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Impact of red blood cell transfusion dose density on progression-free survival in lower-risk myelodysplastic syndromes patients

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Authorship

-Contributions: Design: LdS, SC, MH, AS, SL, DB, and TDW; provision of patients, assembly of data: SC, AS, SL, PF, ASY, JC, EHL, RS, GS, MM, MSH, LM, KM, UG, AT, ASA, AMA, NGS, AGB, OBR, DC, IK, RI, CvM, DB, and TdW; statistical analysis: LdS, SC, AS, DB, and TdW; manuscript writing: all authors; final approval: all authors.

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Key points

- The new outcome parameter 'Transfusion dose density' allows to incorporate longitudinal changes of transfusion intensity in the evaluation of the impact of transfusions on outcome
- Transfusion dependency may be considered as an indicator of inferior progression-free survival, even at relatively low transfusion dose densities

Abstract

Progression-free survival of lower-risk myelodysplastic syndromes patients treated with red blood cell transfusions is usually reduced, but it is unclear whether transfusion dose density is an independent prognostic factor. The European MDS Registry collects prospective data at 6-monthly intervals of newly diagnosed lower-risk myelodysplastic syndromes patients from 16 European countries and Israel. Data on the transfusion dose density - the cumulative dose received at the end of each interval divided by the time since the beginning of the interval in which the first transfusion was received - were analyzed using proportional hazards regression with time-varying co-variates, with death and progression to higher-risk myelodysplastic syndromes /acute myeloid leukemia as events. Of the 1267 patients included in the analyses, 317 patients died without progression, in 162 patients the disease had progressed. Progression-free survival was significantly associated with age, EQ-5D index, baseline WHO classification, bone marrow blast count, cytogenetic risk category, number of cytopenias, and country. Transfusion dose density was inversely associated with progression-free survival ($p<1x10^{-4}$): dose density had an increasing effect on hazard until a dose density of 3 units/16 weeks. The transfusion dose density effect continued to increase beyond 8 units/16 weeks after correction for the impact of treatment with erythropoietin agents, lenalidomide and/or iron chelators. Conclusion: the negative effect of transfusion treatment on progression-free survival already occurs at transfusion densities below 3 units/16 weeks. This indicates that transfusion dependency, even at relatively low dose densities, may be considered as an indicator of inferior progression-free survival.

This trial was registered at www.clinicaltrials.gov as #NCT00600860.

Introduction

Red blood cell transfusions (RBCT) are the major component of the supportive care of patients with myelodysplastic syndromes (MDS). The life expectancy of MDS patients treated with RBCT is usually reduced compared with untransfused patients,[1,2] but whether the impaired outcome is a result of intrinsic deterioration of the underlying disease or a result of external factors related to transfusion *per se* (for example the iron toxicity induced by RBCT) remains an open question. The EUMDS registry has prospectively collected observational data on patients with low and intermediate-1 risk MDS according to IPSS[3], defined as lower-risk MDS (LR-MDS), since December 2007.[4] The majority of LR-MDS patients become transfusion dependent (51% in the EUMDS Registry)[4], usually within 6 months after diagnosis. With an expected median survival of 2.4 to 11.8 years, these patients might be prone to long-term accumulation of iron due to RBCT.[3,5-8] The toxic effects of iron overload in other iron loading diseases, such as hereditary hemochromatosis[9] and the thalassemia syndromes[10], are well known, but the consequences in MDS patients require further clarification. MDS patients are generally older than patients with other iron loading disorders[11]. Their exposure to RBCT may not be long enough to develop classical tissue damage due to iron overload, but they may suffer from oxidative stress caused by toxic iron species, including non-transferrin bound iron (NTBI) and labile plasma iron (LPI), which have been suggested to serve as early indicators of iron toxicity in iron loading anemias, such as thalassemia syndromes.[8,12] Biomarkers of oxidative stress have been found to be increased in patients with MDS and iron overload.[4,13-16] Data from a recently completed study of the EUMDS Registry[17] showed that elevated LPI levels - in contrast to elevated NTBI levels and TSAT - are associated with decreased survival. The risk of dying prematurely in patients with detectable LPI levels occurred too early in this study to explain this risk by classical iron overload due to organ toxicity (lungs, liver and heart) after long term transfusions, and this may suggest a direct effect associated with elevated LPI levels.

The aim of this analysis was to assess the effect of RBCT dose density on progression-free survival (PFS) of patients with LR-MDS. The hypothesis is that transfusional iron may be toxic and associated with oxidative stress, which may lead to BM failure, genetic damage, increased risk for progression or premature death. Two countervailing forces may play a role in this analysis: firstly, patients with symptomatic anemia are more likely to receive more frequent RBCT. Secondly, higher RBCT doses may lead to faster deterioration of LR-MDS or to a higher risk of complications by co-morbidities.

Methods

Lower-risk (IPSS risk low or intermediate-1)[3] patients from 16 European countries and Israel were included in the EUMDS registry, after signed informed consent within 100 days of the initial diagnosis of a MDS according to WHO 2001 criteria[18]. Patients with an IPSS risk intermediate-2 or high, or with therapy-related MDS were excluded, but MDS-specific treatment, started before registration within 100 days after diagnosis, was not a reason for exclusion. Data were collected at baseline and at each 6-monthly outpatient routine follow-up visit. Clinical information was collected on: demographics, anthropometrics, co-morbidities, performance status, quality of life (EQ-5D), concomitant medication, laboratory parameters, diagnostics including information on bone marrow morphology, histology, cytogenetics, red blood cell transfusion episodes, total number of transfused units and simultaneous therapeutic interventions. All subjects were followed prospectively by full reports every 6 months until death, progression to high risk MDS or leukemia, loss to follow-up or withdrawal of informed consent. The registry was approved by each institution's ethics committee according to countries legislations.

Transfusion data available in the EUMDS Registry consists of the number of units received between each reported visit, usually at 6 months intervals. In order to assess the association between transfusions received and progression free survival (PFS), proportional hazards regression with time-varying covariates was employed, adjusting the effect of transfusions by appropriate baseline and time varying variables. For the purposes of the time-to-event analyses, time is measured from date of diagnosis with MDS to date of disease progression or date of death. Progression is defined as increase to either RAEB-2 or to acute leukemia. Patients without disease progression and still alive at time of the analyses were censored at their date of their last visit.

In order to avoid problems with simultaneity of cause and effect assumed by the proportional hazards approach to survival analysis a "dose density" variable was defined for blood transfusions received, in the following way. The cumulative total of units of blood received at the end of each inter-visit time interval was calculated. This was then divided by the time since the beginning of the time interval in which the first post-diagnosis transfusion was received, giving a dose-density measurement. This dose-density was then assigned to each time interval. The value of this variable at each point in time represents the average rate at which the patient has been receiving units of blood since they started transfusions.

Adjusted baseline variables included: age at diagnosis, number of cytopenias and number of units of blood received before diagnosis. Adjusted time-varying variables (with the intention of adjusting for

the condition of the patient over time) were: bone marrow blast count, EQ5D-index, IPSS-R cytogenetic category, platelet and neutrophil counts. Additional analyses were adjusted for the effect of ESA treatment, iron chelation therapy and lenalidomide, taking these treatments to be confounding factors. Finally, a sensitivity analysis was performed in the survival regressions to take into account that the population is not homogeneous but distributed over different centers in several countries, using a random effects frailty term. The random effect, called "frailty", is the term that describes the common risk or the individual heterogeneity, acting as a factor on the hazard function. Missing values in adjustment variables were imputed with last observation carried forward or next observation carried backward.

Results

Patient characteristics

The EUMDS Registry contained data from 2,192 patients diagnosed between December 3rd, 2007 to March 14th, 2017 of which 1,504 patients had three or more visits recorded (visit 3 = landmark at 1 year follow-up). Two patients with RAEB-2 were excluded resulting in the inclusion of 1,502 patients. An additional 235 patients were excluded, as one or more of the following variables had never been measured or the test failed throughout the study: cytogenetics (n=112), EQ-5D (n=101), blast count (n=60), platelets count (n=1) neutrophil count (n=2). The final cohort consisted of 1267 patients, unselected for any type of treatment. In 162 patients the disease had progressed to higher-risk MDS or acute myeloid leukemia (AML) and 317 patients had died without progression. Median survival after disease progression was 5.3 months (95%CI 3.2- 9.8 months). For full details of the exclusions, see the supplementary data. Table 1 and supplementary table 1 show the baseline demographics. For the landmark analysis patients were defined as untransfused if they had never received a transfusion from diagnosis until the end of the study period (death or progression), or if they had received transfusion only once (n=751). Patients were defined as transfused if they had received multiple transfusions (n=516) within the first year of follow-up (visit 3 = landmark). Regular transfusions were initiated usually during the first 6 months. Using visit 3 as the landmark ensured that the majority of patients who received more than one transfusion were correctly identified.

Distribution of transfusion dose density

The distribution of non-zero dose densities at the third visit (the landmark visit) is shown in figure 1. Mean dose density amongst those who had received a transfusion at one year of follow-up was 1.24 units per month, with a median of 0.88 units per month (IQR 0.31 – 1.85). Dose densities of

the transfused patients declined on approach to the final recorded interval, if patient died or progressed to higher-risk MDS during the last interval (supplementary figure 1). This implies that patients received fewer transfusions per month in the interval during which death occurred, than in the preceding intervals. Presumably, the treatment focus switches to palliative care at home on the approach to death. Patients alive at the last recorded visit and no signs of progression did not show an increase of the transfusion density over time (supplementary figure 1).

Outcome of patients stratified according to transfusion status at landmark one year after registration

Characteristics at time of landmark visit 3 stratified according the transfusions status are shown in supplementary table 2. 145 Subjects untransfused at visit 3, went on to have transfusions after the landmark visit. Out of 516 transfused by the time of the landmark 288 subjects were not reported to have received any further transfusions, but out of these 288, 125 subjects did not have any further visits and a further 91 had only one additional visit. Of the 163 who had an additional visit (91+72), 73 received treatment with ESA, 19 lenalidomide, 10 hypomethylating agents, 2 hydroxycarbamide, 3 iron chelators. Unadjusted PFS stratified by transfusion status (transfused n=516, untransfused n=751) at the third visit is presented in figure 2A. The overall PFS of the untransfused patients at visit 3 was significantly better ($p < 0.0001$) compared to the transfused patients.

Transfused patients were divided into those receiving above (high density) or below (low density) the median value (0.87 units per month) of non-zero dose densities. Unadjusted PFS stratified by transfusion status and dose density (untransfused n=751, low dose density n=258, high dose density=258) at the third visit is presented in figure 2B. The overall PFS of the three groups of patients, stratified according to the dose density at visit 3, was significantly different ($p < 0.0001$). We evaluated the time to progression in the three groups of patients by censoring those who died before progression, see figure 2C . The Hazard ratios of the patients in the low and high density group were 1.85 (95% CI 1.24, 2.76), and 3.79 (95% CI 2.65, 5.42) relative to the non-transfused group. The recently revised IWG hematological response criteria in patients with MDS have refined the RBCT burden by dividing patients into 3 categories (non-transfused patients, low transfusion burden (0.75-2 units per month) and high transfusion burden patients ($\geq$ 2 units per months)[19]. Therefore, we repeated the analysis and subdivided the patients into 4 groups: no transfusions, >0 to <0.75 (low transfusion burden), 0.75 to 1.75 (mid transfusion burden) and >1.75 (high transfusion burden). The results are shown in figure 2D. The main effect occurred for low dose densities, such that the outcome of the mid and high transfusion density group was similar. The low transfusion burden

group is almost identical to the low burden group (<0.89 units per month) of figure 2B. MDS-related causes of death increased from 28% in the non-transfused group to 39% and 48% in the mid and high transfusion burden group (data not shown).

Impact of individual prognostic factors

The univariate effect of various covariates on the outcome was investigated in order to discover the appropriate functional form for the covariates (i.e. to discover whether a linear or non-linear form was best) and to discover appropriate ways of adjusting for confounding covariates. Increasing RBCT dose density was associated with inferior PFS ($p<10^{-4}$). The functional form is shown in figure 3A. The effect of the dose density increases until a dose density of about 1 unit per month; thereafter, the effect is flat. Baseline age (as continuous variable) was strongly associated with PFS ($p<1x10^{-4}$) in univariate regression analyses, as well as baseline MDS diagnosis ($p<1x10^{-4}$), quality of life measured by the EQ5D Index ($p<1x10^{-4}$), country ($p=0.002$), bone marrow blast count ($p<1x10^{-4}$), number of cytopenias ($p<1x10^{-4}$), IPSS-R cytogenetic category ($p<1x10^{-4}$), hemoglobin levels ($p<1x10^{-4}$), neutrophil levels ($p<1x10^{-4}$) and platelet levels ($p<1x10^{-4}$). No difference in PFS was detected by sex ($p=0.1$), but PFS in females was superior in the multivariate analyses.

Progression-free survival using time-varying covariates proportional hazards regression analysis

Variables used for adjustment at baseline included age at diagnosis, sex, country of origin, number of cytopenias (and their corresponding blood counts), number of units of blood received before registration. Time varying variables measured longitudinally included: dose density, EQ5D index, components of the IPSS-R, receipt of ESA, iron chelators and lenalidomide.

In multivariate analysis, not adjusting for the effects of ESA, iron chelation and lenalidomide therapy, all variables entered in the regression retained statistical significance. The functional form of the dose density effect ($p<10^{-4}$) was as shown in figure 3B. With a frailty term added for the subject country, all previously significant variables, including the dose density, retained statistical significance, with a dose density p-value of $<10^{-4}$.

Impact of therapeutic interventions on RBCT densities

Treatment with ESAs, lenalidomide and iron chelators may improve erythropoiesis and reduce the need for RBCT. Reduction of the RBCT rate will result in a gradual decrease of the subsequent RBCT dose densities in intervals during the response period. Therefore, we investigated how many of the

transfused patients had been treated with these interventions and calculated the average treatment duration and the number of patients with reduced transfusion densities after starting the intervention. In our cohort of 1267 patients, 679 of them received treatment with ESA and 151 had reduced transfusion densities in the first visit after starting ESA treatment. Supplementary figure 2 gives the individual dose density over time during ESA treatment of the 151 responding patients. Overall, 100 patients received treatment with lenalidomide and of these 53 patients had a reduced transfusion density in the first visit after starting lenalidomide treatment; Supplementary figure 3 shows the individual dose density over time during lenalidomide treatment of the 53 responding patients. Within our study group 186 patients received treatment with iron chelators and 75 patients had a response leading to reduced transfusion densities in the first interval after start of iron chelation treatmen (Supplementary figure 4). In contrast to the dose densities over time during ESA and lenalidomide treatment, the pattern of longer term dose densities during iron chelation appeared to show a more stable pattern. Subjects receiving a certain level of blood transfusion dose when they first receive iron chelation appear to be maintaining that level of dose density. The decline of the dose density is less pronounced, but this might be a reflection of the longer transfusion period before starting chelation treatment when compared with the other two interventions.

The observed patterns of dose density trajectories suggest that receiving ESA, lenalidomide and iron chelation therapy modulates the dose density and therefore we included these variables in the regression model. This analysis resulted in an effect for the dose density similar to the previous analyses (figure 3B), with a p-value of <0.0001 indeed all variables entered in the regression retained statistical significance, except for platelet count (p=0.47) and neutrophil count (p=0.24). However, the dose density effect continues to increase beyond 1 unit per month after correction for the three interventions (ESA, iron chelation and lenalidomide) up until a dose of 6 units per month (figure 3C).

Some patients received more than one intervention simultaneously, including 25 patients who received chelation and lenalidomide and 88 patients who received ESA and chelation. However, no additional impact could be detected over and above the impact of the two individual interventions.

Discussion

This large prospective, observational study confirmed the reported association of transfusion dose density with reduced PFS in patients with LR-MDS.[20] More surprisingly, we showed in this study that this negative association already occurred at a low transfusion rate. In addition, we showed that the risk of progression increased both in the low and high transfusion burden group when compared to the non-transfused patients. We could even show that the deleterious effect of transfusions occurred at a very low transfusion burden (< 0.75 units per month or <3 units per 16 weeks as defined in the revised IWG), when the patients were subdivided according to the revised IWG hematological response criteria[19]. These patients with a very low transfusion burden are considered as untransfused patients using the revised IWG response criteria[19].

The main focus of our study was to analyze the association of transfusion rate with outcome, assuming that regularly transfused patients may be exposed to the postulated toxicity of RBCT at a lower transfusion burden than generally accepted. Several studies have addressed this question using various definitions of transfusion rate. The initial publications describing the impact of RBCT on outcome in MDS compared RBCT dependent patients with RBCT independent patients, using RBCT dependency as a time dependent variable.[1,21] These studies were based on various definitions of RBCT dependency[22,23], including a study using a rigid criterion, which implied a RBCT rate of at least 1 unit per month during a period of 2 months.[24] In this last study, transfusion dependency occurred in a minority of the patients (35% to 44%). The use of this definition implies that patients receiving regularly less than 3 units per 16 weeks are defined as RBCT independent, but these patients might also be subject to the deleterious association with RBCT. In addition, patients may respond to therapeutic interventions, such as ESA, lenalidomide or iron chelators and become RBCT independent again. The conclusion was that the severity of anemia was the leading cause of impaired survival rather than RBCT dependency.[24] However, the definition of severe anemia (<9 g/dL in males and <8 g/dL in females) implies that the majority of these patients were regularly transfused, as confirmed in this study.[24] This study also showed that the transfusion rate was significantly associated with an increased risk of cardiac complications. The risk of cardiac complications was significantly higher in patients with a RBCT intensity of >3 units per months compared to patients transfused with <1 unit per month.[24] In an open forum discussion RBCT dependency was even defined much higher at 2 units per months in a 3-month interval.[25] A Spanish study in 191 transfused patients with MDS used the interval between each transfusion to calculate the transfusion intensity.[26] They concluded that high transfusion intensity was associated with decreased survival and increased risk for development of AML in concordance with our study. Interestingly, the

cumulative transfusion burden was not a prognostic factor when the transfusion intensity was included in the model.[26]

The traditional evaluation of prognostic impact of factors influencing outcome have used standard time-to-event methods based on variables at diagnosis, however, many variables in MDS may change over time. This aspect can be addressed by using proportional hazards regression with time-varying covariates. The EUMDS registry is collecting its observational data at registration of each new patient (within 100 days after diagnosis) and follow-up data at six months intervals. This practice leads to regular visit intervals of six months. For many patients in this dataset, the value of the recorded transfusion rate varies strongly over time, as shown in the supplementary files. Therefore, we calculated the RBC rate at each reported visit during all preceding visit intervals between the date of the first RBCT and the date of the last visit, leading to a "smoothed" variable, defined as dose density. This reflects an average rate of receiving transfusions during the whole observation period with transfusions. The relatively low number of RBCT units per months can be explained by the remarkable variation of the transfusion rate over time, even when using interval visit reports of 6 months duration.

Baseline age, bone marrow percentage category, number of cytopenias, and the EQ5D-index retained their significant prognostic impact in the proportional hazards regression with time-varying explanatory variables. Also, the non-linear component of the dose density effect was retained ($p<10^{-4}$). The unfavorable effect of the dose density increased until a dose density of about two units per month and leveled off thereafter. A similar form and effect was observed when using the cumulative dose of RBCT units over time in an identical multivariate regression model using the same variables (data not shown). The negative impact of the cumulative RBCT dose already starts at time of administering the first transfusion and does not increase any further beyond 30 units RBCTs received (data not shown).

Many patients showed a (temporary) decrease of the RBCT dose density, reflecting response to ESA[27], lenalidomide[28], and/or iron chelators[12] in 22%, 53% and 40% of the treated patients respectively. The observed patterns of dose density trajectories suggest that receipt of ESA, lenalidomide and iron chelation modulate the dose density and therefore we included these variables as confounding variables in the regression model. This analysis showed that the impact of the dose density remained similar to the previous analyses, but in contrast to the previous analyses there is some evidence that the dose density effect continues to increase beyond 2 units per month after correction for the three interventions.

RBCT are usually administered after a certain storage time, but the survival of stored RBC depends on this time.[29] [30] Transfusion of stored RBCs leads to pro-inflammatory reactions, associated with a higher risk of infection and increased levels of circulating iron and in particular non-transferrin bound iron (NTBI) species, which enhance bacterial growth in vitro.[31,32] Infusion of autologous RBC from healthy volunteers after increasing storage up to 6 weeks resulted in increased extravascular hemolysis, decreased RBC survival, elevated NTBI and ferritin levels in units transfused after 6 weeks compared to units transfused after shorter shortage.[33] Excess toxic iron species, including NTBI and especially its component Labile plasma iron (LPI)[34] catalyze the cellular generation of reactive oxygen species. Oxidative stress may lead to pro-inflammatory responses and to oxidation of lipids, proteins and DNA causing cell and tissue damage.[35,36] Elevated NTBI levels after a single unit of RBC stored for 6 weeks normalize within 24 hours.[37] However, in multi-transfused patients (cumulative number of units $\geq$10) with MDS, NTBI and LPI remained elevated up to the next transfusion.[17]

Conclusion The negative association of transfusions on PFS already occurs at low RBCT dose densities below 3 units per 16 weeks. This indicates that the RBCT dependency in patients transfused at relatively low rates, who are usually considered as untransfused patients, may be considered as an indicator of poor prognosis for progression-free survival. This poor prognosis in transfusion dependent patients might be the result of direct toxicity by the toxic iron radicals resulting from the RBCT or the result of concomitant disease progression, including hematopoietic impairment. The data in the chelation study from our group in this issue provides support for the direct toxicity of RBCT density on outcome, because chelated patients have a better outcome, if treated with chelators, which remove toxic iron radicals effectively. Future studies, including interventional studies, are needed to confirm our observations, which may lead to adaptations of the current recommendations.

References

1. Cazzola M, Malcovati L. Myelodysplastic syndromes--coping with ineffective hematopoiesis. N Engl J Med. 2005;352(6):536-538.
2. Malcovati L, Porta MG, Pascutto C, et al. Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: a basis for clinical decision making. J Clin Oncol. 2005;23(30):7594-7603.
3. Greenberg P, Cox C, LeBeau MM, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood. 1997;89(6):2079-2088.
4. de Swart L, Smith A, Johnston TW, et al. Validation of the revised international prognostic scoring system (IPSS-R) in patients with lower-risk myelodysplastic syndromes: a report from the prospective European LeukaemiaNet MDS (EUMDS) registry. Br J Haematol. 2015;170(3):372-383.
5. Zipperer E, Post JG, Herkert M, et al. Serum hepcidin measured with an improved ELISA correlates with parameters of iron metabolism in patients with myelodysplastic syndrome. Ann Hematol. 2013;92(12):1617-1623.
6. Santini V, Girelli D, Sanna A, et al. Hepcidin levels and their determinants in different types of myelodysplastic syndromes. PLoS One. 2011;6(8):e23109.
7. Cuijpers ML, Raymakers RA, Mackenzie MA, de Witte TJ, Swinkels DW. Recent advances in the understanding of iron overload in sideroblastic myelodysplastic syndrome. Br J Haematol. 2010;149(3):322-333.
8. Shenoy N, Vallumsetla N, Rachmilewitz E, Verma A, Ginzburg Y. Impact of iron overload and potential benefit from iron chelation in low-risk myelodysplastic syndrome. Blood. 2014;124(6):873-881.
9. Ambaglio I, Malcovati L, Papaemmanuil E, et al. Inappropriately low hepcidin levels in patients with myelodysplastic syndrome carrying a somatic mutation of SF3B1. Haematologica. 2013;98(3):420-423.
10. Rund D, Rachmilewitz E. Beta-thalassemia. N Engl J Med. 2005;353(11):1135-1146.
11. Gattermann N, Rachmilewitz EA. Iron overload in MDS-pathophysiology, diagnosis, and complications. Ann Hematol. 2011;90(1):1-10.
12. Gattermann N, Finelli C, Della Porta M, et al. Hematologic responses to deferasirox therapy in transfusion-dependent patients with myelodysplastic syndromes. Haematologica. 2012;97(9):1364-1371.
13. Ghoti H, Amer J, Winder A, Rachmilewitz E, Fibach E. Oxidative stress in red blood cells, platelets and polymorphonuclear leukocytes from patients with myelodysplastic syndrome. Eu J Haematol. 2007;79(6):463-467.
14. De Souza GF, Ribeiro HL, Jr., De Sousa JC, et al. HFE gene mutation and oxidative damage biomarkers in patients with myelodysplastic syndromes and its relation to transfusional iron overload: an observational cross-sectional study. BMJ Open. 2015;5(4):e006048.
15. Saigo K, Takenokuchi M, Hiramatsu Y, et al. Oxidative stress levels in myelodysplastic syndrome patients: their relationship to serum ferritin and haemoglobin values. J Int Med Res. 2011;39(5):1941-1945.
16. Bulycheva E, Rauner M, Medyouf H, et al. Myelodysplasia is in the niche: novel concepts and emerging therapies. Leukemia. 2015;29(2):259-268.
17. de Swart L, Reiniers C, Bagguley T, et al. Labile plasma iron levels predict survival in patients with lower-risk myelodysplastic syndromes. Haematologica. 2018;103(1):69-79.
18. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood. 2002;100(7):2292-2302.
19. Platzbecker U, Fenaux P, Ades L, et al. Proposals for revised IWG 2018 hematological response criteria in patients with MDS included in clinical trials. Blood. 2018;133(10):1020-1030.

20. Goldberg SL, Chen E, Corral M, et al. Incidence and clinical complications of myelodysplastic syndromes among United States Medicare beneficiaries. J Clin Oncol. 2010;28(17):2847-2852.
21. Hiwase DK, Singhal D, Strupp C, et al. Dynamic assessment of RBC-transfusion dependency improves the prognostic value of the revised-IPSS in MDS patients. Am J Hematol. 2017;92(6):508-514.
22. Malcovati L, Germing U, Kuendgen A, et al. Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol. 2007;25(23):3503-3510.
23. Waszczuk-Gajda A, Madry K, Machowicz R, et al. Red Blood Cell Transfusion Dependency and Hyperferritinemia Are Associated with Impaired Survival in Patients Diagnosed with Myelodysplastic Syndromes: Results from the First Polish MDS-PALG Registry. Adv Clin Exp Med. 2016;25(4):633-641.
24. Malcovati L, Della Porta MG, Strupp C, et al. Impact of the degree of anemia on the outcome of patients with myelodysplastic syndrome and its integration into the WHO classification-based Prognostic Scoring System (WPSS). Haematologica. 2011;96(10):1433-1440.
25. Gale RP, Barosi G, Barbui T, et al. What are RBC-transfusion-dependence and -independence? Leuk Res. 2011;35(1):8-11.
26. Pereira A, Nomdedeu M, Aguilar JL, et al. Transfusion intensity, not the cumulative red blood cell transfusion burden, determines the prognosis of patients with myelodysplastic syndrome on chronic transfusion support. Am J Hematol. 2011;86(3):245-250.
27. Hellstrom-Lindberg E, Negrin R, Stein R, et al. Erythroid response to treatment with G-CSF plus erythropoietin for the anaemia of patients with myelodysplastic syndromes: proposal for a predictive model. Br J Haematol. 1997;99(2):344-351.
28. Fenaux P, Giagounidis A, Selleslag D, et al. A randomized phase 3 study of lenalidomide versus placebo in RBC transfusion-dependent patients with Low-/Intermediate-1-risk myelodysplastic syndromes with del5q. Blood. 2011;118(14):3765-3776.
29. Luten M, Roerdinkholder-Stoelwinder B, Schaap NP, de Grip WJ, Bos HJ, Bosman GJ. Survival of red blood cells after transfusion: a comparison between red cells concentrates of different storage periods. Transfusion. 2008;48(7):1478-1485.
30. van de Watering L, Lorinser J, Versteegh M, Westendord R, Brand A. Effects of storage time of red blood cell transfusions on the prognosis of coronary artery bypass graft patients. Transfusion. 2006;46(10):1712-1718.
31. Hod EA, Zhang N, Sokol SA, et al. Transfusion of red blood cells after prolonged storage produces harmful effects that are mediated by iron and inflammation. Blood. 2010;115(21):4284-4292.
32. McQuilten ZK, French CJ, Nichol A, Higgins A, Cooper DJ. Effect of age of red cells for transfusion on patient outcomes: a systematic review and meta-analysis. Transfus Med Rev. 2018;32(2):77-88.
33. Rapido F, Brittenham GM, Bandyopadhyay S, et al. Prolonged red cell storage before transfusion increases extravascular hemolysis. J Clin Invest. 2017;127(1):375-382.
34. de Swart L, Hendriks JC, van der Vorm LN, et al. Second international round robin for the quantification of serum non-transferrin-bound iron and labile plasma iron in patients with iron-overload disorders. Haematologica. 2016;101(1):38-45.
35. Rachmilewitz EA, Weizer-Stern O, Adamsky K, et al. Role of iron in inducing oxidative stress in thalassemia: Can it be prevented by inhibition of absorption and by antioxidants? Ann N Y Acad Sci. 2005;1054:118-123.
36. Hershko C, Link G, Cabantchik I. Pathophysiology of iron overload. Ann N Y Acad Sci. 1998;850:191-201.
37. Hod EA, Brittenham GM, Billote GB, et al. Transfusion of human volunteers with older, stored red blood cells produces extravascular hemolysis and circulating non-transferrin-bound iron. Blood. 2011;118(25):6675-6682.

Tables

Table 1: Baseline Characteristics from time of diagnosis & PFS, stratified according to transfusion status at landmark (Visit 3)

	Total	Hazard Ratio (95%CI)	Adjusted Hazard Ratio (95%CI) (95%CI)	Transfusion Status at landmark	
				No	Yes
Total	1267 (100.0)			751 (100.0)	516 (100.0)
Median age at diagnosis	73.0 (18.0 - 95.0)	1.03 (1.02 - 1.04)	1.03 (1.02 - 1.04)	73.0 (18.0 - 91.0)	73.0 (21.0 - 95.0)
Sex:					
Male	757 (59.7)	1	1	445 (59.3)	312 (60.5)
Female	510 (40.3)	0.84 (0.70 - 1.01)	0.76 (0.62 - 0.92)	306 (40.7)	204 (39.5)
WHO Diagnosis:					
RA	218 (17.2)	0.84 (0.64 - 1.10)	0.78 (0.59 - 1.03)	139 (18.5)	79 (15.3)
RARS	214 (16.9)	0.73 (0.56 - 0.96)	0.59 (0.45 - 0.78)	123 (16.4)	91 (17.6)
RCMD	492 (38.8)	1	1	296 (39.4)	196 (38.0)
RCMD-RS	86 (6.8)	1.03 (0.72 - 1.46)	0.91 (0.64 - 1.30)	47 (6.3)	39 (7.6)
RAEB-1	133 (10.5)	1.58 (1.20 - 2.07)	1.86 (1.41 - 2.46)	78 (10.4)	55 (10.7)
MDS-U	41 (3.2)	0.64 (0.34 - 1.22)	0.68 (0.36 - 1.29)	27 (3.6)	14 (2.7)
Deletion 5q	83 (6.6)	0.61 (0.40 - 0.92)	0.54 (0.35 - 0.83)	41 (5.5)	42 (8.1)
MDS Comorbidity Index:					
Low	782 (61.7)	1	1	482 (64.2)	300 (58.1)
Intermediate	411 (32.4)	1.24 (1.02 - 1.50)	1.08 (0.88 - 1.31)	232 (30.9)	179 (34.7)
High	71 (5.6)	1.55 (1.08 - 2.22)	1.30 (0.90 - 1.89)	35 (4.7)	36 (7.0)
Not known	3 (0.2)	-	-	2 (0.3)	1 (0.2)
Karnofsky Status:					
80-100	881 (69.5)	1	1	543 (72.3)	338 (65.5)
50-70	210 (16.6)	1.72 (1.38 - 2.15)	1.40 (1.10 - 1.77)	93 (12.4)	117 (22.7)
10-40	10 (0.8)	2.04 (0.76 - 5.48)	1.89 (0.69 - 5.15)	3 (0.4)	7 (1.4)
Not known	166 (13.1)	1.08 (0.80 - 1.45)	0.99 (0.73 - 1.34)	112 (14.9)	54 (10.5)
Quality of life					
Visual analogue score, mean (sd)	70.5 (19.7)	0.99 (0.98 - 0.99)	0.99 (0.99 - 1.00)	73.1 (18.9)	66.8 (20.2)

	Total	Hazard Ratio (95%CI)	Adjusted Hazard Ratio (95%CI) (95%CI)	Transfusion Status at landmark	
				No	Yes
IPSS category					
Low	680 (53.7)	1	1	460 (61.3)	220 (42.6)
Intermediate	557 (44.0)	1.95 (1.62 - 2.34)	1.71 (1.39 - 2.11)	274 (36.5)	283 (54.8)
Cytogenetics not done	30 (2.4)	0.83 (0.43 - 1.62)	0.74 (0.38 - 1.45)	17 (2.3)	13 (2.5)
Revised IPSS category					
Very low	386 (30.5)	1	1	310 (41.3)	76 (14.7)
Low	571 (45.1)	1.80 (1.41 - 2.29)	1.85 (1.45 - 2.37)	309 (41.1)	262 (50.8)
Intermediate	204 (16.1)	3.19 (2.41 - 4.22)	3.40 (2.55 - 4.52)	89 (11.9)	115 (22.3)
High	39 (3.1)	4.27 (2.72 - 6.71)	4.59 (2.91 - 7.22)	11 (1.5)	28 (5.4)
Very high	3 (0.2)	3.15 (0.78 - 12.82)	4.65 (1.13 - 19.15)	1 (0.1)	2 (0.4)
Not known	64 (5.1)	1.69 (1.07 - 2.68)	1.76 (1.11 - 2.80)	31 (4.1)	33 (6.4)

Legend table 1: Baseline characteristics of the included patients from time of diagnosis and progression-free survival, stratified according to transfusion status at landmark (Visit 3).

[1]Hazard Ratios (HR) & 95% Confidence Intervals (CI) adjusted for all other variables in the table,

[2]Refractory anemia (RA), Refractory anemia with ring sideroblasts (RARS), Refractory cytopenia with multilineage dysplasia (RCMD), Refractory cytopenia with multilineage dysplasia & ring sideroblasts (RARS), Myelodysplastic syndrome, unclassifiable (MDS-U), 5q-syndrome.

[3]Myelodysplastic syndrome-specific co morbidity index (MDS-CI).

[4]Visual analogue scale (VAS).

Figures

Figure 1: Distribution of Dose Densities of all transfused patients in the interval preceding the landmark of one year

Legend figure 1:
Frequency: number of patients in each dose dose density ranging from >0 to 0.2 units per month to >6 units per month

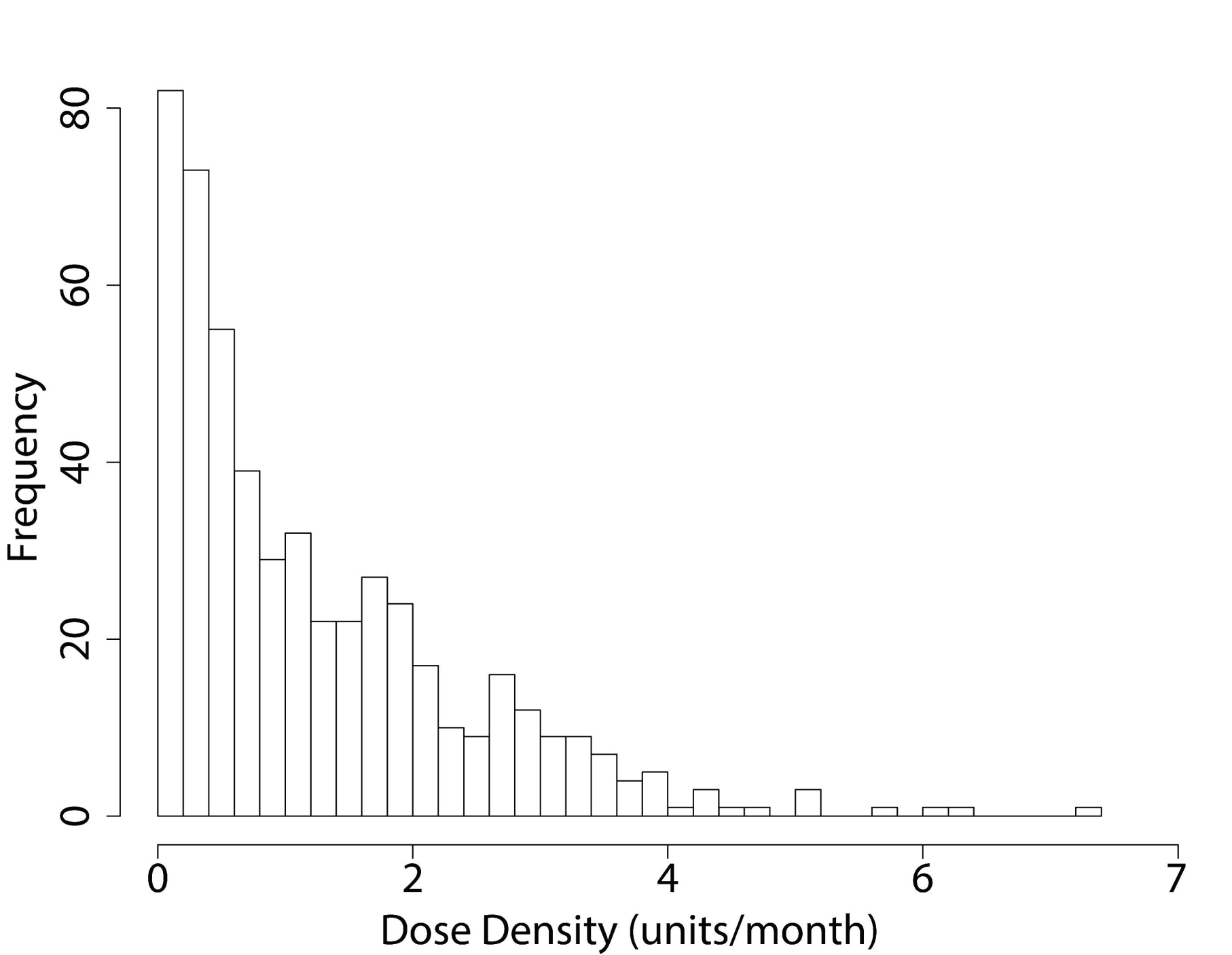
Figure 2: Progression-free survival and risk of progression according to transfusion status at landmark of visit 3 (1 year after registration)

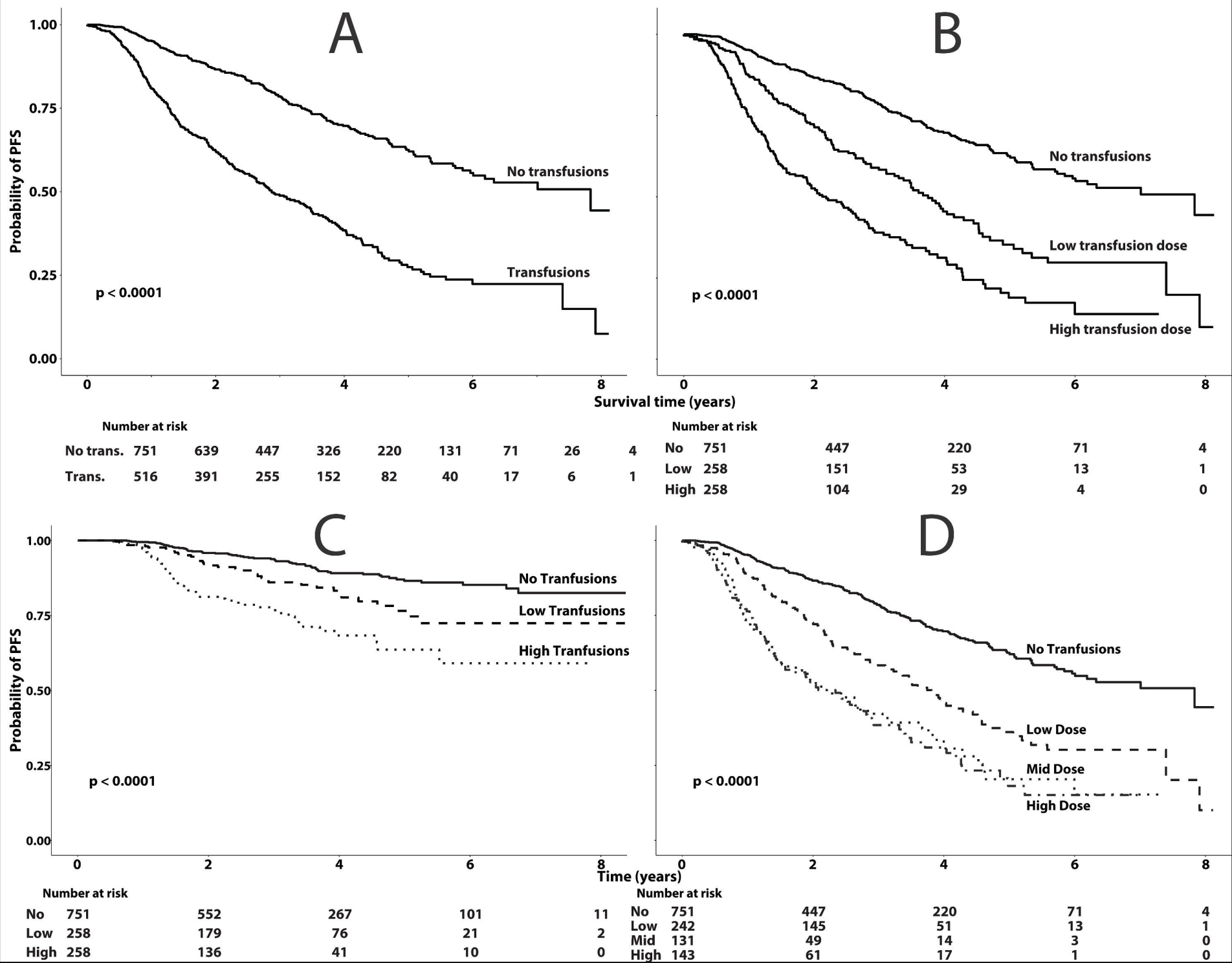
Legend figure 2:

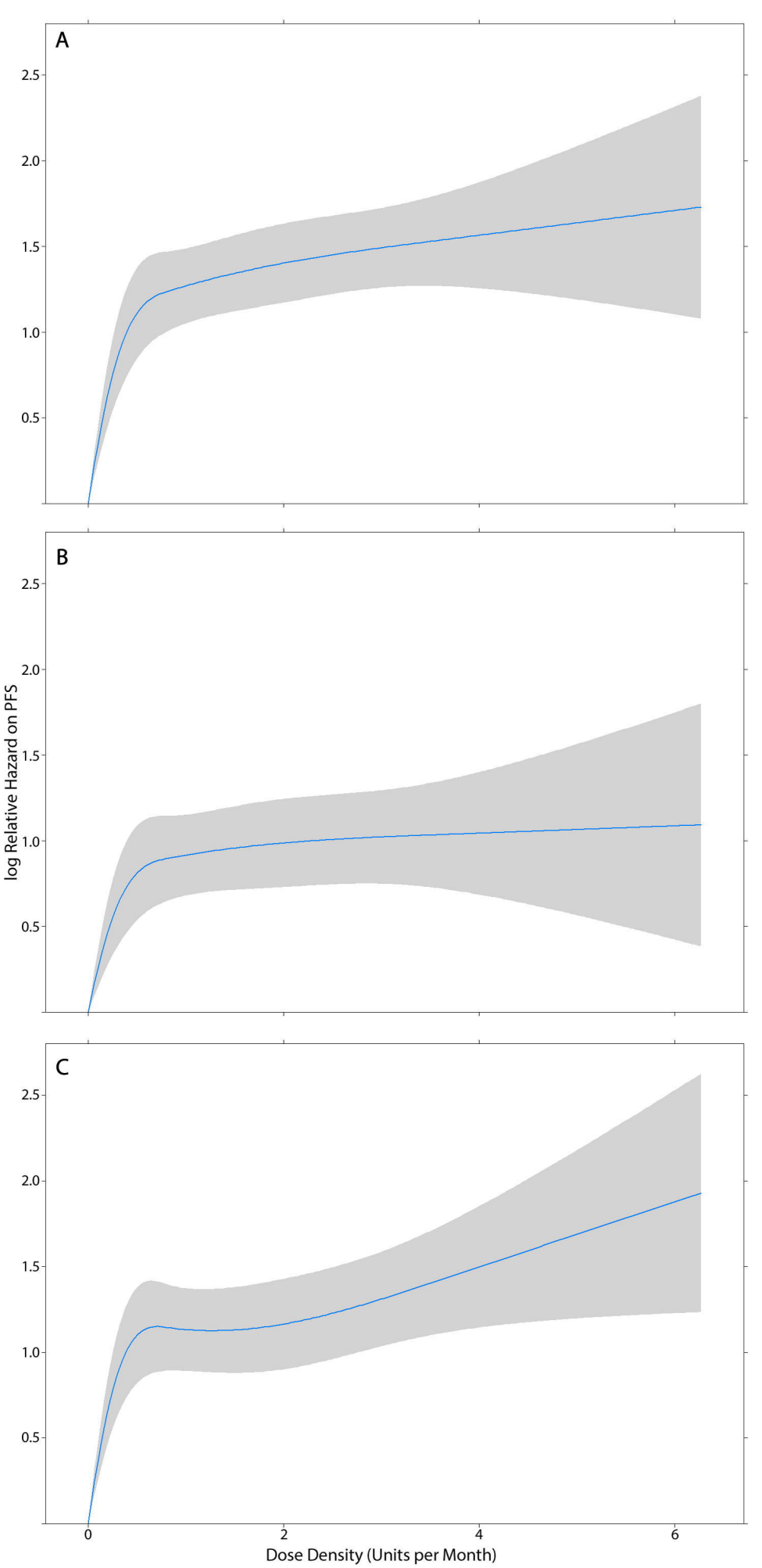
A; Kaplan-Meijer plot of progression-free survival of patients receiving transfusions by landmark (visit 3) versus not receiving transfusions. B; Kaplan-Meijer plot of progression-free survival of patients receiving transfusions at a low density (<0.87 units/month) by landmark or at a high density (>0.87 units/month) versus not receiving transfusions; C; Kaplan-Meijer plot of time to progression of patients surviving till progression subdivided according to transfusion burden or not as in figure B; D; Kaplan-Meijer plot of progression-free survival of patients receiving transfusions at densities according to the revised IWG criteria: low dose density: >0- <0.75 units/month; mid dose density: 0.75 to 1.75 units per months; high dose density >1.75 units per month.

Figure 3: Influence of dose density on progression-free survival

Legend figure 3:
A; Dose density effect on progression-free survival (PFS) in an univariate analysis. B; Dose density effect on PFS in a multivariate regression model unadjusted for the three treatment variables. C; Dose density effect on PFS in a multivariate regression model adjusted for treatment with either ESA, Iron Chelation or Lenalidomide.


Frequency
0
20
40
60
80
0
2
4
6
7
Dose Density (units/month)


A
B
C
D
Probability of PFS
1.00
0.75
0.50
0.25
0.00
No transfusions
Transfusions
p < 0.0001
No transfusions
Low transfusion dose
High transfusion dose
p < 0.0001
No Tranfusions
Low Tranfusions
High Tranfusions
p < 0.0001
No Tranfusions
Low Dose
Mid Dose
High Dose
p < 0.0001
0
2
4
6
8
Survival time (years)
Time (years)
Number at risk
No trans. 751 639 447 326 220 131 71 26 4
Trans. 516 391 255 152 82 40 17 6 1
Number at risk
No 751 447 220 71 4
Low 258 151 53 13 1
High 258 104 29 4 0
Number at risk
No 751 552 267 101 11
Low 258 179 76 21 2
High 258 136 41 10 0
Number at risk
No 751 447 220 71 4
Low 242 145 51 13 1
Mid 131 49 14 3 0
High 143 61 17 1 0


A
B
C
log Relative Hazard on PFS
2.5
2.0
1.5
1.0
0.5
0
2
4
6
Dose Density (Units per Month)

Supplementary Material

Supplementary Method Section

Data Handling and Exclusions

The dataset contained data from 1504 patients having three or more visits recorded. Within this dataset, there were 368 deaths and 181 progressions to high-risk MDS or AML, which were considered as events for this analysis. The following exclusions were made:

- 2 patients with RAEB-2 were removed leaving 1502 patients.
- 1 patient was deleted as the subject had a very long pre-diagnosis transfusion history.

These exclusions leave 1501 patients in the cohort.

We also removed all patients that had completely missing values for any of the following variables: Haemoglobin (0 patients have all observations missing), neutrophils (2 patients), platelets (1 patient), bone marrow blast count (60 patients), cytogenetic risk category (112 patients), EQ5D-index (101 patients).

This left a total of 1267 patients in the analysis dataset, with 407 deaths of which 90 had progression. 72 Patients survived until the end of the study period but progressed to high-risk MDS or AML.

For the analysis dataset, missing values in these variables were imputed with last observation carried forward or next observation carried back.

Handling of missing transfusion data

10 remaining subjects were flagged as having received transfusions before diagnosis, but were missing the number of units received. Those 4 transfused more than a year before diagnosis were set to no transfusions received. Those 6 transfused less than a year before diagnosis were assigned either an imputed 2 units (a plausible figure) or 4 units (the mean number received among those receiving pre-diagnosis transfusion) according to the actual length of time before diagnosis.

Six additional subjects were identified as having received transfusions a very long time before diagnosis (more than 600 days). These patients were set to not having received pre-diagnosis transfusions unless they had received more than 7 units (3 patients).

The Definition of Dose Density

For each patient, the transfusion data available consisted of the number of units received between each visit. This variable, which we call dose, is expressed as an average of "units received per month" in each inter-visit interval.

The analysis used is proportional hazards regression with time-varying covariates. The basic interval on which all variables are defined is the interval between visits. Therefore, covariates are assigned to be piecewise constant on these time intervals.

In standard survival analysis, the hazard at any instant is assumed to be modified by the value of the explanatory variables at that instant. This presented three problems for the analysis with the dose transfusion variable:

- For many patients in this dataset, the value of the recorded transfusion dose variable is very "spiky", varying strongly over time. It seems unlikely that the actual value of the hazard would follow such a form.
- It is unlikely that the hazard will respond instantaneously to the transfusion dose received.
- It is observed that, in this dataset, patients receive fewer transfusions in the interval in which death occurs, than in the intervals before the interval in which death occurs (Supplement figure 1). Presumably, the treatment focus switches to palliative care on the approach to death. This would mean that the event of death is correlated with zero or low values of transfusion dose, leading to a hazard estimate that is high for low values of dose and which reduces as dose increases.

Rather, it seems more likely that the association between transfusions and hazard would be better expressed as an association with some sort of cumulative dose value (reflecting the idea that the hazard at any time is proportional to the total dose received) or with some other "smoothed" variable that reflects an average rate of receiving transfusions.

In order to perform this smoothing, the cumulative total dose at the end of each inter-visit time interval was calculated. This was then divided by the time (in months) since the beginning of the time interval in which the first post-diagnosis transfusion was received, giving a dose-density measurement. This dose-density is then assigned to each time interval. The value of this variable at each point in time represents the average rate at which the patient has been receiving units of blood

since they started transfusions. This main variable of interest, the dose density, was modelled in regression analysis using restricted cubic splines with four knots.

With the dose density defined as such, the hazard is taken as proportional to the number of units received since transfusions started divided by the time since transfusions started. The effect of this is to allow the hazard to depend upon something that has happened in the past; but the strength of the effect will decay as time passes and no further transfusions are received. Contrast this with simply using the cumulative number of units received at any point in time. Here, again, the effect on the hazard is proportional to the dose received in the past, but there is now no decay in the size of the effect.

Results

Supplementary Table 1: Baseline Characteristics from time of diagnosis & PFS, stratified according to transfusion status at landmark (Visit 3)

	Total	Hazard Ratio (95%CI)	Adjusted Hazard Ratio[1] (95%CI) (95%CI)	Transfusion Status at landmark	
				No	yes
Total	1267 (100.0)			751 (100.0)	516 (100.0)
Country:					
Austria	86 (6.8)	0.84 (0.56 - 1.27)	0.76 (0.49 - 1.18)	61 (8.1)	25 (4.8)
Czech Republic	94 (7.4)	0.82 (0.57 - 1.19)	0.89 (0.61 - 1.31)	43 (5.7)	51 (9.9)
Denmark	47 (3.7)	1.94 (1.25 - 3.02)	1.97 (1.26 - 3.07)	17 (2.3)	30 (5.8)
France	313 (24.7)	1	1	200 (26.6)	113 (21.9)
Germany	25 (2.0)	1.09 (0.62 - 1.94)	1.34 (0.75 - 2.40)	17 (2.3)	8 (1.6)
Greece	128 (10.1)	0.85 (0.60 - 1.20)	0.90 (0.63 - 1.30)	81 (10.8)	47 (9.1)
Israel	67 (5.3)	0.83 (0.46 - 1.47)	0.92 (0.51 - 1.64)	47 (6.3)	20 (3.9)
Italy	46 (3.6)	0.47 (0.23 - 0.96)	0.62 (0.30 - 1.27)	33 (4.4)	13 (2.5)
Netherlands	44 (3.5)	0.75 (0.44 - 1.29)	0.96 (0.56 - 1.65)	28 (3.7)	16 (3.1)
Poland	31 (2.4)	1.96 (1.19 - 3.22)	1.45 (0.85 - 2.47)	13 (1.7)	18 (3.5)
Portugal	2 (0.2)	-	-	0 (0.0)	2 (0.4)
Romania	17 (1.3)	0.28 (0.09 - 0.87)	0.15 (0.05 - 0.50)	7 (0.9)	10 (1.9)
Serbia	14 (1.1)	2.02 (0.94 - 4.33)	1.32 (0.61 - 2.87)	5 (0.7)	9 (1.7)
Spain	85 (6.7)	1.01 (0.67 - 1.53)	1.18 (0.77 - 1.79)	54 (7.2)	31 (6.0)
Sweden	88 (6.9)	0.87 (0.61 - 1.24)	0.99 (0.68 - 1.43)	45 (6.0)	43 (8.3)
United Kingdom	180 (14.2)	0.99 (0.75 - 1.32)	1.07 (0.80 - 1.44)	100 (13.3)	80 (15.5)
Ring Sideroblasts:					
No	967 (76.3)	1	1	581 (77.4)	386 (74.8)
Yes	300 (23.7)	0.83 (0.68 - 1.03)	0.78 (0.63 - 0.98)	170 (22.6)	130 (25.2)
IPSS cytogenetic score					
Good	1052 (83.0)	1	1	655 (87.2)	397 (76.9)
Intermediate	170 (13.4)	1.87 (1.49 - 2.35)	1.92 (1.52 - 2.43)	73 (9.7)	97 (18.8)
Poor	15 (1.2)	2.30 (1.18 - 4.46)	2.36 (1.21 - 4.60)	6 (0.8)	9 (1.7)
Cytogenetics not done	30 (2.4)	0.68 (0.35 - 1.32)	0.68 (0.35 - 1.32)	17 (2.3)	13 (2.5)

	Total	Hazard Ratio (95%CI)	Adjusted Hazard Ratio[1] (95%CI) (95%CI)	Transfusion Status at landmark	
				No	yes
Revised IPSS cytogenetic score					
Very good	121 (9.6)	0.94 (0.66 - 1.33)	0.86 (0.60 - 1.23)	87 (11.6)	34 (6.6)
Good	963 (76.0)	1	1	594 (79.1)	369 (71.5)
Intermediate	141 (11.1)	2.56 (2.02 - 3.25)	2.47 (1.93 - 3.15)	55 (7.3)	86 (16.7)
Poor/ Very Poor	23 (1.8)	1.41 (0.75 - 2.65)	1.26 (0.67 - 2.38)	9 (1.2)	14 (2.7)
Not known	19 (1.5)	1.81 (0.99 - 3.29)	1.63 (0.89 - 3.00)	6 (0.8)	13 (2.5)

Legend: Baseline characteristics of the included patients from time of diagnosis and progression-free survival, stratified according to transfusion status at landmark (Visit 3).

[1]Hazard Ratios (HR) & 95% Confidence Intervals (CI) adjusted for all other variables, as described in Table 1 in the manuscript,

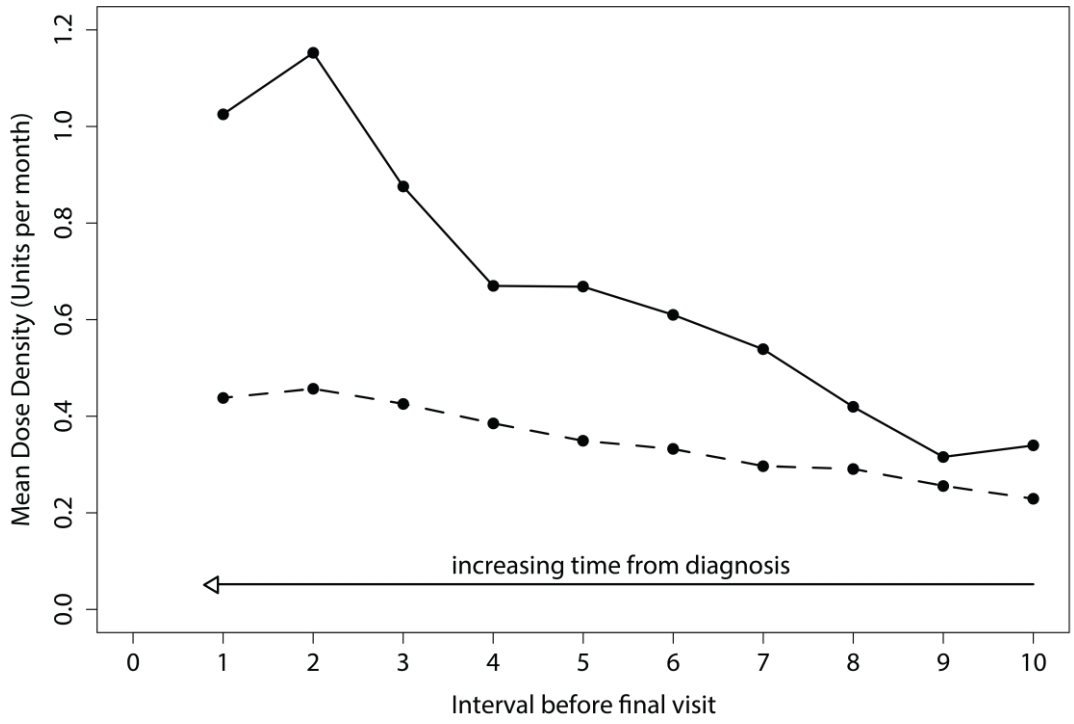
Supplementary Table 2 Characteristics at time of landmark Visit 3 stratified according to transfusion status at landmark (Visit 3)

	Total	Transfusion Status at landmark	
		No	Yes
Total	1267 (100.0)	751 (100.0)	516 (100.0)
Median age at visit 3	74.5 (20.1 - 96.3)	74.2 (20.1 - 92.9)	74.8 (22.2 - 96.3)
WHO Diagnosis[2]:			
RA	130 (10.3)	82 (10.9)	48 (9.3)
RARS	166 (13.1)	91 (12.1)	75 (14.5)
RCMD	328 (25.9)	190 (25.3)	138 (26.7)
RCMD-RS	46 (3.6)	23 (3.1)	23 (4.5)
RAEB-1	103 (8.1)	57 (7.6)	46 (8.9)
RAEB-2	30 (2.4)	5 (0.7)	25 (4.8)
MDS-U	27 (2.1)	21 (2.8)	6 (1.2)
Deletion 5q	70 (5.5)	31 (4.1)	39 (7.6)
Bone Marrow not done	367 (29.0)	251 (33.4)	116 (22.5)
MDS-CI[3]:			
Low	928 (73.2)	583 (77.6)	345 (66.9)
Intermediate	293 (23.1)	154 (20.5)	139 (26.9)
High	35 (2.8)	10 (1.3)	25 (4.8)
Not known	11 (0.9)	4 (0.5)	7 (1.4)
Karnofsky Status:			
80-100	738 (58.2)	496 (66.0)	242 (46.9)
50-70	205 (16.2)	74 (9.9)	131 (25.4)
10-40	25 (2.0)	11 (1.5)	14 (2.7)
Not known	299 (23.6)	170 (22.6)	129 (25.0)
Quality of life			
Visual analogue score, mean (sd)	69.7 (19.0)	73.6 (18.5)	64.1 (18.2)
Revised IPSS category			
Very low	384 (30.3)	305 (40.6)	79 (15.3)
Low	576 (45.5)	314 (41.8)	262 (50.8)
Intermediate	203 (16.0)	88 (11.7)	115 (22.3)
High	45 (3.6)	16 (2.1)	29 (5.6)
Very high	4 (0.3)	1 (0.1)	3 (0.6)
Not known	55 (4.3)	27 (3.6)	28 (5.4)

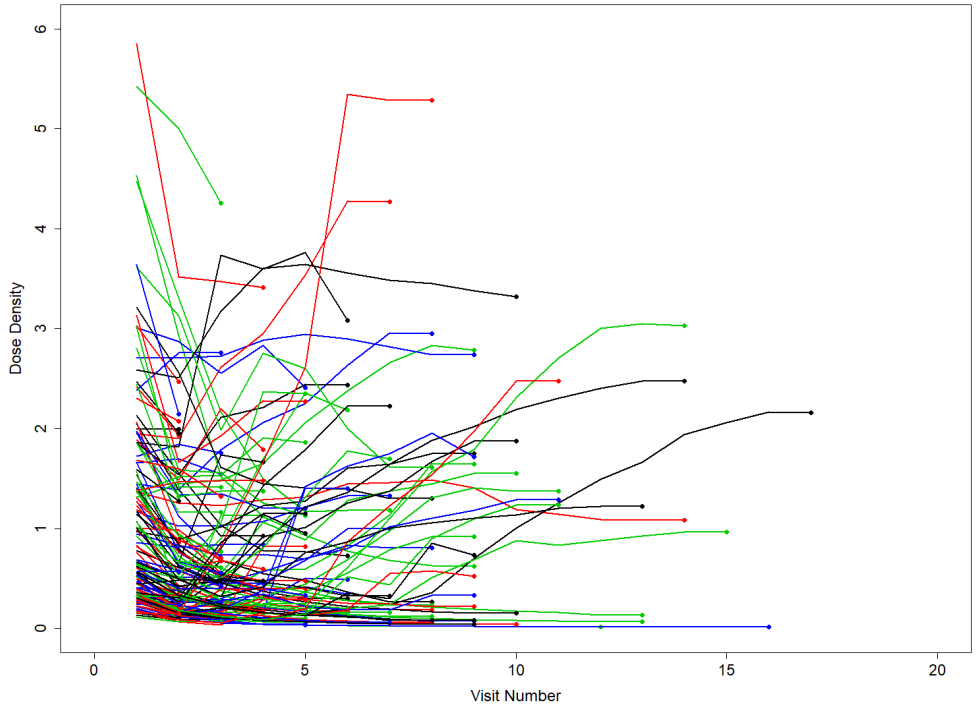
[2]Refractory anemia (RA), Refractory anemia with ring sideroblasts (RARS), Refractory cytopenia with multilineage dysplasia (RCMD), Refractory cytopenia with multilineage dysplasia & ring sideroblasts (RARS), Refractory anemia with excess blasts-1 (RAEB-I), Refractory anemia with excess blasts-2 (RAEB-II), Refractory anemia with excess blasts-2 (RAEB-II), Myelodysplastic syndrome, unclassifiable (MDS-U).

[3]Myelodysplastic syndrome-specific comorbidity index (MDS-CI).

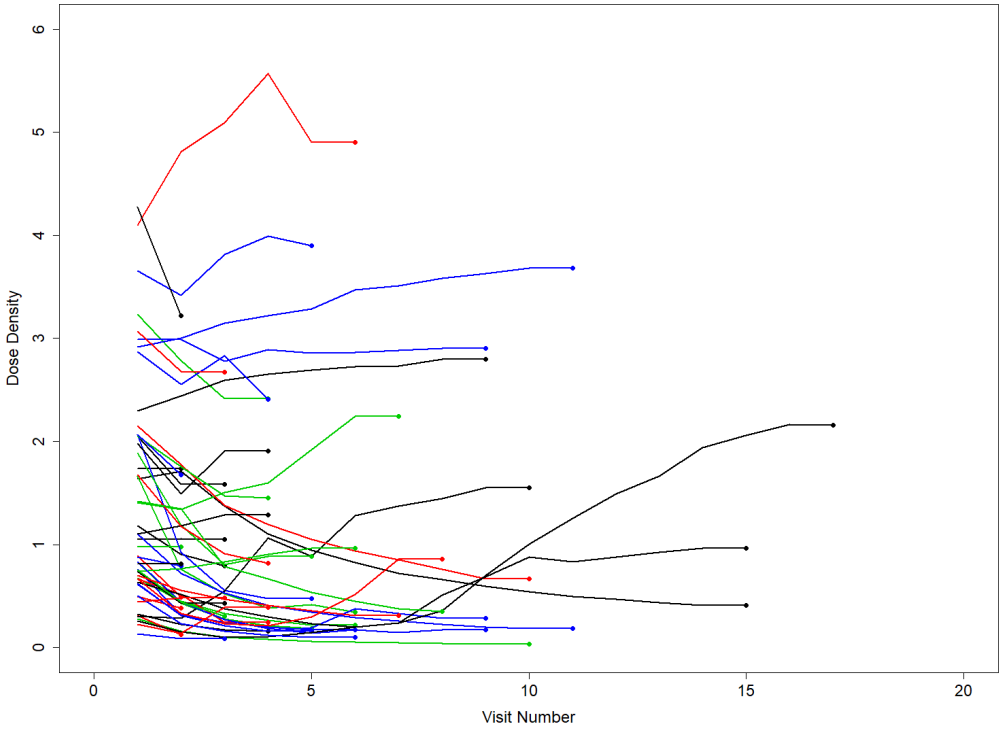
Supplementary figure 1: Mean number of transfused units per month, counting back from the final interval before death, transformation (solid line) or censoring for last interval report, alive and well (broken line).



Supplementary figure 2 Dose Density Trajectories for all Subjects with Initial Response to ESA.



Supplementary figure 3 Dose Density Trajectories for all Subjects with Initial Response to Lenalidomide.



Supplementary figure 4 Dose Density Trajectories for all Subjects with Initial Response to Iron Chelation.