death is higher up to approximately 13 cycles of therapy, where the quadratic parabola of the relationship with G-CSF applications reaches its extreme. Such a declining-rising effect of the number of G-CSF cycles on survival is depicted in SM14. After roughly one year of HMA, when there is a gradual failure of therapy and an

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Table 1. Fitted joint model for the overall survival on the GA vs A and the response to the G-CSF therapy of the GA vs A arm.

|                                | Coefficient | SE   | 95% CI for coefficient | Hazard ratio\(^a\)/Odds ratio\(^b\) | 95% CI for HR/OR | P value |
|--------------------------------|-------------|------|------------------------|-------------------------------------|------------------|---------|
| **Cox PH model for OS time\(^a\)** |             |      |                        |                                     |                  |         |
| Arm (GA vs A)                  | −0.4516     | 0.2078 | −0.8589, −0.0444       | 0.6366                             | 0.4236, 0.9566   | 0.0297  |
| Number of G-CSF cycles (1 cycle increase) | 0.0885     | 0.0395 | 0.0110, 0.1660         | 1.0926                             | 1.0111, 1.1806   | 0.0252  |
| (Number of G-CSF cycles)^2     | −0.0033     | 0.0013 | −0.0057, −0.0008       | 0.9967                             | 0.9943, 0.9992   | 0.0100  |
| Gender (Male vs Female)        | −0.5944     | 0.2073 | −1.0007, −0.1880       | 0.5519                             | 0.3676, 0.8286   | 0.0041  |
| Neutropenia Gr4 in 4 cycles (Yes vs No) | 0.5639     | 0.2479 | 0.0780, 1.0498         | 1.7575                             | 1.0811, 2.8570   | 0.0229  |
| **Ordinal multivariate logistic mixed model for response to the therapy\(^b\)** |             |      |                        |                                     |                  |         |
| Arm (GA vs A)                  | −1.3744     | 0.4139 | −2.1858, −0.5631       | 0.2530                             | 0.1124, 0.5694   | 0.0009  |
| G-CSF injections / 4-cycle (1 inj. increase) | 0.3443     | 0.0659 | 0.2152, 0.4734         | 1.4110                             | 1.2401, 1.6050   | <0.0001 |
| (Number of G-CSF inj. per 4-cycle)^2 | −0.0085   | 0.0022 | −0.0128, −0.0043       | 0.9915                             | 0.9873, 0.9957   | <0.0001 |
| MB% PB                         | −0.1501     | 0.0445 | −0.2373, −0.0629       | 0.8606                             | 0.7888, 0.9391   | 0.0007  |
| PLT                            | 0.0499      | 0.0087 | 0.0329, 0.0670         | 1.0513                             | 1.0335, 1.0690   | <0.0001 |
| HB                             | 0.0073      | 0.0018 | 0.0038, 0.0108         | 1.0073                             | 1.0038, 1.0110   | <0.0001 |

\(\text{P values in bold (far right)}\)

\(\text{SE indicates standard error, CI confidence interval.}\)

\(^a\)A positive (negative) coefficient estimate in the time-varying Cox PH model indicates a higher (lower) risk of death and therefore a shorter (longer) OS.

\(^b\)A positive coefficient estimate in the ordinal multivariate logistic mixed model indicates a remission response to treatment rather than progression.
increase in infectious complications, G-CSF is a rather neutral parameter for survival. In addition to G-CSF, detected DNA variants also influence OS: negative predictors are DNMT3A mutations \( p = 0.0131 \), \( ET6 \) \( p = 0.0012 \), \( EZH2 \) \( p = 0.0044 \), positive: SF3B1 \( p = 0.0005 \). Male patients tend to have a longer OS \( p = 0.0041 \) while Gr4 neutropenia indicates a shorter OS \( p = 0.0229 \). Predicted survival curves include SM15-16.

Response to treatment was assessed according to IWG criteria [15] (SM17, Table1). Overall response rate (ORR, GA vs A) was 77\% vs 61\% \( p = 0.000899 \), CR 31\% vs 23\% \( p = 0.575 \), PR 23\% vs 23\% \( p = 0.554 \), SD with HI 18\% vs 0\% \( p = 0.473 \), SD without HI 8\% vs 13\% \( p = 0.739 \). Progression-free survival (PFS, GA vs A) was 9.7 vs 6.1 months (95\% CI: [254, 831] and [64, 208] days, respectively) \( p = 0.00184 \). Moreover, the positive effect of G-CSF is reinforced by the fact that the presence of Gr4 neutropenia is associated with significantly shorter OS (SM15-16).

Our primary objective of increasing treatment response and survival in the GA versus A arm was confirmed, particularly in patients with initial neutropenia in the first year of HMA treatment. We did not detect an effect of G-CSF on progression to AML, which is also significant. Thus, the administration of G-CSF prior to AZA represents an improvement to the standard AZA regimen in patients with high-risk MDS and oligoblastic AML.

Fig. 1  Kaplan–Meier plot. Survival probability versus time (in days) for both treatment arms (GA vs A) of the clinical trial.

| Arm | Number at risk |
|-----|---------------|
| A   | 31 28 22 20 17 15 12 10 8 6 6 3 3 3 3 2 2 0 0 0 0 0 |
| GA  | 39 37 36 33 31 26 25 23 18 16 14 13 12 8 4 4 4 4 4 3 2 1 1 0 |

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DATA AVAILABILITY
Original data and protocols are available to other investigators upon request.
