

# High transferrin saturation predicts inferior clinical outcomes in patients with MDS

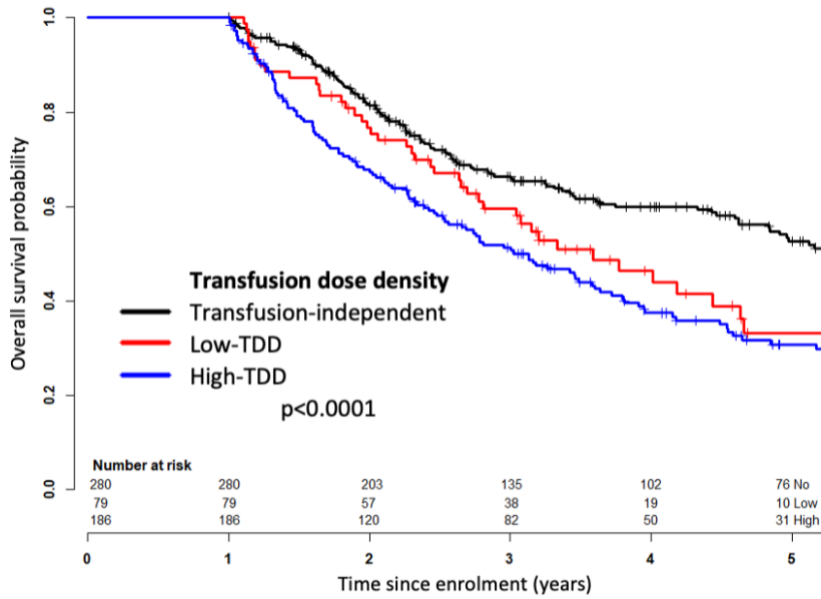
Teichman J, et al.

## Supplementary Material

**Table S1.** Demographic and clinical variables stratified by transfusion density (TD), where TD-low and TD-high were defined as below and above the median TD, respectively.

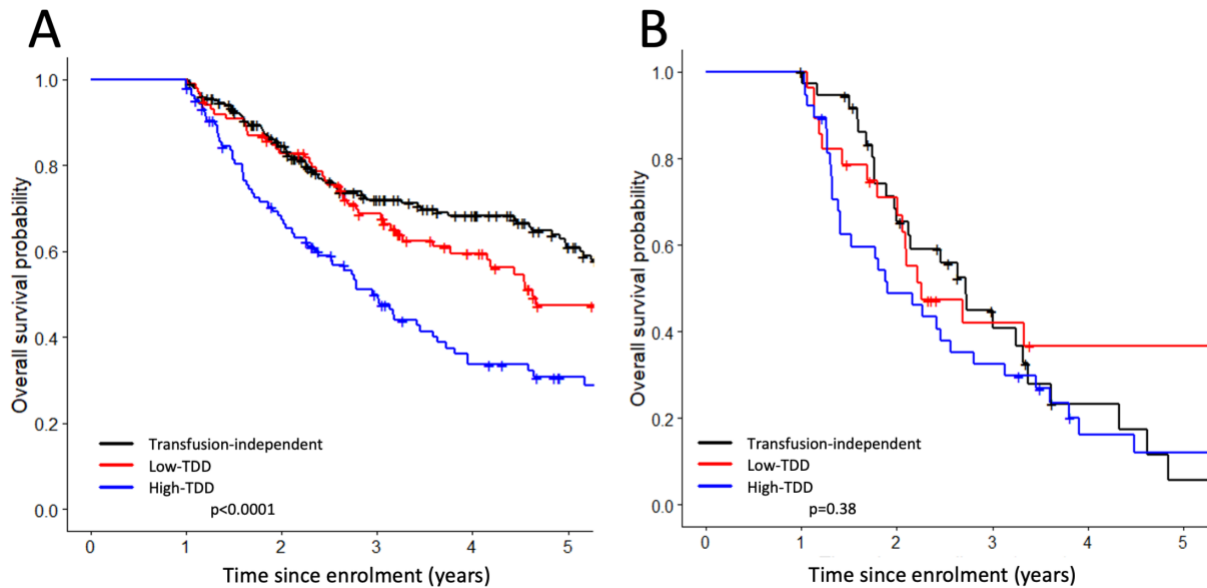
|  | <i>At Landmark Year 1<br/>(calculation from the first transfusion date)</i> |                                     |                                      | <i>p-value</i>   |
|--|---|-------------------------------------|--------------------------------------|------------------|
|  | <i>No transfusions<br/>(n=259)</i>  | <i>Low transfusion dose (n=138)</i> | <i>High transfusion dose (n=148)</i> |                  |
| Age categories                                 |   |                                     |                                      | 0.1573           |
| ≤ 60   | 33 (12.74%)   | 7 (5.07%)                           | 16 (10.81%)                          |                  |
| 61-70  | 65 (25.10%)   | 35 (25.36%)                         | 40 (27.03%)                          |                  |
| > 70   | 161 (62.16%)  | 96 (69.57%)                         | 92 (62.16%)                          |                  |
| Sex  |   |                                     |                                      | 0.1566           |
| M  | 170 (65.89%)  | 77 (56.20%)                         | 95 (64.19%)                          |                  |
| F  | 88 (34.11%)   | 60 (43.80%)                         | 53 (35.81%)                          |                  |
| WHO subtype                                    |   |                                     |                                      | 0.1736           |
| 5q-  | 13 (5.02%)  | 9 (6.52%)                           | 5 (3.38%)                            |                  |
| Secondary AML, AML (previously RAEBT) or T-AML | 8 (3.09%)   | 4 (2.90%)                           | 2 (1.35%)                            |                  |
| MDS-EB1  | 17 (6.56%)  | 16 (11.59%)                         | 24 (16.22%)                          |                  |
| MDS-EB2  | 16 (6.18%)  | 11 (7.97%)                          | 19 (12.84%)                          |                  |
| MDS-MLD  | 80 (30.89%)   | 41 (29.71%)                         | 44 (29.73%)                          |                  |
| MDS-MPN, CMML-0, CMML-1 or CMML2               | 80 (30.89%)   | 8 (5.80%)                           | 13 (8.78%)                           |                  |
| MDS-MPN-RS-T                                   | 3 (1.16%)   | 3 (2.17%)                           | 2 (1.35%)                            |                  |
| MDS-RS-MLD                                     | 13 (5.02%)  | 8 (5.80%)                           | 8 (5.41%)                            |                  |
| MDS-RS-SLD                                     | 23 (8.88%)  | 20 (14.49%)                         | 12 (8.11%)                           |                  |
| MDS-SLD  | 29 (11.20%)   | 8 (5.80%)                           | 11 (7.43%)                           |                  |
| MDS-U  | 15 (5.79%)  | 10 (7.25%)                          | 7 (4.73%)                            |                  |
| Not available                                  | 2 (0.77%)   | 0 (0.00%)                           | 1 (0.68%)                            |                  |
| <b>IPSS-RR category</b>                        |   |                                     |                                      | <b>&lt;.0001</b> |
| Very Low                                       | 53 (21.81%)   | 10 (7.81%)                          | 11 (7.64%)                           |                  |
| Low  | 91 (37.45%)   | 40 (31.25%)                         | 46 (31.94%)                          |                  |
| INT  | 61 (25.10%)   | 50 (39.06%)                         | 49 (34.03%)                          |                  |
| High   | 25 (10.29%)   | 18 (14.06%)                         | 17 (11.81%)                          |                  |
| Very high                                      | 13 (5.35%)  | 10 (7.81%)                          | 21 (14.58%)                          |                  |
| Cytogenetics                                   |   |                                     |                                      | 0.6413           |
| Very good                                      | 8 (3.25%)   | 4 (3.10%)                           | 3 (2.05%)                            |                  |
| Good   | 184 (74.80%)  | 92 (71.32%)                         | 102 (69.86%)                         |                  |
| Intermediate                                   | 36 (14.63%)   | 25 (19.38%)                         | 25 (17.12%)                          |                  |
| Poor   | 8 (3.25%)   | 2 (1.55%)                           | 4 (2.74%)                            |                  |
| Very poor                                      | 10 (4.07%)  | 6 (4.65%)                           | 12 (8.22%)                           |                  |

**Figure S1.** Overall survival of MDS patients based on transfusion dose density, using the revised International Working Group definition of low transfusion density ( $\geq 0.75$  to  $< 2$  units per month) and high transfusion dose density ( $\geq 2$  units per month).

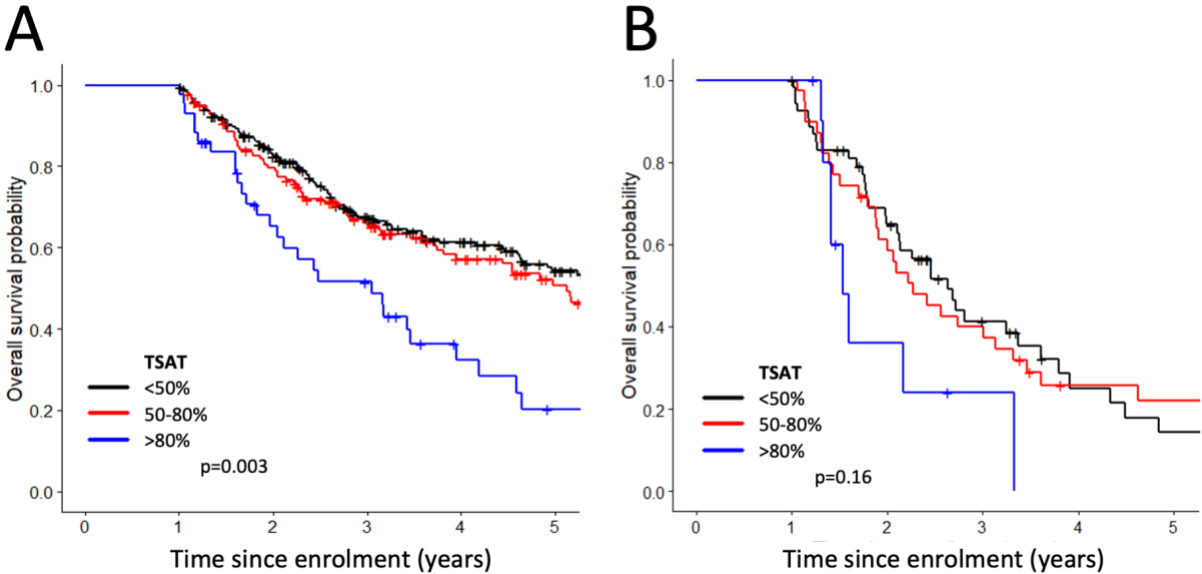


TDD = transfusion dose density.

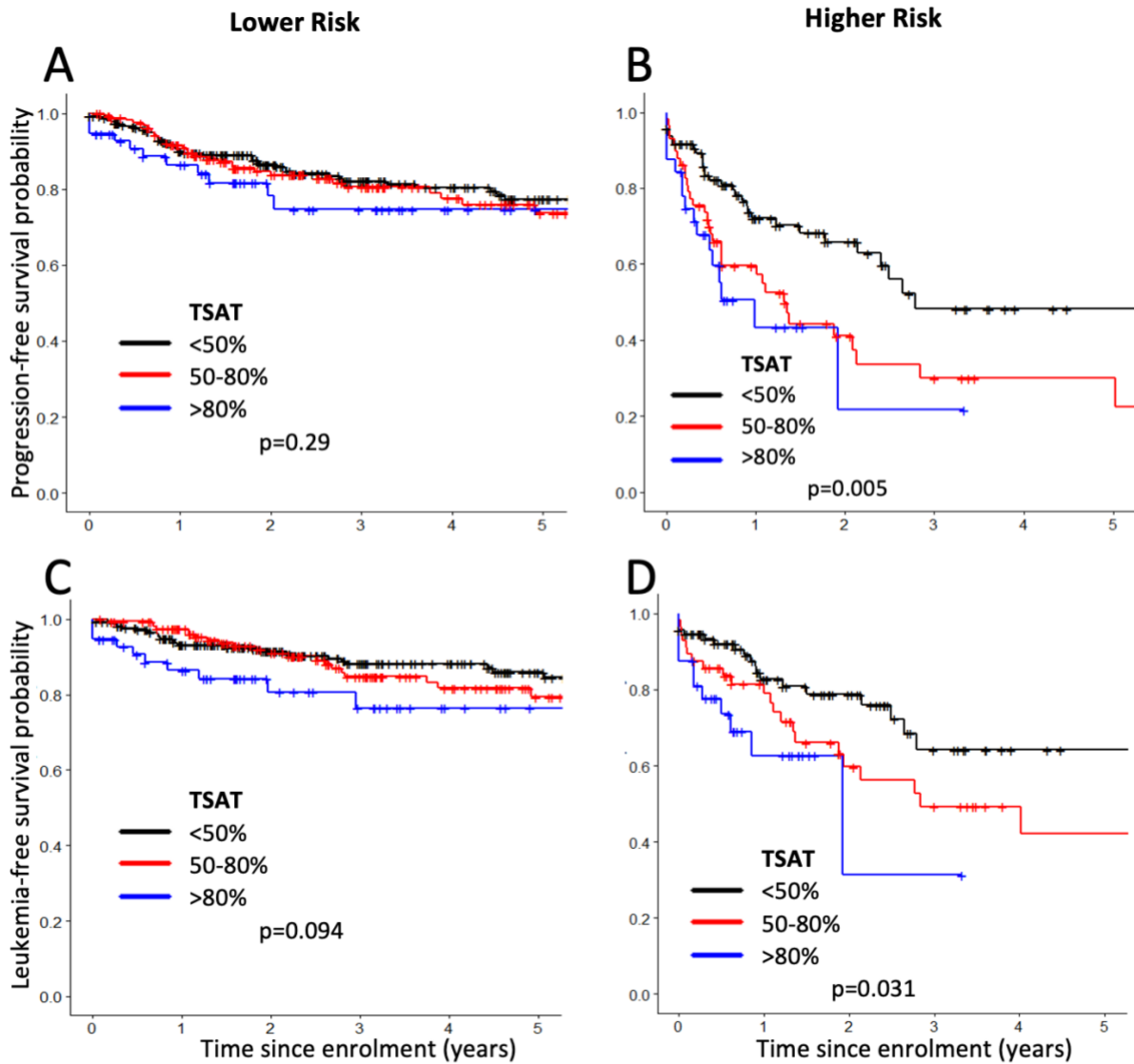
**Figure S2:** Higher transfusion density was significantly associated with inferior OS in lower risk MDS patients (A) but not in higher risk MDS patients (B)



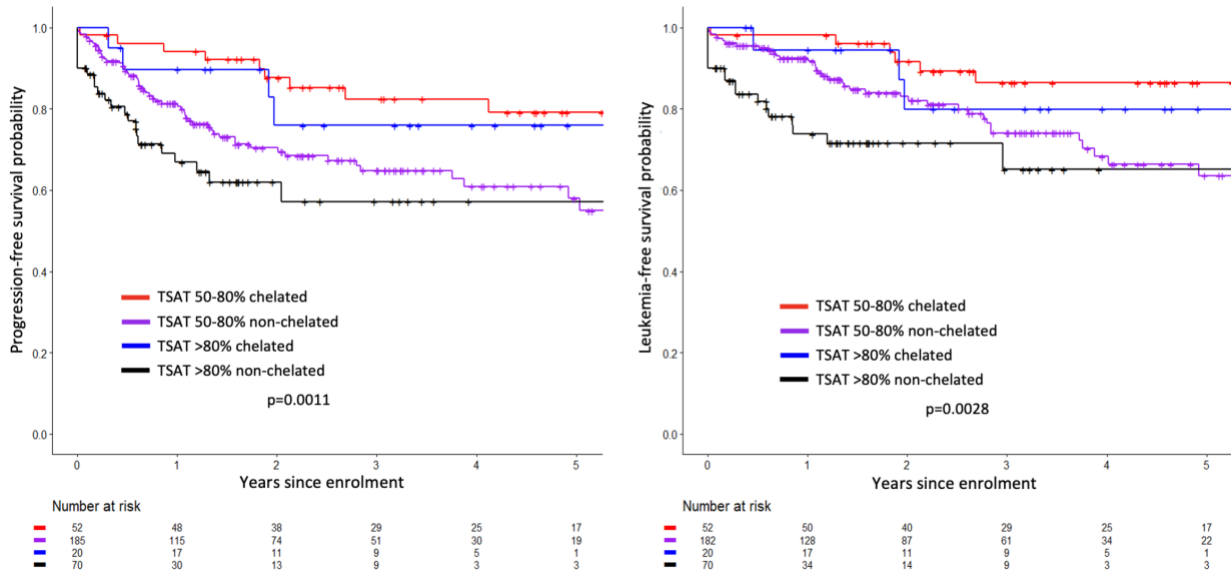
**Figure S3:** Higher TSAT was significantly associated with inferior OS in lower risk MDS patients (A) with a trend toward significance among higher risk MDS patients (B)



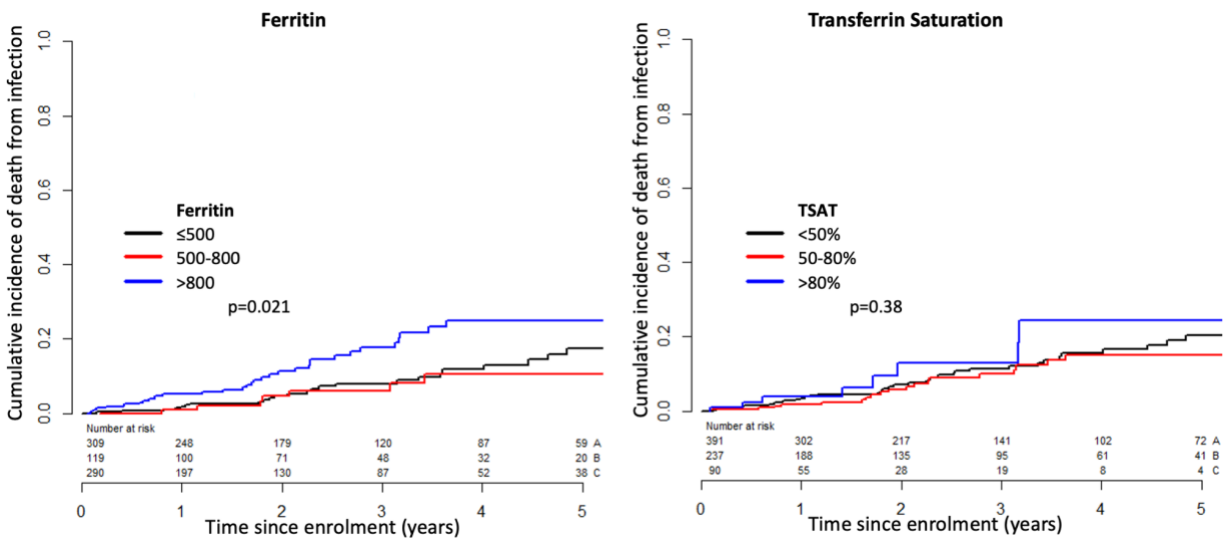
**Figure S4:** Higher TSAT was significantly associated with inferior PFS (B) and LFS (D) in higher risk MDS patients, but not in lower risk MDS (A,C), although a trend toward significance was seen in LFS among lower risk patients (C).



**Figure S5:** Iron chelation therapy had an attenuating effect on the impact of TSAT on progression-free survival and leukemia-free survival, although sample sizes were limited.



**Figure S6.** Cumulative incidence of death from infection according to three TSAT and three ferritin categories, where mean TSAT and mean ferritin were taken over the entire duration of follow-up.



**Table S2.** Univariate Cox proportional hazards analysis of the impact of covariates on overall survival.

| Variable  | p-value       | HR           | 95% CI of HR |       | R <sup>2</sup> (%) |
|---|---------------|--------------|--------------|-------|--------------------|
| Age at baseline (years)                               | <.0001        | <b>1.039</b> | 1.028        | 1.050 | 7.63               |
| IPSS-R value at baseline                              | <.0001        | <b>1.369</b> | 1.303        | 1.438 | 18.88              |
| Blasts categories at baseline                         | <.0001        |              |              |       | 10.10              |
| 5-9% vs. <5%  | <.0001        | <b>1.922</b> | 1.498        | 2.467 |                    |
| ≥10% vs. <5%  | <.0001        | <b>2.947</b> | 2.313        | 3.755 |                    |
| ≥10% vs. 5-9%   | <b>0.0049</b> | <b>1.533</b> | 1.139        | 2.065 |                    |
| ECOG (0-4)  | <.0001        | <b>1.557</b> | 1.362        | 1.780 | 5.37               |
| BMI (kg/m <sup>2</sup> )                              | 0.7516        | 1.003        | 0.984        | 1.023 | 0.02               |
| TD vs. TI at anytime                                  | 0.3575        | 1.092        | 0.906        | 1.316 | 0.12               |
| Frailty value at baseline (continuous)                | <.0001        | <b>1.510</b> | 1.383        | 1.649 | 12.85              |
| Charlson Comorbidity value at baseline (continuous) * | <.0001        | <b>1.502</b> | 1.269        | 1.777 | 3.66               |
| Iron chelation (Yes vs. No)                           | <b>0.0144</b> | <b>0.708</b> | 0.537        | 0.934 | 0.90               |
| Iron saturation averaged value from all measurements  | <b>0.0012</b> | <b>1.007</b> | 1.003        | 1.012 | 1.42               |
| Iron saturation averaged value categories             | <.0001        |              |              |       | 3.21               |
| 50-80% vs. <50%                                       | 0.7049        | 1.041        | 0.845        | 1.282 |                    |
| >80% vs. <50%   | <.0001        | <b>2.031</b> | 1.546        | 2.667 |                    |
| >80% vs. 50-80%                                       | <.0001        | <b>1.951</b> | 1.460        | 2.605 |                    |
| Iron saturation averaged value >80% (Yes vs. No)      | <.0001        | <b>1.999</b> | 1.541        | 2.593 | 3.19               |
| Ferritin averaged value from all measurements *       | <.0001        | <b>1.264</b> | 1.164        | 1.372 | 4.44               |
| Ferritin averaged value categories                    | <.0001        |              |              |       | 4.66               |
| 501-800 vs. ≤500                                      | 0.9316        | 1.013        | 0.757        | 1.355 |                    |
| >800 vs. ≤500   | <.0001        | <b>1.764</b> | 1.438        | 2.163 |                    |
| >800 vs. 501-800                                      | <b>0.0001</b> | <b>1.742</b> | 1.315        | 2.307 |                    |
| Ferritin averaged value >800 (Yes vs. No)             | <.0001        | <b>1.758</b> | 1.458        | 2.119 | 4.66               |
| Ferritin averaged value >1000 (Yes vs. No)            | <.0001        | <b>1.678</b> | 1.389        | 2.027 | 3.82               |
| Transfusion density (>2.7 units/months) categories    | <.0001        |              |              |       | 6.48               |
| High vs. No   | <.0001        | <b>1.955</b> | 1.587        | 2.410 |                    |
| Low vs. No  | 0.5198        | 0.919        | 0.710        | 1.189 |                    |
| High vs. Low  | <.0001        | <b>2.128</b> | 1.641        | 2.760 |                    |

\*Natural log transformation was applied for some covariates to normalize their distributions. P-values < 0.05 were considered statistically significant (bolded rows). Hazard ratios and 95% confidence intervals (CI) of hazard ratio were also calculated for each covariate. The generalized R<sup>2</sup> statistic was calculated based on the likelihood ratio statistic (LRT) for testing the global null hypothesis (see Allison, Paul D. 2010. *Survival Analysis Using the SAS System: A Practical Guide*. Cary, NC: SAS Institute Inc, Second ed. Page 282-283)

**Table S3.** Multivariable cox proportional hazards analysis.

| Model A                                      | p-value | HR    | 95% CI of HR |       | R <sup>2</sup> (%) |
|--|---------|-------|--------------|-------|--------------------|
| Iron saturation averaged value >80% vs. ≤80% | 0.0071  | 1.584 | 1.133        | 2.215 | <b>30.30</b>       |
| Ferritin averaged value >800 vs. ≤800        | 0.0056  | 1.480 | 1.122        | 1.953 |                    |
| Age at baseline (years)                      | 0.0206  | 1.017 | 1.003        | 1.032 |                    |
| IPSS-R value at baseline                     | <.0001  | 1.272 | 1.197        | 1.352 |                    |
| Frailty value at baseline (continuous)       | <.0001  | 1.328 | 1.197        | 1.472 |                    |
| Charlson Comorbidity value at baseline (log) | 0.0356  | 1.230 | 1.014        | 1.493 |                    |
| Iron chelation (Yes vs. No)                  | 0.0022  | 0.581 | 0.410        | 0.822 |                    |

| Model B  | p-value | HR    | 95% CI of HR |       | R <sup>2</sup> (%) |
|--|---------|-------|--------------|-------|--------------------|
| Iron saturation averaged value >80% vs. ≤80%       | 0.0072  | 1.581 | 1.132        | 2.209 | <b>30.35</b>       |
| Transfusion density (>2.7 units/months) categories | 0.0002  |       |              |       |                    |
| High vs. No  | 0.0092  | 1.516 | 1.108        | 2.075 |                    |
| Low vs. No   | 0.1760  | 0.798 | 0.576        | 1.106 |                    |
| High vs. Low                                       | <.0001  | 1.899 | 1.386        | 2.603 |                    |
| Age at baseline (years)                            | 0.0064  | 1.020 | 1.006        | 1.035 |                    |
| IPSS-R value at baseline                           | <.0001  | 1.271 | 1.196        | 1.351 |                    |
| Frailty value at baseline (continuous)             | <.0001  | 1.338 | 1.205        | 1.486 |                    |
| Charlson Comorbidity value at baseline (log)       | 0.0463  | 1.217 | 1.003        | 1.476 |                    |
| Iron chelation (Yes vs. No)                        | 0.0092  | 0.636 | 0.452        | 0.894 |                    |

Potentially significant (p-value <0.10) variables from the univariate analysis were included in the multivariate analysis and backward stepwise selection procedure was used. The above two models emerged with nearly identical R<sup>2</sup> values. While age, IPSS-R, frailty, Charlson Comorbidity, ICT and TSAT remained independent predictors in all three models, only one of ferritin or TDD retained significance in each model, suggesting significant co-linearity among these variables.