blood®
Letters to *Blood*

TO THE EDITOR:

Neutrophil and platelet increases with luspatercept in lower-risk MDS: secondary endpoints from the MEDALIST trial

Guillermo Garcia-Manero,[1] Ghulam J. Mufti,[2] Pierre Fenaux,[3] Rena Buckstein,[4] Valeria Santini,[5] María Díez-Campelo,[6] Carlo Finelli,[7] Osman Ilhan,[8] Mikkael A. Sekeres,[9] Amer M. Zeidan,[10] Rodrigo Ito,[11] Jennie Zhang,[11] Anita Rampersad,[11] Daniel Sinsimer,[11] Jay T. Backstrom,[12] Uwe Platzbecker,[13,*] and Rami S. Komrokji[14,*]

[1]Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX; [2]Department of Haemato-Oncology, King's College London, London, United Kingdom; [3]Service d'Hématologie Séniors, Hôpital Saint Louis, Université Paris 7, Paris, France; [4]Odette Cancer Centre, Sunnybrook Health Sciences Centre, Toronto, ON, Canada; [5]MDS Unit, AOU Careggi, Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy; [6]Hematology Department, Institute of Biomedical Research of Salamanca, University Hospital of Salamanca, Salamanca, Spain; [7]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Istituto di Ematologia "Seràgnoli" Bologna, Bologna, Italy; [8]Department of Hematology, Ankara University School of Medicine, Ankara, Turkey; [9]Sylvester Cancer Center, University of Miami Miller School of Medicine, Miami, FL; [10]Department of Internal Medicine, Yale School of Medicine and Yale Cancer Center, Yale University, New Haven, CT; [11]Bristol Myers Squibb, Princeton, NJ; [12]Acceleron Pharma, Cambridge, MA; [13]Medical Clinic and Policlinic 1, Hematology and Cellular Therapy, University Hospital Leipzig, Leipzig, Germany; and [14]Moffitt Cancer Center, Tampa, FL

Myelodysplastic syndromes (MDS) result in abnormal blood cell development, cytopenias, and risk of progression to acute myeloid leukemia (AML).[1] Most patients with lower-risk MDS (LR-MDS) have anemia, but patients can also have neutropenia and/or thrombocytopenia with significant clinical implications.[2-4] Treatments for anemia include red blood cell (RBC) transfusions, erythropoiesis-stimulating agents (ESAs), hypomethylating agents (HMAs),[5] or lenalidomide.[5] However, RBC transfusions can result in iron overload[6,7]; patients can become resistant to ESAs,[4,8] and HMAs and lenalidomide have been associated with grade 3 or 4 neutropenia and thrombocytopenia.[9,10] Luspatercept is a first-in-class erythroid maturation agent that binds several transforming growth factor-β (TGF-β) superfamily ligands to diminish Smad2/3 signaling and enhance late-stage erythropoiesis.[11] Its efficacy and safety were demonstrated in the phase 3, placebo-controlled MEDALIST trial in RBC transfusion-dependent patients with LR-MDS with ring sideroblasts (RS).[12] In this study, significantly more luspatercept-treated patients achieved RBC transfusion independence for ≥8 weeks during weeks 1 to 24 (37.9% vs 13.2%; $P < .001$).[12] Significantly more patients in the luspatercept arm achieved hematologic improvement-erythroid (HI-E), as per 2006 International Working Group (IWG) criteria,[13] during weeks 1 to 24 (52.9% vs 11.8%; $P < .001$) and weeks 1 to 48 (58.8% vs 17.1%; $P < .001$).[12] Here, we report the effect of luspatercept on lineages outside the erythroid compartment, including platelets and neutrophils, and the HI for these lineages in MEDALIST patients cytopenic at baseline.

Full details of the MEDALIST trial (NCT02631070) have been published.[12] Briefly, 229 adults with LR-MDS (defined as very low-, low-, or intermediate-risk MDS per the Revised International Prognostic Scoring System [IPSS-R][14]) with RS (either ≥15% or ≥5% if *SF3B1* mutation was present), who were refractory to, intolerant of, or unlikely to respond to ESAs (serum erythropoietin >200 U/L) and required RBC transfusions, were randomized 2:1 to receive luspatercept (n = 153) or placebo (n = 76) subcutaneously every 3 weeks for 24 weeks.

Data cutoff for the current analysis was July 1, 2019. The secondary endpoints reported are mean neutrophil and platelet counts; mean neutrophil and platelet changes from baseline; proportions of patients achieving absolute increases in neutrophil and platelet counts of ≥0.5 × 10^9/L and ≥30 × 10^9/L, respectively; proportions of patients achieving HI-neutrophil (HI-N) and HI-platelet (HI-P) during weeks 1 to 24 and 1 to 48; and hematological toxicities (neutropenia and thrombocytopenia). HI-N is defined as neutrophil increase of >0.5 × 10^9/L and ≥100% among patients with pretreatment levels <1 × 10^9/L.[13] HI-P is defined as platelet increase, without platelet transfusion, of ≥30 × 10^9/L (>20 × 10^9/L at baseline), or of >20 × 10^9/L and ≥100% increase (<20 × 10^9/L at baseline) among patients with pretreatment levels <100 × 10^9/L.[13]

Median age of patients in the MEDALIST trial was 71 years; 62.9% were male.[12] Most patients (95.6%) had refractory cytopenia with multilineage dysplasia and RS (RCMD-RS), and 91.0% of those with available data had *SF3B1* mutations (Table 1).[12]

Mean baseline absolute neutrophil count (ANC) was 2.8 × 10^9/L, and 25 patients (10.9%) had neutropenia (neutrophils <1 × 10^9/L per IWG 2006 criteria[13]): 15 (9.8%) of the luspatercept arm and 10 (13.2%) of the placebo arm (Table 1). Mean baseline platelet count was 257 × 10^9/L, and 14 (6.1%) patients had thrombocytopenia (platelets <100 × 10^9/L per IWG 2006 criteria[13]): 8 (5.2%) of the luspatercept arm and 6 (7.9%) of the placebo arm (Table 1). Table 1 also lists characteristics of patients with baseline neutropenia or thrombocytopenia.

Among all randomized patients, 124 (81.0%) vs 39 (51.3%) patients in the luspatercept and placebo arms, respectively, achieved mean absolute increase in neutrophils of ≥0.5 × 10^9/L

Table 1. Baseline patient and treatment characteristics

Characteristic	ITT population			Patients with neutropenia*			Patients with thrombocytopenia†		
	Luspatercept (n = 153)	Placebo (n = 76)	Total (n = 229)	Luspatercept (n = 15)	Placebo (n = 10)	Total (n = 25)	Luspatercept (n = 8)	Placebo (n = 6)	Total (n = 14)
Age, median, y (range)	71 (40-95)	72 (26-91)	71 (26-95)	72 (60-86)	69.5 (43-79)	72 (43-86)	72.5 (58-79)	73.5 (65-80)	73.5 (58-80)
Male, n (%)	94 (61.4)	50 (65.8)	144 (62.9)	9 (60.0)	6 (60.0)	15 (60.0)	5 (62.5)	5 (83.3)	10 (71.4)
MDS WHO 2008 classification, n (%)									
RS and multilineage dysplasia	1 (0.7)	0	1 (0.4)	NA	NA	NA	NA	NA	NA
RCMD-RS	145 (94.8)	74 (97.4)	219 (95.6)	15 (100.0)	9 (90.0)	24 (96.0)	8 (100.0)	6 (100.0)	14 (100.0)
RARS	7 (4.6)	2 (2.6)	9 (3.9)	NA	1 (10.0)	1 (4.0)	NA	NA	NA
Mutated *SF3B1*,‡ n/N with data (%)	138/148 (93.2)	64/74 (86.5)	202/222 (91.0)	12 (80.0)	10 (100.0)	22 (88.0)	5 (62.5)	4 (66.7)	9 (64.3)
ANC, mean, $\times 10^9$/L (SD)	2.8 (2.1)	2.7 (2.0)	2.8 (2.0)	0.8 (0.19)	0.8 (0.11)	0.8 (0.16)	3.6 (5.00)	1.8 (0.96)	2.8 (3.83)
ANC category, n (%)									
$<0.5 \times 10^9$/L	1 (0.7)	0	1 (0.4)	1 (6.7)	0	1 (4.0)	NA	NA	NA
0.5 to $<1.0 \times 10^9$/L	14 (9.2)	10 (13.2)	24 (10.5)	14 (93.3)	10 (100.0)	24 (96.0)	2 (25.0)	2 (33.3)	4 (28.6)
$\geq 1.0 \times 10^9$/L	138 (90.2)	66 (86.8)	204 (89.1)	NA	NA	NA	6 (75.0)	4 (66.7)	10 (71.4)
Platelet count, mean, $\times 10^9$/L (SD)	259 (123)	252 (124)	257 (123)	160.5 (58.13)	179.1 (80.97)	167.9 (67.20)	78.1 (14.16)	84.7 (12.74)	80.9 (13.48)
Platelet count category, n (%)									
$<100 \times 10^9$/L	8 (5.2)	6 (7.9)	14 (6.1)	2 (13.3)	2 (20.0)	4 (16.0)	8 (100.0)	6 (100.0)	14 (100.0)
100-400 $\times 10^9$/L	128 (83.7)	61 (80.3)	189 (82.5)	13 (86.7)	8 (80.0)	21 (84.0)	NA	NA	NA
$>400 \times 10^9$/L	17 (11.1)	9 (11.8)	26 (11.4)	NA	NA	NA	NA	NA	NA
ICT use, n (%)	71 (46.4)	40 (52.6)	111 (48.5)	10 (66.7)	8 (80.0)	18 (72.0)	3 (37.5)	5 (83.3)	8 (57.1)

ANC, absolute neutrophil count; ICT, iron chelation therapy; ITT, intention to treat; NA, not applicable; RARS, refractory anemia with RS; RCMD-RS, refractory cytopenia with multilineage dysplasia and RS; SD, standard deviation; WHO, World Health Organization.

*Patients from the ITT population with neutropenia defined per IWG 2006 criteria as neutrophil level $<1 \times 10^9$/L.

†Patients from the ITT population with thrombocytopenia defined per IWG 2006 criteria as platelet level $<100 \times 10^9$/L.

‡No patients with *SF3B1* mutation had RS <15%.

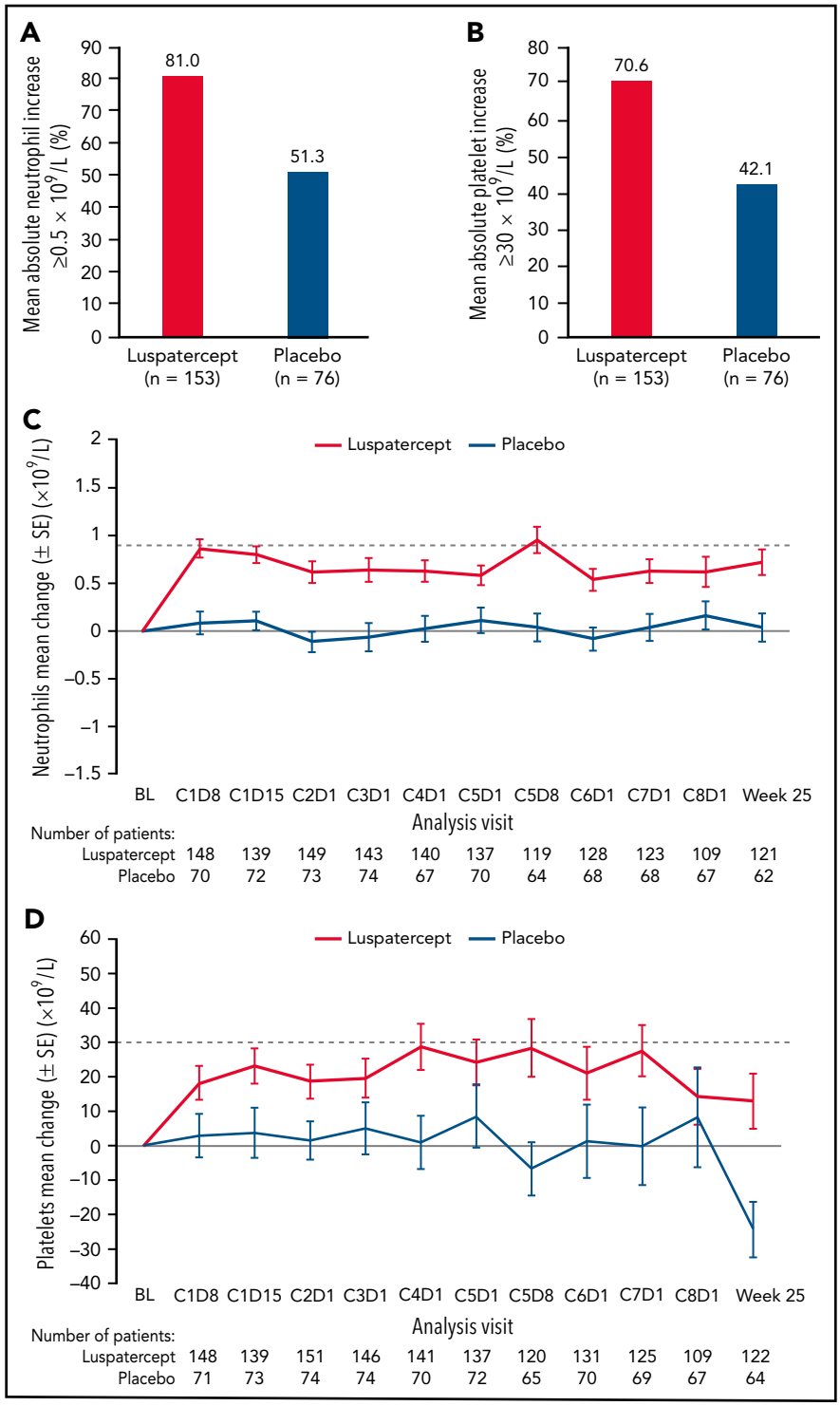

Figure 1. Neutrophil and platelet improvements. Achievement of mean (A) absolute neutrophil increase ≥0.5 × 10^9/L and (B) absolute platelet increase ≥30 × 10^9/L. Mean change from baseline in (C) neutrophils and (D) platelets over time. Mean counts of (E) neutrophils and (F) platelets. Dashed lines indicate (C) a mean change from baseline of 0.9 × 10^9/L and (D) a mean change from baseline of 30 × 10^9/L. BL, baseline; C, cycle; D, day; SD, standard deviation; SE, standard error.

for 56 consecutive days compared with baseline (Figure 1A). Similarly, 108 (70.6%) vs 32 (42.1%) patients in the luspatercept vs placebo arms achieved mean absolute increase in platelets of ≥30 × 10^9/L (Figure 1B), maintained through week 25. By cycle 5, day 8, mean change from baseline in neutrophils was 0.95 × 10^9/L vs 0.04 × 10^9/L in the luspatercept and placebo arms, respectively (Figure 1C). By cycle 4, day 1, mean change from baseline in platelets was 28.7 × 10^9/L in the luspatercept arm and 0.9 × 10^9/L in the placebo arms (Figure 1D). Mean neutrophil and platelet counts are presented in Figure 1E-F. Although the increased levels of both neutrophils and platelets were maintained throughout luspatercept treatment (weeks 1-24), they did

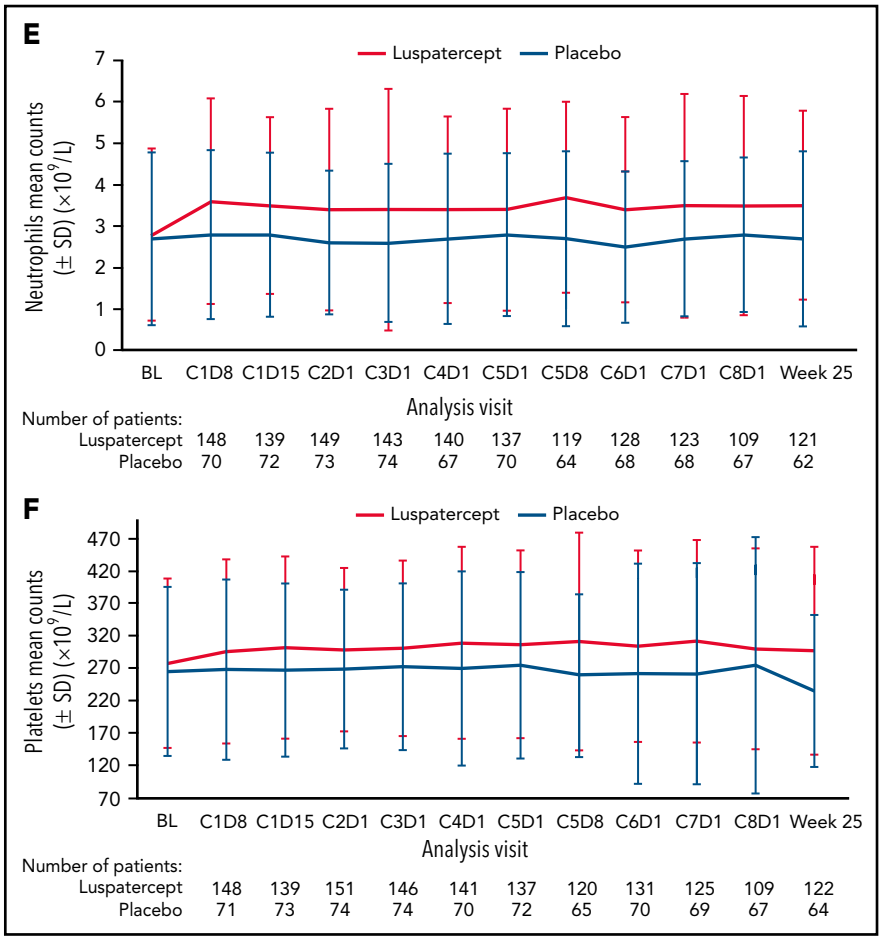

Figure 1. (Continued)

not exceed the upper-limits-of-normal values for adults to be considered a safety concern. The observed mean absolute increases in neutrophils and platelets were not dose-dependent.

Of the 25 patients evaluable for HI-N, more of those randomized to luspatercept vs placebo achieved HI-N during weeks 1 to 24 (13.3% vs 0.0%) and weeks 1 to 48 (20.0% vs 10.0%). Similarly, of the 14 patients evaluable for HI-P, more luspatercept- vs placebo-treated patients achieved HI-P during weeks 1 to 24 (50.0% vs 33.3%) and weeks 1 to 48 (62.5% vs 33.3%). These findings potentially support the use of luspatercept to treat patients with LR-MDS with RS who are often neutropenic and/or thrombocytopenic and anemic. However, the HI-N and HI-P responses in the placebo arm might highlight the normal oscillations seen in blood counts of patients with LR-MDS. Coupled with the low numbers of patients evaluable for HI-N and HI-P, these results should be interpreted with caution.

Treatment-emergent grade 3 or 4 neutropenia was infrequently reported, with lower incidence in the luspatercept vs the placebo group (7/153 [4.6%] vs 6/76 [7.9%]) and may have represented normal fluctuations in patients' blood counts. No grade 3 or 4 treatment-emergent thrombocytopenia was reported in either treatment arm. These rates of grade 3 or 4 cytopenias are much lower than those observed with other therapies for MDS, including decitabine,[15] azacytidine,[16] and lenalidomide, which in a phase 3, randomized, placebo-controlled trial in patients with lower-risk non-del(5q) MDS showed high rates of grade 3 or 4 neutropenia (61.9% vs 12.7%) and thrombocytopenia (35.6% vs 3.8%).[10]

Despite the increase in neutrophil counts, there was a slight increase in infection rate with luspatercept compared with placebo. Infection was reported in 4 of 9 (44.4%) and 3 of 7 (42.9%) luspatercept- and placebo-treated patients, respectively, who experienced neutropenia (any grade) during the study. Overall infection rates for luspatercept and placebo patients were 53.6% and 40.8%, respectively. The infections were not opportunistic and were mostly grade 1 to 2 in severity. The differences in infection rates were not assessed, as this study was not designed or powered for this purpose. Bleeding was not reported in any luspatercept- or placebo-treated patients who experienced thrombocytopenia (any grade) on study. Among patients who achieved HI-N or HI-P, 1 patient in the luspatercept arm progressed to higher-risk MDS, but none progressed to AML.

Although only a minority of patients were evaluable for HI-P/HI-N, luspatercept treatment resulted in a mean increase from baseline in platelet and neutrophil counts in most patients overall vs placebo. Mean neutrophil and platelet count increases were observed early on luspatercept treatment and persisted to

week 25. This could be associated with the positive effect of luspatercept on hematopoietic stem and progenitor cell expansion by modulating the structure of extracellular matrix[17] or by direct inhibition of transforming growth factor-β signaling.[18] In the 25 patients with baseline neutropenia and 14 patients with baseline thrombocytopenia, higher proportions of patients in the luspatercept vs placebo arms achieved HI-N and HI-P during weeks 1 to 24 and weeks 1 to 48. As meaningful statistical analyses were not possible because of small sample sizes, these results should be treated with caution.

Acknowledgments

The authors thank all the patients who participated in the study.

This study was sponsored by Celgene, a Bristol Myers Squibb Company, Princeton, New Jersey in collaboration with Acceleron Pharma. The authors received writing support in the preparation of this report from Karolina Lech of Excerpta Medica, supported by Bristol Myers Squibb.

The authors are fully responsible for all content and editorial decisions. All the patients in the MEDALIST study provided written informed consent.

Authorship

Contribution: G.G.-M., V.S., U.P., and R.S.K. designed the study; G.G.-M., G.J.M., P.F., R.B., V.S., M.D-C., C.F., O.I., M.A.S., A.M.Z., U.P., and R.S.K. collected data; G.G-M., G.J.M., P.F., R.B., V.S., M.D-C., C.F., O.I., M.A.S., A.M.Z., U.P., R.S.K., R.I., J.Z., A.R., D.S., and J.T.B. analyzed and interpreted the data; R.I. and A.R. supervised the clinical study; and J.Z. performed statistical analysis.

Conflict-of-interest disclosure: G.G-M. served in a consulting or advisory role for Acceleron Pharma, Astex Pharmaceuticals, Bristol Myers Squibb, Helsinn Therapeutics, and Jazz Pharmaceuticals; received honoraria from AbbVie, Acceleron Pharma, Astex Pharmaceuticals, Bristol Myers Squibb, and Helsinn Therapeutics; and received research funding from AbbVie, Amphivena Therapeutics, Astex Pharmaceuticals, Bristol Myers Squibb, H3 Biomedicine, Helsinn Therapeutics, Merck, Novartis, and Onconova Therapeutics. G.J.M. served in a consulting or advisory role for AbbVie and Novartis; and received research funding from Bristol Myers Squibb and Novartis. P.F. served in a consulting or advisory role for and received honoraria from AbbVie, Bristol Myers Squibb, Janssen, and Jazz Pharmaceuticals; and received accommodations, expenses, travel from Jazz Pharmaceuticals. R.B. served in a consulting or advisory role for and received honoraria and research funding from Bristol Myers Squibb and TAIHO; and received research funding from Takeda. V.S. received honoraria from Bristol Myers Squibb and Novartis; and served in a consulting or advisory role for Astex, Bristol Myers Squibb, Geron, Gilead, Menarini, and Novartis. M.D.-C. served in a consulting or advisory role for, received honoraria and research funding from, and had membership on an entity's board of directors or advisory committee of Bristol Myers Squibb and Novartis. C.F. served in a consulting or advisory role for and on the speakers bureau of Bristol Myers Squibb, Janssen, Novartis, and Takeda; and received research funding from Bristol Myers Squibb. M.A.S. served in a consulting or advisory role for Bristol Myers Squibb, Millennium, and Syros Pharmaceuticals; and received research funding from Pfizer and Takeda. A.M.Z. served in a consulting or advisory role for and received honoraria from AbbVie, Acceleron Pharma, Agios, Astellas, Beyond Spring, Boehringer-Ingelheim, Bristol Myers Squibb, Cardiff Oncology, Cardinal Health, Daiichi Sankyo, Epizyme, Incyte, Ionis, Jazz Pharmaceuticals, Novartis, Pfizer, Otsuka, Seattle Genetics, Taiho, Takeda, and Trovagene; and received other from AbbVie, ADC Therapeutics, Aprea, Astex, Boehringer-Ingelheim, Bristol Myers Squibb, Incyte, MedImmune/AstraZeneca, Novartis, Pfizer, Takeda, and Trovagene; Cardiff Oncology, CCITLA, and Leukemia and Lymphoma Society. R.I. ended employment in the past 24 months with and received stock and other ownership interests from Bristol Myers Squibb; and is employed by and received stock and other ownership interests from Eli Lilly and Company. J.Z. is employed by and received stock and other ownership interests in Bristol Myers Squibb. D.S. ended employment in the past 24 months with Bristol Myers Squibb; and is employed by CRISPR Therapeutics. A.R. is employed by and received travel, accommodations, expenses, stock, and other ownership interests from Bristol Myers Squibb. J.T.B. is employed by and received stock and other ownership interests from Acceleron Pharma; and received stock and other ownership interests from Bristol Myers Squibb. U.P. served in a consulting role for and received honoraria from AbbVie, Bristol Myers Squibb, and Novartis. R.S.K. served in a consulting or advisory role for Agios, Bristol Myers Squibb, Daiichi Sankyo, Incyte, Janssen, Novartis, and Pfizer; served on the speakers bureau for Alexion Pharmaceuticals, Jazz Pharmaceuticals, and Novartis; and received stock and other ownership interests from AbbVie. O.I. declares no competing financial interests.

ORCID profiles: V.S., 0000-0002-5439-2172; M.D.-C., 0000-0002-1467-6779; C.F., 0000-0001-9372-1197; U.P., 0000-0003-1863-3239; R.S.K., 0000-0002-1876-5269.

Correspondence: Guillermo Garcia-Manero, Department of Leukemia, The University of Texas MD Anderson Cancer Center, 515 Holcombe Blvd, Houston, TX 77030, USA; e-mail: ggarciam@mdanderson.org.

Footnotes

Submitted 26 May 2021; accepted 15 October 2021; prepublished online on *Blood* First Edition 10 November 2021.

*U.P. and R.S.K. are joint senior authors.

REFERENCES

1. Platzbecker U. Treatment of MDS. *Blood*. 2019;133(10):1096-1107.
2. Bryan J, Jabbour E, Prescott H, Kantarjian H. Thrombocytopenia in patients with myelodysplastic syndromes. *Semin Hematol*. 2010;47(3):274-280.
3. Toma A, Fenaux P, Dreyfus F, Cordonnier C. Infections in myelodysplastic syndromes. *Haematologica*. 2012;97(10):1459-1470.
4. Adès L, Itzykson R, Fenaux P. Myelodysplastic syndromes. *Lancet*. 2014;383(9936):2239-2252.
5. Fenaux P, Haase D, Santini V, Sanz GF, Platzbecker U, Mey U; ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org. Myelodysplastic syndromes: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2021;32(2):142-156.
6. Gattermann N. Iron overload in myelodysplastic syndromes (MDS). *Int J Hematol*. 2018;107(1):55-63.
7. Moukalled NM, El Rassi FA, Temraz SN, Taher AT. Iron overload in patients with myelodysplastic syndromes: an updated overview. *Cancer*. 2018;124(20):3979-3989.
8. Fenaux P, Adès L. How we treat lower-risk myelodysplastic syndromes. *Blood*. 2013;121(21):4280-4286.
9. Zeidan AM, Kharfan-Dabaja MA, Komrokji RS. Beyond hypomethylating agents failure in patients with myelodysplastic syndromes. *Curr Opin Hematol*. 2014;21(2):123-130.
10. Santini V, Almeida A, Giagounidis A, et al. Randomized phase III study of lenalidomide versus placebo in RBC transfusion-dependent patients with lower-risk non-del(5q) myelodysplastic syndromes and ineligible for or refractory to erythropoiesis-stimulating agents. *J Clin Oncol*. 2016;34(25):2988-2996.

11. Suragani RN, Cadena SM, Cawley SM, et al. Transforming growth factor-β superfamily ligand trap ACE-536 corrects anemia by promoting late-stage erythropoiesis. *Nat Med*. 2014;20(4):408-414.

12. Fenaux P, Platzbecker U, Mufti GJ, et al. Luspatercept in patients with lower-risk myelodysplastic syndromes. *N Engl J Med*. 2020;382(2): 140-151.

13. Cheson BD, Greenberg PL, Bennett JM, et al. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. *Blood*. 2006;108(2):419-425.

14. Greenberg PL, Tuechler H, Schanz J, et al. Revised international prognostic scoring system for myelodysplastic syndromes. *Blood*. 2012; 120(12):2454-2465.

15. Lübbert M, Suciu S, Baila L, et al. 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*. 2011;29(15):1987-1996.

16. Platzbecker U, Middeke JM, Sockel K, et al. Measurable residual disease-guided treatment with azacitidine to prevent haematological relapse in patients with myelodysplastic syndrome and acute myeloid leukaemia (RELAZA2): an open-label, multicentre, phase 2 trial. *Lancet Oncol*. 2018;19(12):1668-1679.

17. Wobus M, Mies A, Magno V, et al. Altered structure and function of mesenchymal stromal cell-derived extracellular matrix in MDS can be restored by luspatercept [abstract]. *Blood*. 2019;134(suppl 1). Abstract 1699.

18. Kubasch AS, Fenaux P, Platzbecker U. Development of luspatercept to treat ineffective erythropoiesis. *Blood Adv*. 2021;5(5):1565-1575.

DOI 10.1182/blood.2021012589

TO THE EDITOR:

Aged healthy mice acquire clonal hematopoiesis mutations

Desmond Wai Loon Chin,[1,*] Tetsuichi Yoshizato,[1,*] Stina Virding Culleton,[1] Francesca Grasso,[1] Magdalena Barbachowska,[1] Seishi Ogawa,[1,2] Sten Eirik W. Jacobsen,[1,3-5,†] and Petter S. Woll[1,3,†]

[1]Department of Medicine Huddinge, Center for Hematology and Regenerative Medicine, Karolinska Institutet, Stockholm, Sweden; [2]Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [3]Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden; [4]Karolinska University Hospital, Stockholm, Sweden; and [5]MRC Molecular Hematology Unit, MRC Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom

Recent studies have revealed the presence of clonally expanded cells with somatically acquired cancer-associated mutations within normal human tissues,[1-5] including in blood from healthy elderly individuals, where these identify individuals with clonal hematopoiesis (CH).[3-5] Among the most prevalent CH mutations are those seen in *DNMT3A*, *TET2*, *ASXL1*, and *TP53*,[3-5] implicated as initiating mutations in myeloid malignancies.[6,7] CH confers increased risk for later development of myeloid malignancies.[3-5] However, most CH cases never develop any malignancy, and mechanisms enhancing transformation risk and clonal advantage of CH mutations remain unclear.[5] Unraveling these mechanistic aspects of CH could greatly benefit from studies in genetically modified mice. Such studies have already provided some insights, but with conflicting results.[8-10] Because the relevance of mice for modeling of CH mutations, myeloid malignancies, and cancer in general has been questioned,[5,9-11] it would be important to establish to what degree mutations seen in human CH also occur spontaneously and promote clonal expansion in normal-aged mice. CH mutations have yet to be described in mice screened for spontaneous oncogeneic mutations,[12] potentially because of the few mice investigated and sequencing strategies with insufficient sensitivity to detect small clones[12] as human CH mutations, often occur early in life, but are often first detected in aged individuals (>70 years of age) when the clones have become large enough for detection with existing methodology.[5,13] The much lower number of mouse hematopoietic stem cells (HSCs)[14,15] and their shorter lifespan (2 to 3 years) suggest that CH mutations would occur at a much lower rate in mice and prove more difficult to detect than in human. However, the much smaller size of the mouse and fewer HSCs could potentially enable detection of CH clones in aged mice. We screened (supplemental methods, available on the *Blood* Web site) for the most common CH mutations in up to 24-month-old wild-type C57BL/6j mice, the most extensively used mouse strain for studies of normal and malignant hematopoiesis, including genetically modified mice with CH mutations.[8-10] DNA isolated from single aged human (70 to 75 years; n = 6) or mouse (24 months; n = 6) HSC-derived cells was subjected to whole-genome sequencing, and bulk bone marrow (BM) of aged (n = 97) and transplanted (n = 88) mice to digital droplet polymerase chain reaction (ddPCR) analysis and error-corrected targeted DNA sequencing (ECTS). Similar to cultured fibroblasts,[16] but not previously investigated for HSCs, we observed a significantly higher mutation rate (8.5-fold) in mouse compared with human HSCs (Figure 1A; supplemental Figure 1A). In line with previous reports,[14,17] aged human HSCs contained ~1000 mutations (Figure 1B). Although a 2-year old laboratory mouse approximates a 70-year-old human individual in relative lifespan, the increased mutation rate in mouse HSCs did not result in comparable mutational accumulation, as aged mouse HSC mutations were fivefold lower than in aged human HSCs (Figure 1B). Despite their differences in lifespan, mutations in aged mouse HSCs were distributed among similar genomic regions, dominated by the aging-associated COSMIC signature 1 featured by enrichment of C>T transitions at CpG dinucleotides[18] in both aged human and mouse HSCs[14,17] (Figure 1C-E; supplemental Figure 1B-C). Together with the estimated HSC pool size in mice vs humans,[14,15] this suggests that although mutations targeted to CH-associated genes would be much more frequent in aged