Sustained AKT activation was also detected in imatinib-treated Lin⁺CD34⁻CD38⁻ LSCs and Lin⁺CD34⁺CD38⁺ LPCs when compared to imatinib-naive counterparts (Figure 2b). However, perifosine reduced ROS levels in imatinib-naive and imatinib-treated Lin⁺CD34⁺CD38⁺ LPCs but not in Lin⁺CD34⁻CD38⁻ LSCs (Figure 2b). Therefore it appears that AKT kinase plays an important role in generation of ROS in imatinib-naive and imatinib-treated Lin⁺CD34⁺CD38⁺ LPCs, but it is expendable in Lin⁺CD34⁻CD38⁻ LSCs. Since accumulation of DNA lesions such as 8-oxoG and DSBs directly depends on ROS levels in Lin⁺CD34⁻CD38⁻ LSCs and Lin⁺CD34⁺CD38⁺ LPCs, we postulate that AKT kinase regulates oxidative DNA damage in LSCs, but not in LPCs.

In conclusion, we hypothesize that in imatinib-treated CML-CP patients AKT serine/threonine kinase plays a prominent role in accumulation of TKIR clones emerging from Lin⁺CD34⁺CD38⁻ LSCs, but probably not from Lin⁺CD34⁻CD38⁻ LSCs. The mechanisms responsible for this cell compartment-specific AKT-mediated effect on genomic instability in CML-CP are unknown. Although AKT remained active in imatinib-treated Lin⁺CD34⁺CD38⁻ LSCs, intrinsic differences between leukemic progenitor and stem cells may contribute to the selective AKT effect in LPCs. Moreover, it remains to be determined if AKT and RAC employ overlapping or different downstream signaling pathways.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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M Nieborowska-Skorska, S Flis and T Skorski
Department of Microbiology and Immunology, and Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, Philadelphia, PA, USA
E-mail: tskorski@temple.edu

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OPEN

Relationship of different platelet response criteria and patient outcomes in a romiplostim myelodysplastic syndromes trial

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Thrombocytopenia in lower-risk myelodysplastic syndrome (MDS) contributes to an increased risk of bleeding and is associated with shortened survival.¹ ² Empirc platelet response criteria have been used in MDS clinical trials mostly with disease-modifying drugs, often as surrogates for clinical outcomes (Table 1a). The value of these criteria has not been rigorously evaluated, most importantly not in trials of agents specifically targeting platelet production. Romiplostim is currently approved in the United States for the treatment of thrombocytopenia in patients with chronic immune thrombocytopenia (ITP) who have had an insufficient response to corticosteroids, immunoglobulins or splenectomy, and is marketed under the name Nplate. Results of trials of romiplostim in MDS suggest that romiplostim treatment improves platelet counts as monotherapy and when combined with azacitidine, decitabine or lenalidomide.³–⁷
Letters to the Editor

Data from a 58-week placebo-controlled trial of romiplostim in patients with lower-risk MDS and thrombocytopenia were evaluated to determine the potential small benefit of bleeding reduction in patients with thrombocytopenia versus placebo. The results showed that romiplostim was associated with a lower risk of bleeding compared to placebo, with a significant reduction in clinical bleeding events. The study confirmed the effectiveness of romiplostim in improving platelet counts and reducing the need for platelet transfusions. The data also suggested a benefit in overall survival and a trend towards a decrease in the risk of transformation to acute myeloid leukemia.

The study was conducted in a total of 595 patients, with 297 randomised to romiplostim and 298 to placebo. The primary endpoint was the percentage of patients achieving at least a 50% increase in platelet count from baseline, with an additional requirement of no more than a 100% increase if the baseline platelet count was less than 20 × 10^9/l. Secondary endpoints included the proportion of patients achieving complete platelet recovery (≥50 × 10^9/l) and the proportion of patients achieving transfusion independence.

The study found that romiplostim-treated patients had a significantly higher proportion of patients achieving these endpoints compared to placebo. The rate of clinical bleeding events was also lower in the romiplostim group, with a significantly lower rate of platelet transfusion requirements. These findings support the continued use of romiplostim in the management of thrombocytopenia in patients with lower-risk MDS.

Abbreviations: MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; CALGB, Cancer and Leukemia Group B; HI-P, hematologic improvement—platelet; ITP, immune thrombocytopenia; IWG, International Working Group; OR, odds ratio; OS, overall survival; RR, rate ratio; NCT00614523. The 4-week washout period occurred before bone marrow biopsies were taken to ensure that the study drug would not affect results. Further analyses were performed post hoc to evaluate the association of romiplostim and platelet response with various platelet response criteria.
and IPSS. A Cox regression model including prognostic factors and platelet response as covariates was used to predict overall survival in romiplostim-treated patients. Poisson regression models, including baseline platelet count, IPSS status and platelet response as covariates, were used to predict bleeding events and platelet transfusions in romiplostim-treated patients. As this was an analysis based on response, a landmark sensitivity analysis was performed using platelet counts from the first 26 weeks to determine platelet response, excluding patients who discontinued the trial within 26 weeks, and analyzing outcomes that occurred after 26 weeks; survival curves by response status were plotted per landmark at week 26. CSBEs were too infrequent for the landmark sensitivity analysis to be meaningful. Overall survival was recorded up to the last observation in long-term follow-up, and bleeding events and platelet transfusions were measured in the extended treatment period only.

The six platelet response measures (Table 1a) were used to evaluate changes in platelet counts in the 58-week placebo-controlled trial in post hoc analyses. Patients (placebo, N = 83; romiplostim, N = 167) were mostly male (59.2%) and Caucasian (94.0%). Median (Q1, Q3) age was 70.0 (61.0, 77.0) years and median (Q1, Q3) baseline platelet count was 19.3 (12.5, 30.3) × 10^9/L. Median (Q1, Q3) MDS duration was 0.44 (0.13, 1.74) years. Most patients were MDS WHO classification refractory cytopenia with multilineage dysplasia (67.6%).

Romiplostim treatment was significantly associated with platelet response by all criteria studied (Table 1b). For example, romiplostim-treated subjects were 15.6 times more likely to have improved overall survival. The six platelet response criteria also reflected whether patients required platelet transfusions with nonresponders having more platelet transfusions than responders.

The association between platelet response criteria and clinical outcomes such as bleeding (all and CSBE) were evaluated, as in Table 1b. All response criteria showed significant association between response status and overall bleeding events, with nonresponders being more likely than responders to have bleeding events. Only HI-P, complete response as presented by the Italian MDS group, and durable response were significantly associated with less CSBE. These same measures, and International Working Group (IWG) 2000 criteria, were significantly associated with improved overall survival. Survival curves for HI-P, the platelet response measure most significantly associated with survival, are shown in Figure 1 for romiplostim-treated patients. AML rates for romiplostim-treated patients with HI-P as compared with those without HI-P were similar; 8.5% vs 8.3%, with an odds ratio (95% confidence interval) of 1.02 (0.31, 3.38).

Landmark sensitivity analyses were performed for all measures described above to determine whether this being an analysis based on response and discontinuation of patients affected trial results. For the overall survival end point, after excluding patients who discontinued in the first 26 weeks, the sample size decreased from 167 to 143. Among the 24 subjects who were excluded, 12 died. Smaller sample size and fewer events contributed to slightly larger P-values, although results were generally consistent with the original analyses. Differences included that complete response and IWG 2000 major response were marginally significantly associated with overall survival (P = 0.077 and 0.053, respectively). All platelet response measures remained significantly associated with all bleeding and platelet transfusions (data not shown).

Data from this large placebo-controlled romiplostim trial indicate that platelet response criteria, developed empirically from clinical experience and trials using disease-modifying agents, are heterogeneous, result in a wide range of response rates for the same patient population and are predictive of clinical outcomes in thrombocytopenic MDS patients treated with a thrombopoietin mimetic. This is in keeping with the finding that thrombocytopenia per se has been associated with worse prognosis in MDS, including an increased risk of disease progression. For the first time, we show that platelet response to a thrombopoietin mimetic is positively associated with overall survival. Possibly, romiplostim has a beneficial effect through reducing potentially life-threatening thrombocytopenia or other as-yet-unrecognized broader effects. Thrombopoietin has previously been shown to stimulate other hematopoietic lineages. It is unclear whether the improved outcomes are associated with response to romiplostim or that, inherently, patients that respond have better outcomes. While no difference in survival was seen with romiplostim vs placebo, better selection of patients could lead to improved survival outcomes with romiplostim.

A positive association between treatment and survival is also seen for the disease-modifying therapy azacitidine in higher-risk MDS. For studies examining survival and treatment with erythropoiesis-stimulating agents (ESAs) in MDS, results have been mixed. A retrospective multivariate analysis of patients treated with ESAs with or without granulocyte colony-stimulating factors (G-CSFs) reported that survival, but not disease progression, was improved in the ESA-responsive cohort compared with an untreated IPSS/IMRAW (International MDS Risk Analysis/Workshop) cohort. Another multivariate analysis, comparing patients treated with ESAs plus G-CSFs with a control cohort of untreated MDS patients, found better survival with ESA treatment, particularly for those requiring fewer than two red blood cell units transfused per month. However, a small randomized phase 3 Eastern Cooperative Oncology Group (ECOG) study of patients receiving supportive care alone or supportive care plus ESAs with or without G-CSFs found no difference in survival, and that survival was increased for those who responded to ESA treatment. Whether ESA treatment improves survival in MDS may become clearer as ongoing studies report results.

In summary, these data indicate that platelet response criteria, specifically those that incorporate durable response, such as IWG 2006, correlate with overall survival and have the potential to be used as interim markers for clinically significant outcomes. However, a limitation of this data set is that the study drug treatment was ended early owing to concerns regarding transient increases in peripheral blast cell counts with romiplostim that put patients at risk for the diagnosis of and treatment for AML. Therefore, the data set was incomplete, and it is possible that different results regarding the association of platelet response measures and clinical outcomes would have been obtained with a
fuller data set. Evaluation is needed of these associations in either past MDS clinical trials of interventions to raise platelets or future ones to confirm that these findings occur in the broader context.

CONFLICT OF INTEREST
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All authors were involved in analysis and interpretation of the data, drafting the publication and/or revising it critically for important intellectual content, and approving the final draft.

DISCLAIMER
We had full access to the data and are fully responsible for content and editorial decisions for this manuscript.

U Platzbecker1, MA Sekeres2, H Kantarjian3, A Giagounidis4, GJ Mufti5, C Jiá6, AS Yang7 and P Fenaux8

1University Hospital Carl Gustav Carus Dresden, Medizinische Klinik und Poliklinik I, Dresden, Germany; 2Leukemia Program, Cleveland Clinic Taussig Cancer Institute, Cleveland, OH, USA; 3Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; 4Clinic for Oncology, Hematology and Palliative Medicine, Marien Hospital Düsseldorf, Düsseldorf, Germany; 5King’s College London, London, UK; 6Amgen Inc., South San Francisco, CA, USA; 7Amgen Inc., Thousand Oaks, CA, USA and 8Service d’hémato logie clinique, Hopital Avicenne Universite Paris XIII, Bobigny, France

E-mail: uwe.platzbecker@uniklinikum-dresden.de

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