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CORRESPONDENCE

SFLT-1 levels in COVID-19 patients: Association with outcome and thrombosis

To The Editor:

We read with great interest the recent paper by Giardini et al. (2020) regarding endothelial dysfunction in coronavirus disease 2019 (Covid-19) patients and appreciate that the authors recognize similarities with the pre-eclampsia syndrome model.[1] The article addresses a very timely and important topic with a high impact on clinical practice and management of the patients. Covid-19 is not only a worldwide emergency but also an insidious disease. The virus accesses host cells via the angiotensin-converting enzyme 2, which is very abundant in the alveolar epithelial cells and other cell types, including endothelial cells. The clinical spectrum ranges from asymptomatic to critically ill cases, characterized by pneumonia, which can worsen rapidly to respiratory failure and acute respiratory distress syndrome.[2] The mortality rate is high, and over 50% of severe cases die due to complications. In this context, early identification of high-risk patients could facilitate appropriate supportive care and reduce the mortality rate. Therefore, it is crucial to identify reliable biomarkers associated with shortened survival.

A growing number of data shows that Covid-19 mortality is partially due to a severe form of coagulopathy. Furthermore, the most common comorbidities with a significant impact on survival are hypertension, diabetes, and obesity, all conditions characterized by chronic endothelial dysfunction.[3] Varga et al. recently demonstrated histologically diffuse endothelial inflammation in various organs due to direct viral infection.[4] Therefore, it is essential to study better the role of angiogenic and anti-angiogenic factors in the onset of endothelial dysfunction. As pointed out in the article, pre-eclampsia syndrome is a well-known model of angiotensin II-mediated endothelial dysfunction. For this reason, we recently conducted a single-center study focused directly on the vascular damage impact on survival with a similar approach to Giardini's group; however, our primary goal was to assess factors associated with survival in patients with moderate/severe Covid-19.

The study was approved by the institutional review board and conducted according to the Declaration of Helsinki principles. All patients provided written, informed consent, and data were collected and analyzed by the investigators. The study population included 105 inpatients with Covid-19 diagnosis, confirmed by RT-PCR SARS-CoV-2 assay on nasopharyngeal and oropharyngeal swabs, and followed to death or until recovery. The variables evaluated included standard clinical and laboratory variables, placental growth factor (PlGF), soluble fms-like tyrosine kinase-1 (sFlt-1), and interleukin-6 (IL-6). The blood samples were collected 5 to 8 days from admission to the hospital. The Cobas Roche platform was used to determine IL-6, PlGF, and sFlt-1 levels. Significant differences among groups in the distribution of continuous or nominal variables were determined with standard statistical methods. A significant *P* value was <.05.

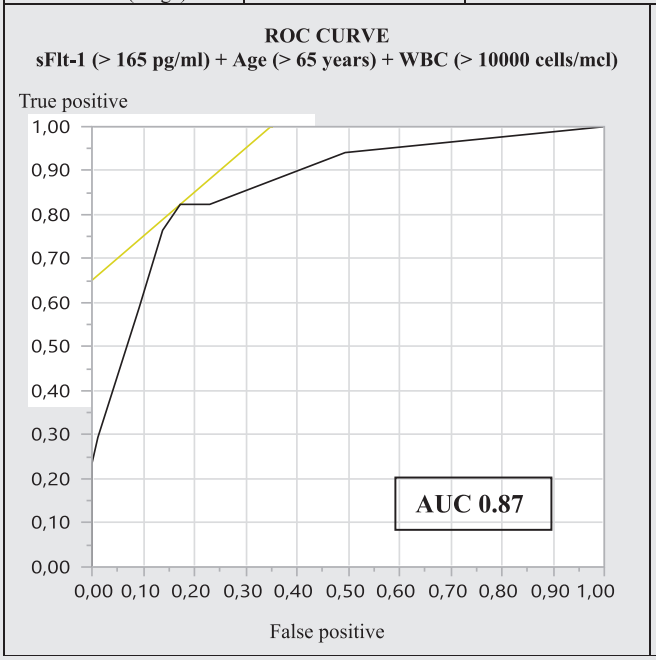
Univariate analysis (Table 1) showed significant differences in distribution of eight variables between the survivors and the deceased: age (*P* = .001), white blood cell count (*P* = .005), and the levels of N-terminal prohormone of brain natriuretic peptide (*P* = .039), albumin (*P* = .003), ferritin (*P* = .043), lactate dehydrogenase (*P* = .020), procalcitonin (*P* = .033), and sFLT-1 (*P* = .001). Notably, neither PlGF nor sFlt-1/PlGF ratio were significantly different between the two groups of the patients. In multivariable analysis only three risk factors retained significance: age (*P* = .018), white blood cell count (*P* = .022), and sFlt-1 levels (*P* = .003). The best threshold for each variable was determined with logistic regression: sFlt-1 > 165 pg/mL; Age > 65 years; WBC > 10 000 cells/μL. The predictive accuracy of the three new binomial categories combined was evaluated with the ROC curve: AUC 0.87. To better understand the endothelial damage role, we decided to rerun the analysis using only sFLT-1 > 165 pg/mL category: AUC = 0.75, OR = 11.61 (3.74-39.67). The same threshold was used to analyze the rate of major thrombotic events (deep vein thrombosis, pulmonary embolism, stroke) during hospitalization in the study population (data not shown). The results (TableS1-S3) showed that elevated sFlt-1 levels are significantly associated with thrombosis (*P* = .020), especially in the subgroup not treated with anticoagulant therapy (*P* = .023).

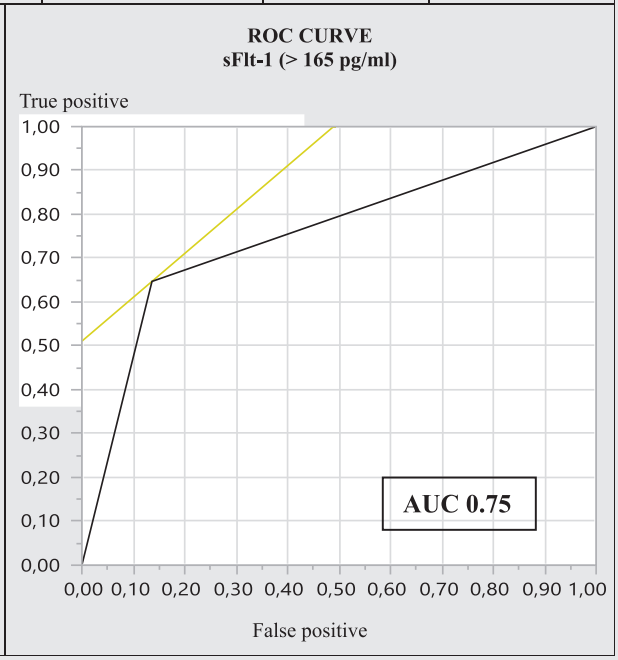
Whereas data from Giardini et al. propose sFlt-1/PlGF ratio as a tool to stratify the severity of angiotensin II-mediated endothelial dysfunction and discern the patients with Covid-19 associated pneumonia from patients with non-Covid-19 pneumonia, the utility of the sFlt-1/PlGF ratio for stratification of endothelial dysfunction among Covid-19 patients only and outcome prediction is unclear. Our study, on a larger population, confirms the presence of endothelial damage in Covid-19 and furthers these results by showing that sFlt-1 is a more reliable biomarker than the sFlt-1/PlGF ratio to predict survival and thrombotic accidents in Covid-19 patients. Increased sFlt-1 levels are associated not only with pre-eclampsia, but also with other conditions characterized by severe endothelial dysfunction like sepsis, and acute pancreatitis.[5,6] The same severe endothelial dysfunction is likely present in Covid-19 patients with a significant impact on survival.

Furthermore, the vascular dysfunction role most likely overarches the impact of inflammation on survival in Covid-19, as testified by the not significant association of poor outcome with IL-6 levels on multivariate analysis. The clinical implication is that up-regulated sFlt-1 levels may represent a useful marker for predicting

TABLE 1 Univariate and multivariate logistic regression on mortality in COVID-19 patients, and area under the receiver operating characteristic curve analysis

Variables	Total patients (n=105)	Survivors (n=88)	Deceased (n=17)	Univariable analysis *P value*	Multivariable analysis *P value*
Age (years); median (range)	65 (18 – 97)	61 (18 – 89)	75 (45 – 97)	***0.001***	***0.018***
Sex (male); *n* (%)	56 (53%)	48 (55%)	8 (47%)	*0.572*	
Hemoglobin (g/dl); median (range)	13.4 (8.6 – 16.9)	13.4 (9.4 – 16.8)	12.7 (8.6 – 16.9)	*0.088*	
Platelets (x10^3/mcl); median (range)	202 (86 – 604)	195.5 (86 – 604)	242 (118 – 537)	*0.093*	
WBC (cells/mcl); median (range)	5825 (900 – 18630)	5590 (900 – 16590)	6510 (4200 – 18630)	***0.005***	***0.022***
D-Dimer (ng/ml); median (range)	1107 (209 – 40000)	1040 (209 – 40000)	3103 (258 – 15786)	*0.524*	
Creatinine (mg/dl); median (range)	0.9 (0.37 – 6)	0.89 (0.37 – 6)	0.92 (0.57 – 3.1)	*0.466*	
NT-proBNP (pg/ml); median (range)	278 (25 – 33903)	203 (25 – 33903)	1445 (193 – 27618)	***0.039***	____
Albumin (g/dl); median (range)	3.91 (2.46 – 4.49)	3.93 (2.83 – 4.49)	3.6 (2.46 – 4.22)	***0.003***	____
AST (U/l); median (range)	43 (12 – 467)	43 (13 – 467)	43 (12 – 149)	*0.781*	
ALT (U/l); median (range)	34.5 (9 – 1123)	37 (9 – 1123)	28 (12 – 261)	*0.767*	
Ferritin (mcg/l); median (range)	631.9 (53.7 – 3667.1)	578 (53.7 – 3667.1)	1327 (168 – 2997)	***0.043***	____
LDH (mU/ml); median (range)	640 (295 – 1699)	616 (295 – 1699)	764 (408 – 1656)	***0.020***	____
PCT (ng/ml); median (range)	0.22 (0.04 – 5.31)	0.21 (0.04 – 5.31)	0.38 (0.08 – 4.54)	***0.033***	____
PCR (mg/l); median (range)	13.8 (0.81 – 129.7)	12.63 (0.81 – 37.4)	17.04 (2.62 – 129.7)	*0.080*	
IL-6 (pg/ml); median (range)	37.85 (1.5 – 4770)	32.4 (1.5 – 4770)	89.1 (6 – 533.7)	*0.790*	
sFlt-1/PlGF (pg/ml); median (range)	7.58 (2.14-211.4)	7.285 (2.93-23.5)	9.44 (2.14-211.4)	*0.2*	____
PlGF (pg/ml); median (range)	18.26 (5.83-69.48)	17.96 (5.96-40.18)	18.77 (5.83-69.48)	*0.4*	____
sFlt-1 (pg/ml); median (range)	131.33 (67.54 – 1233)	124.2 (67.54 – 253.3)	175.7 (99.07 – 1233)	***0.001***	***0.003***





Note: The values in bold indicate a statistically significant difference ($P < .05$).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; LDH, lactate dehydrogenase; IL-6, interleukin-6; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; PCR, C-reactive protein; PCT, Procalcitonin; sFlt-1, soluble Fms-like tyrosine kinase-1; WBC, white blood cell count.

and assessing Covid-19 progression to death. Moreover, the higher rate of thrombosis in the population with elevated sFlt-1 levels and not treated with anticoagulant therapy underscores the importance of starting anticoagulant therapy in this group of patients. In conclusion, sFlt-1 could be a reliable tool to monitor endothelial dysfunction in Covid-19 patients: increased biomarker levels could induce the clinicians to attempt a more aggressive treatment therapy or change the management of the patient to increase the chances of survival.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIUTIONS

A.N. conceived the project. A.F. wrote the first draft of the paper. A.N. and A.F. supplied clinical material and collected samples. D.P. performed the statistical analysis. L.B. and A.Z. performed lab tests. P.G.G. supervised results. A.N. and D.P. finalized the manuscript. All authors approved the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Aurelio Negro[1†], Angelo Fama[2†], Domenico Penna[2,3], Lucia Belloni[4], Alessandro Zerbini[4], Pasquale Gianluca Giuri[1]

[1]*Internal Medicine and Secondary Hypertension Center, Azienda Unità Sanitaria Locale - IRCCS di Reggio Emilia, Sant'Anna Hospital, Castelnovo ne' Monti, Italy*

[2]*Hematology Unit, Azienda Unità Sanitaria Locale – IRCCS, Reggio Emilia, Italy*

[3]*PhD Program in Clinical and Experimental Medicine, University of Modena and Reggio Emilia, Modena, Italy*

[4]*Clinical Immunology, Allergy and Advanced Biotechnologies Unit, Azienda Unità Sanitaria Locale-IRCCS di Reggio Emilia, Reggio Emilia, Italy*

Correspondence

Domenico Penna, Hematology Unit, Azienda Unità Sanitaria Locale - IRCCS, Via Amendola, 2-42122 Reggio Emilia, Italy.

Email: penna.domenico@ausl.re.it; domenico.penna@ausl.re.it

[†]Drs Aurelio Negro and Angelo Fama contributed equally to the article

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ORCID

Aurelio Negro https://orcid.org/0000-0002-0185-0803

Domenico Penna https://orcid.org/0000-0002-3102-422X

REFERENCES

1. Giardini V, Carrer A, Casati M, Contro E, Vergani P, Gambacorti-Passerini C. Increased sFLT-1/PlGF ratio in COVID-19: a novel link to angiotensin II-mediated endothelial dysfunction. *Am J Hematol*. 2020; 95:E188-E191. https://doi.org/10.1002/ajh.25882
2. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. 2020;395(10229):1054-1062.
3. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) oubreak in China: summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention. *JAMA*. 2020;323:1239-1242.
4. Varga Z, Flammer AJ, Steiger P, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet*. 2020;395(10234):1417-1418.
5. Palmer KR, Tong S. Kaitu'u-Lino TJ. Placental specific sFlt-1: role in pre-eclamptic pathophysiology and its translational possibilities for clinical prediction and diagnosis. *Mol H um Reprod*. 2017;23:69-78.
6. Shapiro NI, Schuetz P, Yano K, et al. The association of endothelial cell signaling, severity of illness, and organ dysfuntion in sepsis. *Crit Care*. 2010;14:R182.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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High dose romiplostim as a rescue therapy for adults with severe bleeding and refractory immune thrombocytopenia

To the Editor:

Life-threatening bleeding is a rare event in immune thrombocytopenia (ITP) and intracranial hemorrhage (ICH) occurs in less than 1% but is associated with a mortality rate of 44% in adults.[1] Even if no controlled study has been conducted in patients with life-threatening bleeding, high-dose corticosteroids, IVIg and platelet transfusions are widely used. In an emergency with uncontrolled bleeding, starting high dose of TPO-RAs can be considered to rapidly increase the platelet count to a level where the risk of severe bleeding is minimized.[2] Such off-label strategy has not been systematically evaluated. To evaluate the safety of this "rescue therapy" we carried out a multicenter retrospective cohort study of ITP patients treated with maximal dose of romiplostim for bleeding emergency from 2017 to 2019 in centers belonging to French national network for adult ITP. We

included patients who fulfilled the following criteria: (a) Platelet count <30 × 10^9/L; (b) Severe bleeding manifestations defined as a score > 8 according to the bleeding score previously reported by our group which takes into account cutaneous, mucosal and visceral bleeding[3]; (c) No response to corticosteroids and IVIg; (d) Rescue therapy with maximal dose of romiplostim (ie, 10 μg/kg body weight). Response (R) and Complete response (CR) were defined according to standardized international criteria.[2]

We included 30 patients (15 men) with ITP and severe bleeding manifestations (Table S1, Figure S1). The median age was 65 years [range 18-77]. Twenty-seven patients had newly diagnosed ITP and three chronic ITP. All patients had severe bleeding symptoms, characterized by skin and mucosal bleedings. The median bleeding score was 20 [range 8-33], including intracranial hemorrhage (n = 9), visceral hemorrhage — metrorrhagia, macroscopic hematuria, gastrointestinal or peritoneal bleeding (n = 16) (some patients had several manifestations). Twelve patients had received red blood cell transfusions because of severe anemia related to bleeding. The median platelet count was 2 × 10^9/L [range 0-10] at the time of severe bleeding.

All 30 patients had received corticosteroids and IVIg treatment [median dose 2 g/kg.bw, range 1-4] as first-line therapy, and had failed to achieve any response. Twenty patients (67%) received platelet transfusions. Twenty-six patients had received intravenous vinca alkaloid (VA), in median 8 days (range 3-36) after initial bleeding symptoms: either concomitantly (ie,:1-3 days to romiplostim administration) in 20 cases; or 4-14 days before in six cases (8 mg vinblastine, n = 24 and 1 mg/m2 vincristine n = 2). Four patients did not receive VA including two patients who had a contra-indication to VA. Eight patients had received one or two other concomitant therapies. Romiplostim was started at a median of 9 days [range 3-40] after the first bleeding episode. At that time, bleeding symptoms were severe (bleeding score > 8[3]), and for all but two patients, the platelet count was ≤20 × 10^9/L. These two patients received repeated platelet transfusions because of cerebral bleeding, with platelet counts 63 and 47 × 10^9/L, respectively. All patients received a starting dose of romiplostim at 10 μg/kg.bw/week from the first injection (n = 26), or from the second administration after a first attenuated dose of 5-8 μg/kg.bw (n = 4). During the first month of follow-up, the median peak platelet count among responders was 531 × 10^9/L [range 70-3840]; for 16 patients, the platelet count was >500 × 10^9/L and for nine it was >1000 × 10^9/L. Patients with a platelet count >1000 × 10^9/L received empirically low-dose aspirin. Three serious adverse events were noted. One 52-year-old woman who was bedridden because of a muscular hematoma, experienced deep-vein thrombosis with an asymptomatic pulmonary embolism 13 days after IVIg treatment and 5 days after the first romiplostim injection; the platelet count was 629 × 10^9/L at the time of thrombosis. One 70-year-old woman was diagnosed with ischemic stroke in the left parietal and right temporal lobes 13 days after IVIg administration and 10 days after the first romiplostim injection; at that time. Her platelet count was then in normal range (239 × 10^9/L). No predisposing condition for arterial thrombo-embolic events was found. Lastly, one 67-year-old patient who presented with cerebellar hemorrhage when diagnosed with ITP, died of cerebellar oedema 1 month after initial surgical treatment with external ventricular drains which had to be replaced several times because of infection or occlusion. At that time, cerebellar hemorrhage was stable and complete response was achieved 3 weeks before.

We then evaluated response in the 20 patients who received romiplostim and concomitant VA treatment (VA + ROMI group) and compared to an historical cohort of 22 subjects with severe bleeding with absence of response to steroids and IVIg and who received VA without romiplostim (VA group) from 2008 to 2014. The two groups were not closely similar since patients in the VA + ROMI group had more severe bleeding manifestations, with a higher bleeding score (18 [range 8-33] vs 10 [range 8-28]), more ICH (30% vs 5%) and visceral hemorrhage (58% vs 36%) (Table 1). The day of VA administration was considered the starting point for evaluation. During the 14 days period of analysis, steroids were maintained at stable dose in all patients. Additional treatments administered are detailed in Table S1. At day 7 after VA +/− ROMI initiation, there was no significant difference in terms of response between the two groups (VA + ROMI: 70% vs VA: 48%, P = .15), but complete response was significantly higher in the group of patients who received VA + ROMI compared to those who received VA alone [60% vs 29%, P < .05]. Between day 7 and day 14, 13 patients (65%) received a second administration of ROMI in the VA + ROMI group; and 6 (30%) and 9 (41%) patients received a second administration of VA, respectively in the VA + ROMI and in the VA group. At day 14, both response and complete response were significantly higher in the VA + ROMI group compared to VA group [R: 80% vs 43%, P < .05; and CR: 70 vs 17%, P < .0001] (Figure 1). In the group receiving VA alone, one patient died at day 5 from uncontrolled bleeding and hemorrhagic shock, four experienced severe adverse events (agranulocytosis, n = 2, and urinary tract infection, n = 2). No thrombotic event was observed.

Our work raises safety concerns, as major thrombotic events (TEs) occurred in two patients out of 30. It is noteworthy that in the present series, the platelet count was >500 × 10^9/L at time of thrombosis in one patient but <250 × 10^9/L in the other. This is in line with previous observations showing that thrombosis with TPO-RAs occurred independently from the platelet count[4,5] and may result from platelet activation. We cannot rule out a deleterious pro-thrombotic synergistic effect with other treatments, especially with IVIg which can itself promote TEs when other risk factors are present.[6] Thrombocytosis with a platelet count over 1000 × 10^9/L was observed in a third of the patients, none of whom experienced a symptomatic thrombotic event. However, all were empirically treated with low dose aspirin.

Our study strongly suggests that romiplostim given at a maximal dose is effective for treating patients with refractory ITP in emergency bleeding situations. Patients included in this study had life-threatening hemorrhage despite first-line therapy with corticosteroids + IVIg and with platelet transfusion in about two thirds of patients. Failure to respond to this first-line rescue therapy has been found to be associated with the risk of intracranial hemorrhage which occurred in 32% of our patients. Regarding the initial response, most patients received concomitant therapies that may have interfered with the interpretation of platelet response. In particular, vinca alkaloids were used in most

TABLE 1 Characteristics of patients receiving VA plus ROMI and VA alone

	VA + ROMI (n = 20)	VA (n = 22)
Median age (range)	56 (18-93)	53 (22; 93)
Gender: women / men	9 (45%) / 11 (55%)	14 (64%) / 8 (36%)
Newly diagnosed	18 (90%)	18 (82%)
Secondary ITP	4 (20%)[a]	3 (14%)[b]
Median platelet count, 109/L (range)	1 (0–5)	3 (1-10)
Bleeding manifestations		
• Median bleeding score	18 (9-33)	10 (8–28)
• Skin and mucosal bleeding	20 (100%)	22 (100%)
• Visceral hemorrhage	11 (58%)	8 (36%)
• Intracranial hemorrhage	6 (30%)	1 (4,5%)
Red blood cell transfusions	8 (40%)	7 (32%)
Treatment received prior to alkaloid/romiplostim initiation		
• Steroids	20 (100%)	22 (100%)
• IVIg	20 (100%)[c]	22 (100%)[d]
• Other specific treatments	5 (25%)[e]	8 (36%)[f]
• Platelet transfusions	14 (70%)	10 (46%)

[a]Systemic lupus erythematosus (n = 2), EBV Infection (n = 1), Evan's syndrome (n = 1).
[b]Systemic lupus (n = 1), hepatitis C (n = 1), chronic lymphoid leukemia (n = 1).
[c]Median dosage 2 g/kg.bw (range 2–4).
[d]Median dosage 2 g/kg.bw (range 2-9).
[e]rituximab (n = 3), hydroxychloroquin (n = 1), eltrombopag (n = 3).
[f]rituximab (n = 2), splenectomy (n = 1), danatrol (n = 2), disulone (n = 2), hydroxychloroquin (n = 1), anti-D gammaglobulins (n = 1); IVIg: Intraveinous immunoglobulin.

patients, with an expected time to initial response that is similar to that of romiplostim.[4] To minimize the risk of an interpretation bias, we focused our efficacy analysis on those patients who received romiplostim and VA concomitantly and compared their outcome to an historical group of patients who were treated only with VA in the same situation. We found a significantly higher overall response rate at day 14 in the ROMI + VA group and a significantly higher CR rate at days 7 and 14.

The use of romiplostim rather than eltrombopag was preferred firstly because the subcutaneous route of administration was more suitable for critically ill patients with life-threatening bleeding, especially in the intensive care unit, and secondly because the range of dose of romiplostim [1-10 μg/kg/week] is higher than that of eltrombopag [25-75 mg daily]. However, there is no reason to speculate that eltrombopag at 75 mg/day would not also have been effective. The use of a lower dose of romiplostim (ie, 5 μg/kg) could potentially decrease the risk of thrombocytosis but also the efficacy rate.

The main limitation of this study is the retrospective design and potential bias of analysis.

In conclusion, this study shows that the use of romiplostim at maximal dosage as rescue therapy for ITP patients with severe bleeding not responding to standard rescue therapy is feasible and effective. The risk of thrombo-embolic events and the risk over benefit ratio should however be carefully assessed. Therefore, we suggest that this strategy should be restricted to life-threatening situations while awaiting more robust safety data.

CONFLICT OF INTEREST

MMa received research grants from GSK, and meeting attendance grants from GSK and Amgen. GM received research grants form CSL Behring, Novartis, Grifols, and meeting attendance grants from Amgen and Novartis. LG participated to educational boards for GSK. BG received research grant from Amgen, and B.G. served as an expert for Amgen, Novartis, LFB and Roche. ME has participated in advisory boards for Amgen, Grifols, GSK and Novartis.

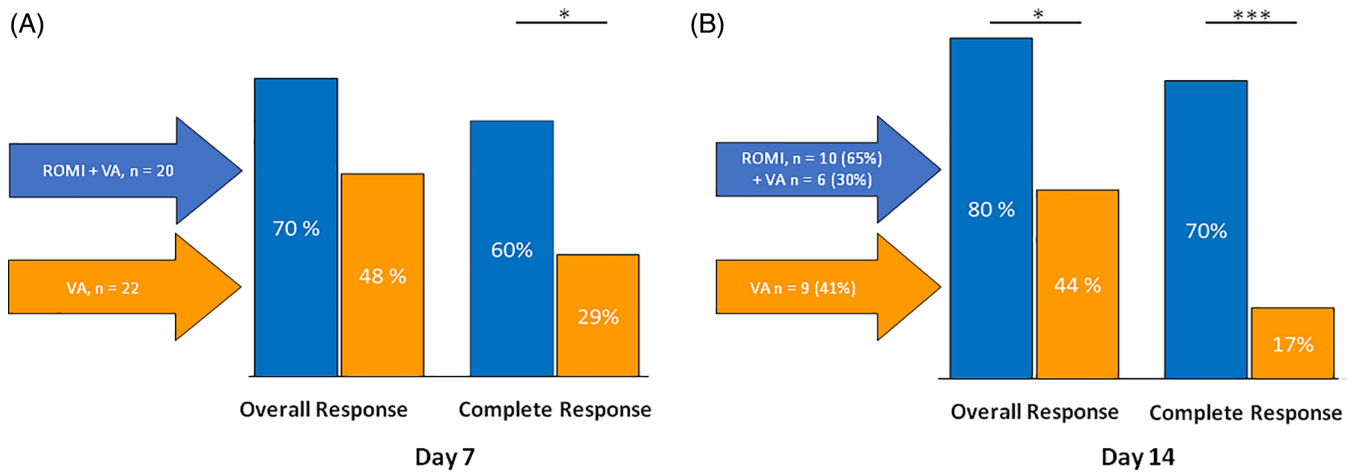

FIGURE 1 Initial response to VA plus ROMI or VA in ITP patients refractory to IVIg and steroids. We compared the response at day 7 and 14 in the 20 patients who received romiplostim (ROMI) and concomitant vinca-alkaloid treatment (VA + ROMI) and compared to a historical cohort of 22 subjects receiving vinca-alkaloids without romiplostim (VA). (* $P < .05$, *** $P < .001$)

AUTHOR CONTRIBUTIONS

M.Ma designed the study and initiated this work; B.G., M.M., M.R and S.LB wrote the report; all authors made substantial contributions to acquisition of data, revised the article critically and gave final approval of the manuscript to be submitted.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Mathilde Roumier[1,2], Sébastien Le Burel[1,3], Sylvain Audia[4], Adrien Chauchet[5], Marie Gousseff[6], Mohammed Hamidou[7], François Liferman[8], Guillaume Moulis[9], Bertrand Lioger[10], Lionel Galicier[11], Mickael Ebbo[12], Jonathan London[13], Solène Poutrel[14], Louis Terriou[15], Virginie Zarrouk[16], Marc Michel[1], Bertrand Godeau[1], Matthieu Mahevas[1]

[1]*Department of Internal Medicine, National Referral Center for Adult'Immune Cytopenias Henri Mondor University Hospital, Assistance Publique Hôpitaux de Paris, Université Paris-Est Créteil, Créteil, France*

[2]*Departmanet of Internal Medicine, Foch Hospital, Suresnes, France*

[3]*Departmanet of Internal Medicine, Bégin Hospital, Saint-Mandé, France*

[4]*Department of Internal Medicine and Clinical Immunology, Bocage Central University Hospital, CR INSERM 1098, Dijon, France*

[5]*Department of Hematology, Besancon University Hospital, Besancon, France*

[6]*Department of Internal Medicine, Centre Hospitalier Bretagne Atlantique, Vannes, France*

[7]*Department of Internal Medicine, Hôtel Dieu University Hospital, Nantes, France*

[8]*Departmanet of Internal Medicine, Centre Hospitalier de Dax, Dax, France*

[9]*Department of Internal Medicine, Purpan University Hospital, Toulouse, France*

[10]*Departmanet of Internal Medicine, Saint-Louis Hospital, Paris, France*

[11]*Department of Clinical Immunology, Saint Louis University Hospital, Assistance Publique Hôpitaux de Paris, Paris, France*

[12]*Department of Internal Medicine, La Conception Hospital, Assistance Publique Hôpitaux de Marseille, Université Aix-Marseille, Marseille, France*

[13]*Department of Internal Medicine, Cochin University Hospital, Assistance Publique Hôpitaux de Paris, Paris, France*

[14]*Department of Internal Medicine, Edouard Herriot Hospital, Hospices Civils de Lyon, Lyon, France*

[15]*Department of Internal Medecine and Clinical Hematology, Claude-Huriez University Hospital, Université Lille Nord de France, Lille, France*

[16]*Department of Internal Medicine, Beaujon Hospital, Assistance Publique Hôpitaux de Paris, Clichy, France*

Correspondence

Matthieu Mahevas, Centre de référence des cytopénies auto-immunes de l'adulte, Hôpital Henri Mondor, Assistance Publique-Hôpitaux de Paris, Université Paris Est Créteil, Créteil, France 94000.

Email: matthieu.mahevas@hmn.aphp.fr

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ORCID

Matthieu Mahevas https://orcid.org/0000-0001-9182-1434

REFERENCES

1. Melboucy-Belkhir S, Khellaf M, Augier A, et al. Risk factors associated with intracranial hemorrhage in adults with immune thrombocytopenia: a study of 27 cases. *Am J Hematol*. 2016;91(12):E499-E501. https://doi.org/10.1002/ajh.24529.
2. Provan D, Arnold DM, Bussel JB, et al. Updated international consensus report on the investigation and management of primary immune thrombocytopenia. *Blood Adv*. 2019;3(22):3780-3817. https://doi.org/10.1182/bloodadvances.2019000812.
3. Khellaf M, Michel M, Schaeffer A, Bierling P, Godeau B. Assessment of a therapeutic strategy for adults with severe autoimmune thrombocytopenic purpura based on a bleeding score rather than platelet count. *Haematologica*. 2005;90(6):829-832.
4. Ghanima W, Cooper N, Rodeghiero F, Godeau B, Bussel JB. Thrombopoietin receptor agonists: ten years later. *Haematologica*. 2019;104(6):1112-1123. https://doi.org/10.3324/haematol.2018.212845.
5. Rodeghiero F, Stasi R, Giagounidis A, et al. Long-term safety and tolerability of romiplostim in patients with primary immune thrombocytopenia: a pooled analysis of 13 clinical trials. *Eur J Haematol*. 2013;91(5):423-436. https://doi.org/10.1111/ejh.12181.
6. Moulis G, Audemard-Verger A, Arnaud L, et al. Risk of thrombosis in patients with primary immune thrombocytopenia and antiphospholipid antibodies: a systematic review and meta-analysis. *Autoimmun Rev*. 2016;15(3):203-209. https://doi.org/10.1016/j.autrev.2015.11.001.

SUPPORTING INFORMATION

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A phase I clinical trial of avelumab in combination with decitabine as first line treatment of unfit patients with acute myeloid leukemia

To the Editor:

Despite considerable efforts, treatment of acute myeloid leukemia (AML) remains challenging. Prognosis for elderly patients or patients who are unfit for intensive chemotherapy is particularly poor as treatment options for them are very limited. Recent success using reagents

targeting immune checkpoints, such as PD-1, offers great promise for effective cancer therapy.[1,2] Several agents blocking the PD-1 pathway have been FDA approved for treating multiple solid tumors and Hodgkin lymphoma. It has been demonstrated that hypomethylating agent (HMA) enhances the PD-1 pathway in MDS and AML patients,[3,4] providing a strong rationale for combining HMA and PD-1 inhibition in AML treatment. Avelumab is a PD-L1 antibody that has been FDA approved for treating Merkel cell carcinoma, renal cell carcinoma, and urothelial carcinoma. Decitabine is a HMA that is commonly used in physicians' practice for treating AML patients who are unfit for intensive chemotherapy.

We performed a single arm, open label phase I study to evaluate safety and tolerability of avelumab in combination with decitabine in patients with untreated AML, who are unfit for intensive chemotherapy (NCT03395873). The trial was approved by the Institutional Review Board of Penn State University College of Medicine (STUDY7889). Written informed consent was obtained from all patients before enrollment. An initial stage (3 + 3 design) followed by an expansion stage of nine additional patients were designed. Patients in the initial stage cohort were monitored for dose-limiting toxicity (DLT). The observation period for a DLT was a minimum of 28 days post induction therapy. The primary objective was to determine the safety of combinational treatment. Secondary objectives were to evaluate the complete remission (CR) rate and the overall survival (OS). Detailed information of patient selection, study design, treatment, and safety and response assessment is provided in Appendix S1.

Patient enrollment started January 2018, seven patients were enrolled by December 2018, at which time the accruement was discontinued (per the recommendation of Penn State University College of Medicine data and safety monitoring committee [DSMC]) for the best interest of patients due to the newly FDA approval of venetoclax, a novel treatment for the same patient population. However, all enrolled patients in this study continued treatment and a follow-up was performed as per protocol defined. Table S1 summarizes the patients' characteristics. The median age was 71 years. Most patients (86%) carried adverse cytogenetics. All seven patients received at least one dose of avelumab and were included in the assessment of safety and survival. Two patients died of sepsis before response assessment by bone marrow biopsy, therefore five patients were evaluable for response.

No DLT was observed in the patient cohort of the initial stage. Two patients experienced grade three pneumonitis that was considered to be related to avelumab. One was in the initial cohort and the pneumonitis developed after the second cycle of treatment (beyond DLT evaluation period). The other was in the extension cohort. In both cases, pneumonitis resolved upon steroid treatment. However subsequent avelumab treatments were discontinued per protocol. The AEs were evaluated in all seven patients, Table S2 lists the non-hematologic AEs observed in more than one patient (>14%). The most common grade three or grade four AEs were febrile neutropenia (86%), hypoxia (57%), heart failure (29%), and pneumonitis (29%). Two patients died within 60 days after starting treatment. Both were due to sepsis, of which cellulitis was the infection source for one patient and dental abscess for the other.

Among the five patients who were evaluable for response, one patient (20%) achieved CR, one (20%) experienced progression of disease (PD), and three patients (60%) were with stable disease (SD) as the best response during the treatment course and follow up. All seven patients were assessed for survival. With a median follow-up of 23.1 months, the median overall survival (OS) was 3.2 months (95% confident interval [CI], 1.2-NR).

Comprehensive correlative studies were performed using blood samples collected from each patient. To investigate the effect of avelumab on immune response, we conducted complex flow cytometry-based immune assays on samples prior vs 1 month post treatment. We observed no alteration in the frequency of each immune component (NK, NKT, B cells, DCs, monocytes, CD4 T cells, CD8 T cells, and Treg) upon avelumab and decitabine treatment (Figure 1A). When T cell differentiation subsets were examined based on the surface expression of CD45RA and CCR7, we found a significant increase in effector memory CD8 T cells. There was a trend of decreased terminal differentiated subsets, although no statistical significance was achieved likely due to limited sample size (Figure 1B). We next performed phenotypic and functional analysis of CD8 T cells. We observed a strong trend of up-regulation of activation markers and co-stimulatory receptors (CD69, CD226, CD38, ICOS and 4-1BB) on CD8 T cells post treatment of avelumab and decitabine. In contrast, the expression of inhibitory molecules, including TIGIT, TIM-3, CD160, LAG-3, 2B4 and BTLA, were lower. Consistently, CD8 T cell function was enhanced in majority of patients, manifested by higher expression of granzyme B, perforin and Ki67, as well as more cytokine release (IFN-γ and TNF-α) upon in vitro TCR engagement (Figure 1C, D). Importantly, avelumab is likely the major contributor for the positive regulatory effect as studies on samples from patients who received decitabine alone did not show the same trend (Figure S1).

We observed grade three pneumonitis in two patients. In addition, five patients died of severe septic shock quickly after infections developed. Although neutropenic sepsis is common in AML patients under chemotherapy, the high severity of the inflammatory response is unusual. We hypothesize that avelumab-mediated immune activity may contribute to the severity of patients' reaction. Proinflammatory cytokines are important regulators for the immune response during sepsis. To evaluate the impact of avelumab on the cytokine production by immune cells in response to infections, we performed ex vivo studies co-culturing PBMCs with lipopolysaccharide (LPS), a major component of the outer membrane in Gram-negative bacteria. Intracellular production of cytokines including TNF-α, IL-6, and IL-8 was assessed by flow cytometry. Among all the immune components tested, we observed that cytokines were predominantly produced by monocytes upon LPS stimulation (Figure 1E). We then examined cytokine production by monocytes from samples collected post vs prior to the combination treatment. We found that upon ex vivo LPS stimulation, monocytes from most patients post avelumab and decitabine treatment had increased cytokine production compared to that of prior to treatment. Statistical significance was achieved in TNF-α and IL-8 (Figure 1F). We conducted the same study in samples from patients received decitabine alone treatment and found no impact of

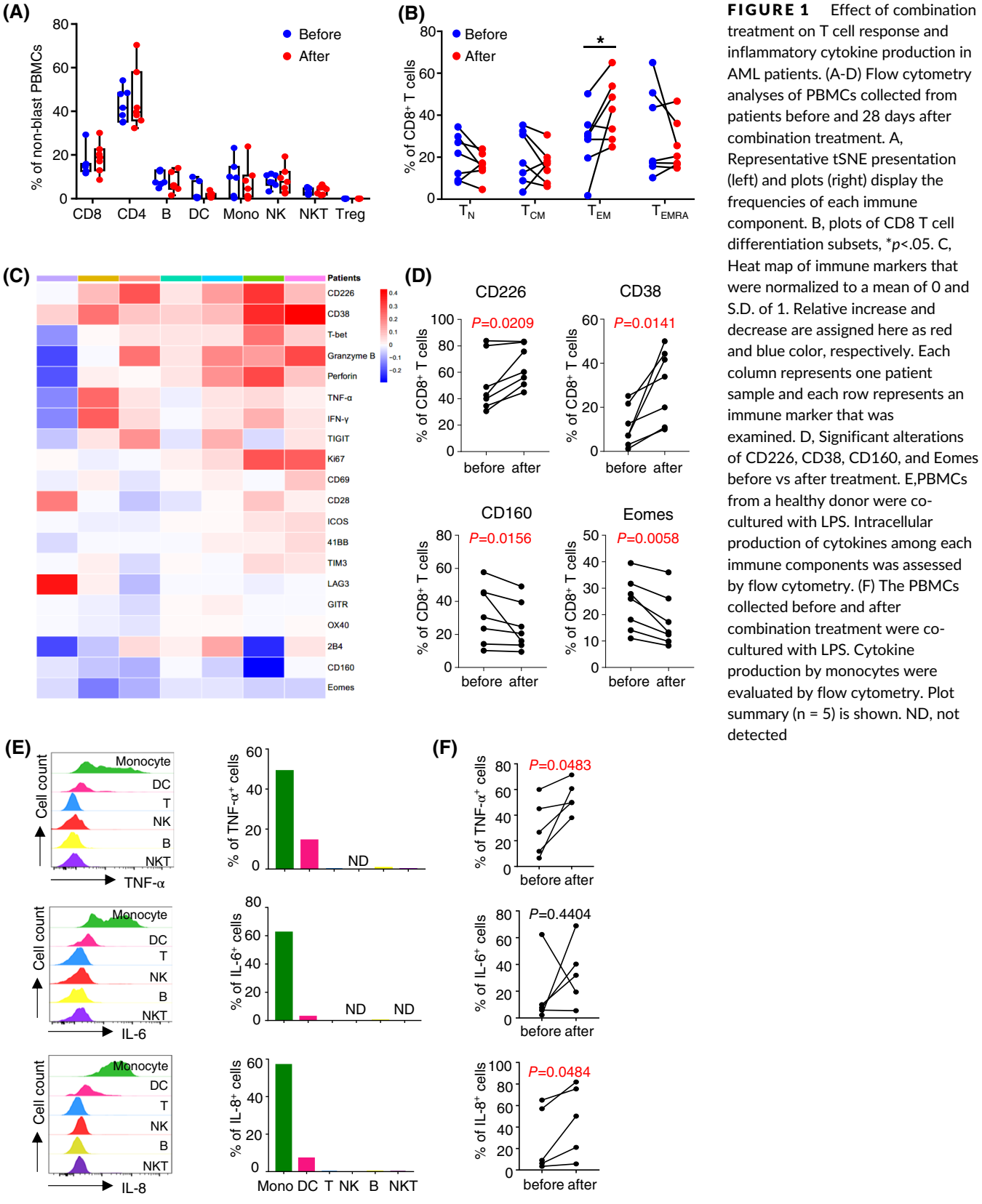

FIGURE 1 Effect of combination treatment on T cell response and inflammatory cytokine production in AML patients. (A-D) Flow cytometry analyses of PBMCs collected from patients before and 28 days after combination treatment. A, Representative tSNE presentation (left) and plots (right) display the frequencies of each immune component. B, plots of CD8 T cell differentiation subsets, *p<.05. C, Heat map of immune markers that were normalized to a mean of 0 and S.D. of 1. Relative increase and decrease are assigned here as red and blue color, respectively. Each column represents one patient sample and each row represents an immune marker that was examined. D, Significant alterations of CD226, CD38, CD160, and Eomes before vs after treatment. E,PBMCs from a healthy donor were co-cultured with LPS. Intracellular production of cytokines among each immune components was assessed by flow cytometry. (F) The PBMCs collected before and after combination treatment were co-cultured with LPS. Cytokine production by monocytes were evaluated by flow cytometry. Plot summary (n = 5) is shown. ND, not detected

decitabine on the cytokine release (Figure S2). These data suggest that avelumab may increase the proinflammatory cytokine production during sepsis.

Comparing with historical data in AML patients treated with decitabine alone,[5] we didn't observe an optimal clinical outcome in our patient cohort. Of note, the majority of patients (86%) enrolled in our study had AML with adverse risk per cytogenetic stratification, two patients (29%) had TP53 mutation and three with complex karyotype, both of which are considered very poor prognostic features. Our data is consistent with the results from the phase II study of Zeidan et al that azacitidine combined with durvalumab failed to show clinical benefit as the front-line treatment for unfit AML patients.[6] These observations highlight the need of optimal designs of clinical studies targeting PD-1 for AML treatment. For instance, appropriate dose and timing of PD-1 agents, as well as defining predictive biomarkers, are essential to improve clinical outcome for the combination treatment.

Inhibition of the PD-1 pathway can effectively treat multiple cancers mainly through reversing T cell exhaustion and improving antitumor T cell response. Consistently, we observed a positive impact of avelumab on T cell immune response in our cohort of AML patients. However, this improvement of T cell response didn't translate to a better clinical outcome. We made important findings that monocytes from patients treated with avelumab produce more proinflammatory cytokines upon ex vivo LPS stimulation. We suspect that avelumab-caused high inflammatory response may contribute to the early death of the five patients who suffered neutropenic sepsis. In contrast to patients with solid tumors, whose blood counts including neutrophils are largely normal, AML patients frequently suffer infections due to persistent neutropenia. Infection-triggered inflammatory response may turn severe in the presence of PD-1Inhibition. This may explain why blockade antibodies to the PD-1 pathway are successful in treating multiple solid tumors but their benefit to AML patients is limited.

In summary, although DLT was not detected in this phase I study, no clinical benefit was achieved in AML patients receiving avelumab and decitabine as first-line treatment. In contrast, significant sepsis-related death was observed. These data argue against the combination treatment at current design. Our correlative studies demonstrate that CD8 T cell response trends up upon avelumab treatment. However, the increased proinflammatory cytokines production during infections may exacerbate severe septic shock. Further mechanistic studies for better controlling the profound inflammation while maintaining anti-leukemia T cell activity are essential to optimize PD-1-trageting treatment for AML.

KEYWORDS

AML, avelumab, decitabine, PD-1, T cell exhaustion

CONFLICT OF INTEREST

Dr. Hong Zheng received research funds from Pfizer for conducting the correlative studies of this trial.

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

KEYWORDS

AML, avelumab, decitabine, PD-1, T cell exhaustion

Hong Zheng[1], Shin Mineishi[1], David Claxton[1], Junjia Zhu[2], Chenchen Zhao[1], Bei Jia[1], W. Christopher Ehmann[1], Witold B. Rybka[1], Seema Naik[1], Natthapol Songdej[1], Joseph J. Drabick[1], Raymond J. Hohl[1]

[1]*Penn State Cancer Institute, Penn State University College of Medicine, Hershey, Pennsylvania*

[2]*Department of Public Health Sciences, Penn State University College of Medicine, Hershey, Pennsylvania*

Correspondence

Hong Zheng, Penn State Hershey Cancer Institute, Penn State University College of Medicine, Hershey, PA 17033.
Email: hzheng@pennstatehealth.psu.edu

Raymond J. Hohl, Penn State Hershey Cancer Institute, Penn State University College of Medicine, Hershey, PA 17033.
Email: rhohl@pennstatehealth.psu.edu

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ORCID

Hong Zheng https://orcid.org/0000-0002-5024-5538

Natthapol Songdej https://orcid.org/0000-0001-6545-7931

REFERENCES

1. Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell*. 2015;161:205-214.
2. Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer Cell*. 2015;27:450-461.
3. Yang H, Bueso-Ramos C, DiNardo C, et al. Expression of PD-L1, PD-L2, PD-1 and CTLA4 in myelodysplastic syndromes is enhanced by treatment with hypomethylating agents. *Leukemia*. 2014;28:1280-1288.
4. Ørskov AD, Treppendahl MB, Skovbo A, et al. Hypomethylation and up-regulation of PD-1 in T cells by azacytidine in MDS/AML patients: a rationale for combined targeting of PD-1 and DNA methylation. *Oncotarget*. 2015;6:9612-9626.
5. Kantarjian HM, Thomas XG, Dmoszynska A, et al. Multicenter, randomized, open-label, phase III trial of decitabine versus patient choice, with physician advice, of either supportive care or low-dose cytarabine for the treatment of older patients with newly diagnosed acute myeloid leukemia. *J Clin Oncol*. 2012;30:2670-2677.

6. Zeidan A, Cavenagh J, Voso MT, Taussig D, Tormo M, Boss I. Efficacy and Safety of Azacitidine (AZA) in Combination with the Anti-PD-L1 Durvalumab (durva) for the Front-Line Treatment of Older Patients (pts) with Acute Myeloid Leukemia (AML) Who Are Unfit for Intensive Chemotherapy (IC) and Pts with Higher-Risk Myelodysplastic Syndromes (HR-MDS): Results from a Large, International, Randomized Phase 2 Study. *Blood*. 2019;134(suppl 1):829.

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Clinical outcomes and influence of mutation clonal dominance in oligomonocytic and classical chronic myelomonocytic leukemia

To the Editor:

Chronic myelomonocytic leukemia (CMML) is a myelodysplastic/myeloproliferative neoplasm characterized by peripheral blood (PB) monocytosis, defined as an absolute monocyte count (AMC) $\geq 1 \times 10^9$/L with ≥10% monocytes, along with myelodysplastic and myeloproliferative bone marrow features.[1] Prognosis of patients with CMML is heterogeneous,[2] with certain clinical, disease phenotype, and genomic features being associated with high risk of progression to acute myeloid leukemia (AML) and short overall survival. Although the current World Health Organization (WHO) definition requires presence of persistent absolute monocytosis for the diagnosis of CMML,[1] several groups have proposed that oligomonocytic CMML (O-CMML), defined by presence of clinical and pathological features of CMML in the presence of an AMC of 0.5-0.9 $\times 10^9$/L[3] and ≥ 10% monocytes, should be recognized as a new entity. Although current available data support that the clinical, morphological, immunophenotypical, and molecular features of O-CMML are overall similar to that of patients with classical CMML,[3,4] there are scarce data on the clonal architecture, optimal therapeutic management, and survival outcomes of these patients.

In order to evaluate further if O-CMML should be recognized as a new entity, we evaluated all previously untreated patients who met proposed diagnostic criteria of O-CMML treated at the University of Texas MD Anderson Cancer Center (MDACC) from 2000 to 2020, and compared their clinical and genomic features with a cohort of 271 patients with classical CMML and 86 patients with MDS and ≥ 10% monocytes without otherwise meeting criteria for O-CMML. O-CMML cases were variably classified as myeloid neoplasms, but met all of the WHO criteria for CMML except for PB AMC of 0.5-0.9 $\times 10^9$/L; the diagnosis of 0-CMML was defined using the criteria proposed by Geyer et al[3] Whole bone marrow DNA was subject to 81 gene-targeted next-generation sequencing (NGS) analysis in a subset of patients (Supplemental Methods).

Thirty patients met criteria for O-CMML. Patient characteristics are detailed in Supplemental Table S1 in Appendix S1. Compared to classical CMML, there were no significant differences in cytogenetic abnormalities based on CMML-specific prognostic scoring system (CPSS).[5] Patients with O-CMML had significantly lower WBC (4.0×10^9/L vs 11.7×10^9/L, $P < .001$) and absolute neutrophil counts (ANC) (1.65×10^9/L vs 6.20×10^9/L, $P < .001$) and lower frequency of bone marrow erythroid dysplasia (40% vs 59%, $P = .046$). Compared to MDS, patients with O-CMML were younger (median age 66 vs 71 years, $P = .035$) and had lower Hgb (9.7 vs 11.4 g/dL, $P < .001$).

Targeted NGS was available in 10 (33%) patients with O-CMML. Frequency of identified mutations and their variant allele frequencies (VAF) are shown in Figure 1A,B. Similar to CMML, the highest frequencies of mutations were noted in *TET2*, *SRSF2*, *RAS* pathway genes, *ASXL1*, and *RUNX1* genes. Frequencies of mutations in *ASXL1* (50% vs 49.1%, $P = .958$), *SRSF2* (60% vs 39.4%, $P = .198$), *TET2* (70% vs 49.1%, $P = .199$), and *RUNX1* (20% vs 19.4%, $P = .965$) were similar in O-CMML and CMML, with RAS pathway mutations (*NRAS*, *KRAS*, *CBL*, *NF1*, *SETBP1*, *PTPN11*) being more frequent in CMML compared to O-CMML (51.4% vs 20%, $P = .053$). The median number of mutations was 4 (range 1-12), and 4 (range 0-8) in O-CMML and CMML ($P = .578$), respectively. Compared to MDS patients, O-CMML had higher frequency of *ASXL1* (50% vs 20%, $P = .046$), *SRSF2* (60% vs 14%, $P = .03$), and *TET2* (70% vs 27%, $P = .009$) mutations. No significant differences in median VAFs for *ASXL1*, *SRSF2*, *TET2*, *RUNX1* and *RAS* pathway mutations were observed between O-CMML and CMML, or between O-CMML and MDS (Figure 1B). *TET2/SRSF2* and *TET2/SRSF2/ASXL1* co-mutations were observed in similar frequencies among O-CMML and CMML (40% vs 23%, $P = .260$; 20% vs 12%, $P = .360$) but were more frequent in O-CMML compared to MDS (40% vs 6%, $P = .006$; 20% vs 4%, $P = .083$). In order to determine the likely clonal dominance of identified mutations, VAF estimates were used to evaluate clonal relationships using Pearson goodness-of-fit tests. Clones with the highest VAF or with VAF close to 40% were defined as dominant, and those present at VAF <20% in the presence of another dominant clone were defined as minor. Mutations in *RAS* pathway were more likely to appear as minor clones in patients with O-CMML compared to CMML (71% vs 50%, $P < .001$) (Figure 1C).

Sequential NGS was available in four patients with O-CMML. Transplant was associated with mutation clearance in one patient (UPN2) (Figure S1 in Appendix S1). In one patient, transformation to AML was associated with expansion of a previously not detectable *ASXL1*, *IDH1*, *RUNX1*, and *NRAS* mutations (UPN1) (Figure S1 in Appendix S1). Therapy with decitabine was associated with clearance of *CBL* and *FLT3* mutations as well as reduction in clonal size of an *ASXL1* mutation at the time of best response, with subsequent

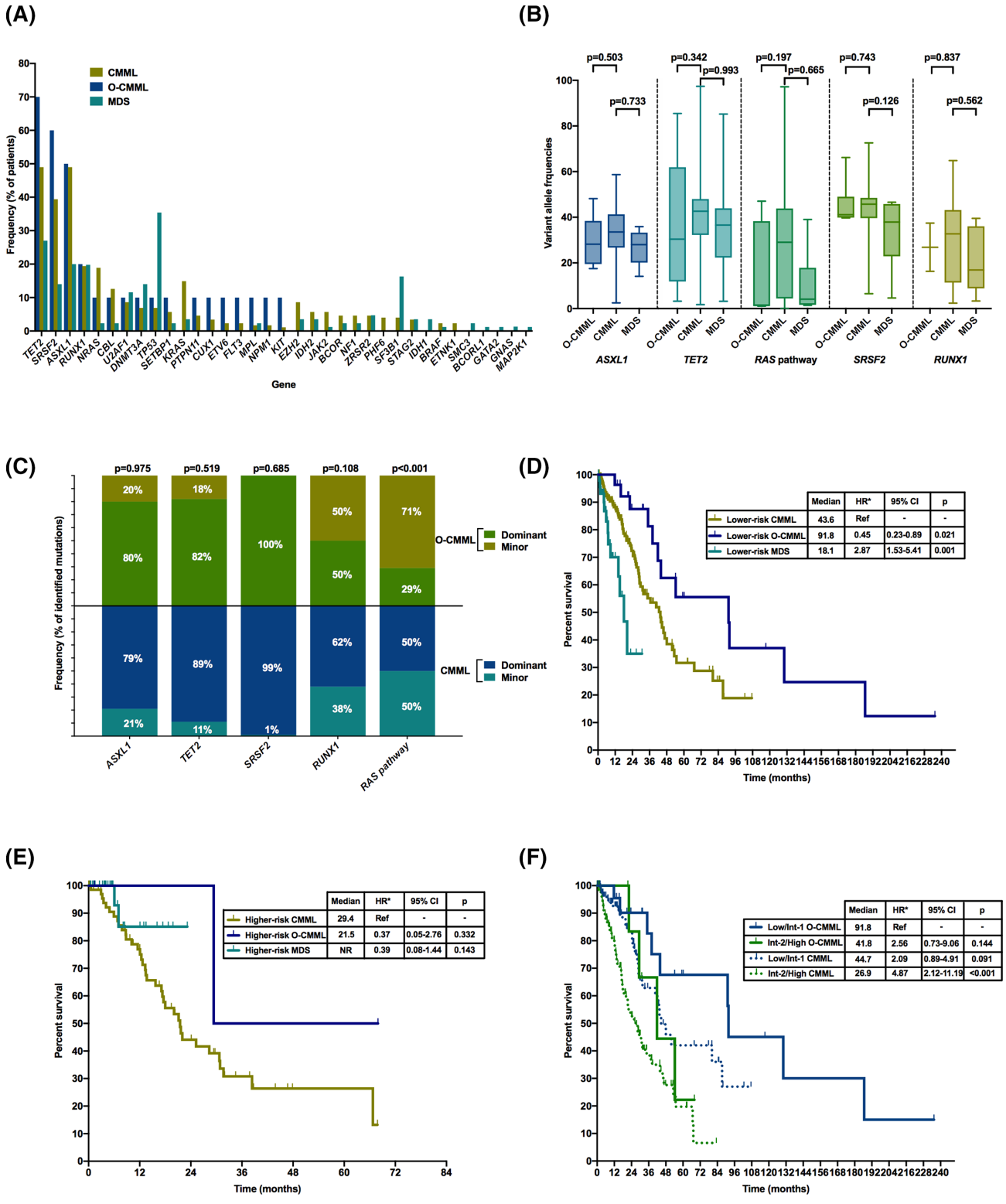

FIGURE 1 Mutational and clonal landscape and clinical outcomes of O-CMML compared to CMML. A, Frequency of identified mutations in patients with O-CMML, CMML, and MDS. B, Median VAF and range of identified mutations among patients with CMML, O-CMML, and MDS. Median VAF are displayed within the interior graph. RAS pathway mutations include *NRAS*, *KRAS*, *CBL*, *SETBP1*, *NF1*, and *PTPN11* mutations. C, Frequency of mutations appearing as dominant or minor events in patients with O-CMML and CMML. VAF estimates were used to evaluate clonal relationships within each individual sample using Pearson goodness-of-fit tests and VAF differences. Clones with the highest VAF or with VAF close to 40% were defined as dominant, and those present at VAF <20% in the presence of another dominant clone were defined as minor. D, Kaplan-Meier estimate survival curves for overall survival comparing outcomes of lower risk (very low, low, or intermediate) IPSS-R O-CMML, MDS, or CMML. E, Kaplan-Meier estimate survival curves for overall survival comparing outcomes of higher risk (high or very high) IPSS-R O-CMML, MDS, or CMML. F, Kaplan-Meier estimate survival curves for overall survival comparing outcomes of O-CMML and CMML based on CPSS risk category

expansion of *STAG2*, *RUNX1*, and *NRAS* mutations at the time of progression in another patient (UPN3) (Figure S1 in Appendix S1). Acquisition of monosomy 7 and expansion of a *RUNX1* mutation was observed at the time of transformation in one patient (UPN4) (Figure S1 in Appendix S1).

Among the O-CMML patients, seven (23%) were followed with observation, all of which had low/intermediate-1 risk by CPSS, and 23 (77%) received therapy with a median time from diagnosis to first treatment of 1.8 months (range 0-62 months) compared to 2.1 months (range 0-60 months) in the CMML cohort ($P = .483$). Therapy consisted of hypomethylating agents (HMA) in 17 (57%) patients, intensive chemotherapy in two (9%) patients, and other investigational agents in four (17%) patients. Response outcomes were evaluated following the MDS/MPN IWG response criteria.[6] Patients with O-CMML had a higher complete response rate (53% vs 27%, $P = .032$) and significantly longer median response durations compared to those with CMML (14.4 vs 6.6 months, $P = .046$) (Table S1 in Appendix S1). A total of six (20%) patients underwent allogeneic stem-cell transplantation. Twelve (40%) patients progressed to overt CMML within a median of 13.5 months (range 3.8-102.8 months). Three (10%) patients experienced transformation to AML compared to 40 (15%) in the control CMML cohort ($P = .480$), with a trend to longer median time to transformation (33.3 vs 13.2 months, $P = .490$). With a median follow up of 59.1 months, the median survival for patients with O-CMML was 91.2 months (95% CI 40.2-142.1 months). Survival outcomes based on diagnosis and IPSS-R category are shown in Figure 1D. Compared to MDS, patients with O-CMML had significantly better outcomes among those with very low, low, or intermediate IPSS-R, with no differences among high or very high categories. Univariate analysis for survival evaluating the O-CMML and CMML cohorts revealed that a diagnosis of O-CMML (HR 0.39, 95% CI 0.21-0.75, $P = .04$), particularly when compared to MP-CMML (Figure S2 in Appendix S1), age (HR 1.06, 95% CI 1.00-1.13, $P = .039$), and CPSS category of intermediate-2/high (HR 2.59, 95% CI 1.72-3.89, $P < .001$) predicted for OS. By multivariate analysis for OS, both age (HR 1.03, HR 1.01-1.51, $P = .003$) and CPSS category intermediate-2/high (HR 2.63, 95% CI 1.73-3.98, $P < .001$), but not O-CMML (HR 0.59, 95% CI 0.31-1.14, $P = .120$) retained their significance. Patients with intermediate-2/high CPSS O-CMML had overlapping outcomes to those with low/intermediate-1 CMML (Figure 1E). Patients with low/intermediate-1 O-CMML had a trend to improved outcomes, and those with intermediate-2/high CMML had significantly worse survival (Figure 1F). Similar findings were observed when comparing MD-CMML with O-CMML (Figure S3 in Appendix S1).

Similar to previous reports,[3,4] our data confirms that O-CMML has similar clinical and mutational features to CMML, although currently not a recognized entity by the WHO. Unlike in the study by Calvo et al[4] we observed lower prevalence of dyserythropoiesis in O-CMML. Similar to their reports, we did not identify differences in cytogenetic abnormalities or mutational landscape between O-CMML and CMML, with the exception of lower frequency of RAS pathway mutations. By studying clonal relationships of identified mutations, we observed that the clonal dominance of common CMML mutations is equally represented among O-CMML and CMML, with exception of RAS pathway mutations, suggesting that mutations in these signaling genes might be responsible for the increased proliferation and monocytosis observed in classical CMML. By comparing to a cohort of MDS patients with ≥10% monocytes but otherwise no other criteria for O-CMML, we could confirm that O-CMML shares clinical and genomic features closer to CMML than to MDS. In addition, although patients with O-CMML tended to present with lower CPSS risk categories and showed improved clinical outcomes when compared to CMML, these survival differences were not significant when corrected by relevant clinical and cytogenetic features, and risk of transformation to AML was similar in both populations. Finally, therapy with HMAs was effective and associated with similar ORR with higher CR and median response duration as compared to classical CMML. Although this might be driven by small patient numbers, it may also suggest that early intervention in these patients prior to the disease becoming proliferative might be associated with improved response outcomes. We acknowledge that this study has several limitations, including its retrospective nature, small numbers of patients with O-CMML (partly as an effect of its low frequency), and absence of NGS in all included patients. However, we believe our findings suggest that the AMC threshold for the diagnosis of CMML should be revised to enable identification of O-CMML patients and facilitate their enrollment in clinical trials for CMML. These data also support the idea that clinical and mutational features should guide confirmation of the diagnosis of CMML.

KEYWORDS

chronic myelomonocytic leukemia, clinical outcomes, mutational dominance, olygomonocytic

CONFLICT OF INTERESTS

Koji Sasaki: This author declares an advisory role with Pfizer Japan.

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AUTHOR CONTRIBUTIONS

Guillermo Montalban-Bravo and Guillermo Garcia-Manero: Concept and design; administrative support; provision of study materials and patients; data collection, analysis, interpretation; and manuscript writing and final approval. Sherry Pierce: Provision of study materials; data collection, and final approval. Rashmi Kanagal-Shamanna, Veronica Guerra, Jorge Ramos-Perez, Danielle Hammond, Paul Shilpa, Kiran Naqvi, Koji Sasaki, Elias Jabbour, Courtney DiNardo, Koichi Takahashi, Marina Konopleva, Naveen Pemmaraju, Tapan Kadia, Farhad Ravandi, Naval Daver, Gautam Borthakur, Zeev Estrov, Joseph D. Khoury, Sanam Loghavi, Carlos Bueso-Ramos, Keyur Patel, and Hagop Kantarjian: Collection and assembly of data; data analysis and interpretation; manuscript writing; and final approval of manuscript.

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DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are not publicly available due to patient privacy concerns but are available from the corresponding author on reasonable request.

KEYWORDS

chronic myelomonocytic leukemia, clinical outcomes, mutational dominance, olygomonocytic

Guillermo Montalban-Bravo[1], Rashmi Kanagal-Shamanna[2], Veronica Guerra[1], Jorge Ramos-Perez[1], Danielle Hammond[1], Paul Shilpa[3], Kiran Naqvi[1], Koji Sasaki[1], Elias Jabbour[1], Courtney DiNardo[1], Koichi Takahashi[1], Marina Konopleva[1], Naveen Pemmaraju[1], Tapan Kadia[1], Farhad Ravandi[1], Naval Daver[1], Gautam Borthakur[1], Zeev Estrov[1], Joseph D. Khoury[2], Sanam Loghavi[2], Sherry Pierce[1], Carlos Bueso-Ramos[2], Keyur Patel[2], Hagop Kantarjian[1], Guillermo Garcia-Manero[1]

[1]*Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, Texas*

[2]*Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, Texas*

[3]*Department of Pharmacy, The University of Texas MD Anderson Cancer Center, Houston, Texas*

Correspondence

Guillermo Montalban-Bravo, Department of Leukemia, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit 428, Houston, TX 770030, USA.

Email: gmontalban1@mdanderson.org

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ORCID

Guillermo Montalban-Bravo https://orcid.org/0000-0002-4533-5176

Rashmi Kanagal-Shamanna https://orcid.org/0000-0001-7829-5249

Koji Sasaki https://orcid.org/0000-0002-9140-0610

Elias Jabbour https://orcid.org/0000-0003-4465-6119

Courtney DiNardo https://orcid.org/0000-0001-9003-0390

Marina Konopleva https://orcid.org/0000-0002-9347-2212

Naveen Pemmaraju https://orcid.org/0000-0002-1670-6513

Tapan Kadia https://orcid.org/0000-0002-9892-9832

Naval Daver https://orcid.org/0000-0001-7103-373X

Gautam Borthakur https://orcid.org/0000-0001-7679-6453

Joseph D. Khoury https://orcid.org/0000-0003-2621-3584

Sanam Loghavi https://orcid.org/0000-0001-8980-3202

Hagop Kantarjian https://orcid.org/0000-0002-1908-3307

Guillermo Garcia-Manero https://orcid.org/0000-0002-3631-2482

REFERENCES

1. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391-2405.
2. Patnaik MM, Itzykson R, Lasho TL, et al. ASXL1 and SETBP1 mutations and their prognostic contribution in chronic myelomonocytic leukemia: a two-center study of 466 patients. *Leukemia*. 2014;28(11):2206-2212.
3. Geyer JT, Tam W, Liu YC, et al. Oligomonocytic chronic myelomonocytic leukemia (chronic myelomonocytic leukemia without absolute monocytosis) displays a similar clinicopathologic and mutational profile to classical chronic myelomonocytic leukemia. *Mod Pathol*. 2017;30(9):1213-1222.
4. Calvo X, Garcia-Gisbert N, Parraga I, et al. Oligomonocytic chronic myelomonocytic leukemia (O-CMML) and chronic myelomonocytic leukemia (CMML) show similar clinical, morphological, immunophenotypic and molecular features. *Blood*. 2019;134(suppl 1):4266.
5. Such E, Germing U, Malcovati L, et al. Development and validation of a prognostic scoring system for patients with chronic myelomonocytic leukemia. *Blood*. 2013;121(15):3005-3015.
6. Savona MR, Malcovati L, Komrokji R, et al. An international consortium proposal of uniform response criteria for myelodysplastic/myeloproliferative neoplasms (MDS/MPN) in adults. *Blood*. 2015;125(12):1857-1865.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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Revisiting the non-transfusion-dependent (NTDT) vs. transfusion-dependent (TDT) thalassemia classification 10 years later

To the Editor:

Clinical classification of patients with thalassemia is the key to making management and follow up decisions. Patients were commonly classified as having a thalassemia major, intermedia or minor phenotype based on clinical presentation and genotype correlates. Around 10 years ago, we recommended the introduction of an alternate classification system / terminology for clinically relevant forms of thalassemia (excluding thalassemia carriers and minor/trait patients) based on transfusion requirements, since receipt of transfusions in this chronic disease modulates the underlying pathophysiology and informs management needs.[1,2]

The terms non-transfusion-dependent (NTDT) and transfusion-dependent (TDT) thalassemia were introduced and are now widely applied in clinical practice and research; including recent international management guidelines and eligibility criteria for clinical trials with novel therapies.[3,4] We do recognize, however, that some confusion remains with regards to the background and implications of using such classification. In our original recommendation, primarily based on expert opinion, we had intended to restrict the use of the term TDT to those patients who are "dependent" on transfusions for survival. These are usually patients with a thalassemia major phenotype, characterized by early presentation to clinical care with severe anemia that prompts physicians to initiate regular and lifelong transfusions. The mainstay of care in these patients is a tailored transfusion program that maintains pre-transfusion hemoglobin levels between 9-10 g/dL, with adequate management of secondary iron overload. The NTDT patients were defined as those who are not dependent on transfusions for survival, including thalassemia intermedia patients with clinical presentation opposite to that aforementioned for TDT. We did caution, however, that these patients may still require transfusions at the discretion of the treating physician either occasionally, when acute or subacute drops of hemoglobin levels are anticipated (eg, during surgery, pregnancy, or infections); or even frequently - yet for defined and short periods of time - to improve the underlying anemia which may be negatively impacting growth and development in children or causing clinical morbidity in adults. The concern with iron overload in NTDT patients is attributed to increased intestinal absorption stemming from ineffective erythropoiesis.

The NTDT/TDT classification may be straightforward when looking at the historic and global picture of an individual patient but may become subject to various interpretations when considered cross-sectionally or during narrow time windows in later stages of the disease. This becomes even more challenging considering changes in the underlying pathology overtime especially those induced by novel therapies. Therefore, we would like to highlight two main scenarios that we believe our colleagues may be puzzled with (Figure 1).

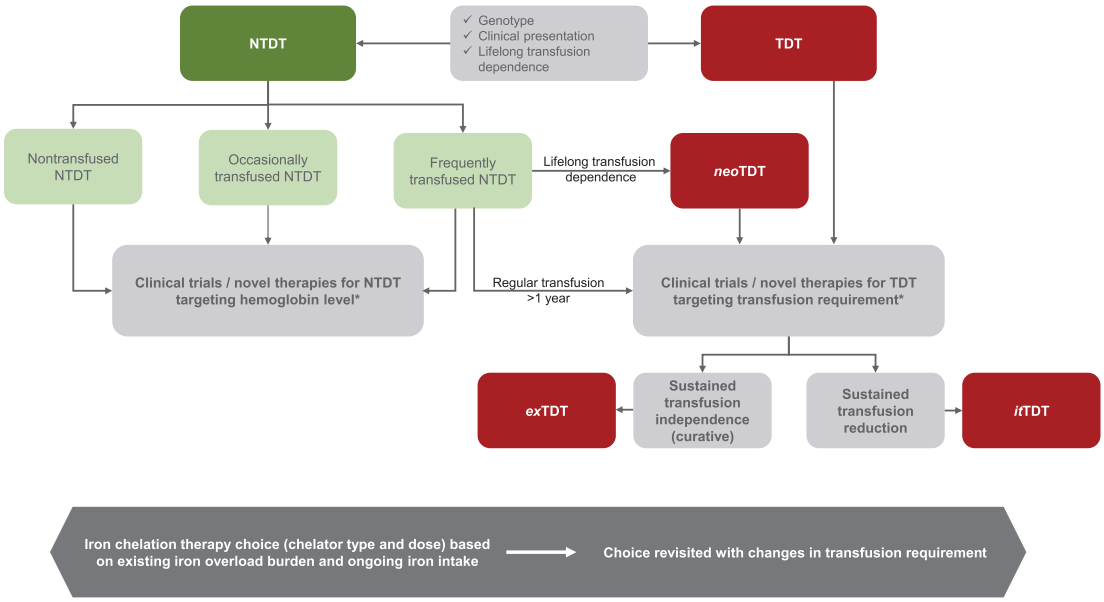

FIGURE 1 Considerations for novel therapies and conventional management with iron chelation based on the clinical classification of thalassemia. *Provided patient meets trial/product defined criteria for transfusion history in prior 6 months. NTDT, non-transfusion-dependent thalassemia; TDT, transfusion-dependent thalassemia; *it*TDT, intermittently transfused TDT

1 | FREQUENTLY TRANSFUSED NTDT PATIENTS

As mentioned earlier, one may encounter NTDT patients that are actually receiving frequent and even regular transfusions during a specific period of time. Ideally, these patients should not be labeled as TDT, as the latter is designed to reflect the underlying severity of disease since diagnosis and implies long-term (past) and lifelong (future) exposure to iron overload and chelation therapy with associated implications in various organs. One exception to this may be in patients who now became definitively dependent on transfusions for the remainder of their disease. In this special scenario, patients may be classified as TDT and managed as such, but with additional designation to clarify that this is a new transformation compared to patients who have had TDT since diagnosis. For this purpose, we recommend the use of the term *neo*TDT. That said, there are few important questions to ask on frequently transfused NTDT which are directly related to the objective of management. Should patients be treated based on NTDT or TDT guidelines and prescribing information for iron chelation therapy? Iron chelation decisions are based on baseline iron overload status and ongoing iron intake, as well as functional status of vital organs from a safety standpoint. Other historic attributes of the disease are less relevant in this setting, and the patient status at the time of iron chelation decision should inform choices. Levels of systemic, hepatic, and/or cardiac iron indices at "baseline" and the rate of transfusion therapy and subsequent iron intake in the past 6 months should be taken into consideration to decide on the choice and dose of iron chelator; while taking into consideration relevant organ function measures for the liver, kidney, and heart. Since regular transfusion therapy is often temporary in this group of patients, iron chelation decisions should be revisited every time there is a considerable change in transfusion requirement.

Another important question is whether these patients should be included in clinical trials of novel therapies designed for NTDT or TDT patients. The answer to this question is trickier. In our opinion, since the goal for most novel therapies is to modify the disease or its management (transfusion) needs permanently, or over an extended period of time, patient eligibility should rely on a long rather than short period of historic observation prior to trial inclusion. For instance, the concern here is that an NTDT patient on regular transfusions for the last 3-6 months to treat an extramedullary hematopoietic tumor, who was anyhow meant to discontinue transfusions shortly after tumor response, would end up being included in a TDT trial aiming to reduce transfusion requirement. Although most ongoing clinical trials only look at the patient's transfusion requirement in the last 6 months prior to enrollment to define NTDT vs TDT status (commonly using cut-offs of 4-6 blood units), there are usually additional eligibility criteria that allow better assessment of the patients underlying phenotype since diagnosis. Terminology put aside, investigators should also exercise their own judgment on whether patients are suitable or not for a specific trial, by looking back at how long the patient has been on regular transfusions or expected to be in the future. If this exceeds a year in either direction, then this patient should probably be considered for therapies aiming to reduce transfusion requirement; although we recommend retaining the terminology of frequently transfused NTDT rather than TDT for the aforementioned reasons. Lastly, although these patients may not be eligible for NTDT trials targeting hemoglobin level, they may be considered in the future once their regular transfusion therapy is tapered or discontinued. Our key message here is that patients need to be in a relatively "steady-state" before any such management or research decisions are made.

2 | TDT PATIENTS RECEIVING NOVEL THERAPIES

Novel therapies for TDT mainly target transfusion requirement aiming for reduction or independence. This leads to a scenario opposite to that mentioned earlier for NTDT. The key question here is whether responding patients to such therapies should be reclassified to NTDT. The short answer is "no". If patients received curative therapy and the disease was permanently and fundamentally modified leading to sustained transfusion independence, they should be classified as *ex*TDT; such may be the case with gene therapy or editing and bone marrow transplantation. Patients who only achieved reduction in their transfusion requirement should be labeled as intermittently transfused TDT (*it*TDT), and considerations for iron chelation therapy and further inclusion in novel therapy trials should follow the same rationale mentioned above for frequently transfused NTDT.

The underlying molecular profile of TDT patients also remains relevant in considerations of novel therapies targeting transfusion requirement. The genetic heterogeneity of TDT implies that some patients may carry mutations or modifiers associated with very low red blood cell (RBC) synthesis, while others with higher numbers of RBCs (although insufficient to be considered NTDT). Regular transfusion therapy may mask these differences due to the intrinsic ability of blood transfusion to suppress endogenous erythropoiesis.[5] Therefore, the hemoglobin levels these patients are able to produce with new treatments may reflect a combination of the intrinsic ability of each patient to produce RBCs in the absence of intervention as well as the ability of the drug itself to increase hemoglobin levels. Thus, clinicians and investigators should not only rely on a TDT classification but also consider the ability of individual patients to produce RBCs based on the genetic profile during management decisions on novel therapies.

Despite its close association with a diagnosis of thalassemia, transfusion therapy remains an intervention that is not only driven by underlying disease severity but also physician choices, which leaves room for considerable variability in practices at the regional and international levels. Clinical classification in thalassemia should remain individualized and rely on evidence from laboratory and clinical observations throughout the patient journey. We hope that our modified recommendations can help classify patients based on historic and evolving transfusion needs to help tailor care and research.

CONFLICT OF INTEREST

K.M.M. has been or is a consultant for Novartis, Celgene Corp (Bristol Myers Squibb), Agios Pharmaceuticals, CRISPR Therapeutics and Vifor Pharma. M.D.C. has been or is a consultant for Novartis, Celgene Corp (Bristol Myers Squibb), Vifor Pharma and Ionis Pharmaceuticals; and received research funding from Novartis, Celgene Corp (Bristol Myers Squibb), La Jolla Pharmaceutical Company, Roche, Protagonist Therapeutics and CRISPR Therapeutics. V.V. has been or is a consultant for Agios Pharmaceuticals, Celgene Corp (Bristol Myers Squibb), Roche, Novartis and Protagonist Therapeutics; and received research funding from Agios Pharmaceuticals, Celgene Corp (Bristol Myers Squibb), Roche, Novartis and Protagonist Therapeutics. AK. has been or is a consultant for Celgene Corp (Bristol Myers Squibb), Novartis, Apopharma/Chiesi Farmaceutici, Vifor Pharma, Ionis Pharmaceuticals, Agios Pharmaceuticals, CRISPR Therapeutics/Vertex Pharmaceuticals and Gilead; and received research funding from Novartis. S.R. is a member of the scientific advisory board of Ionis Pharmaceuticals, Meira GTx, Incyte and Disc Medicine and owns stock options from Disc Medicine and Meira GTx. He has been or is consultant for Cambridge Healthcare Res, Celgene Corp (Bristol Myers Squibb), Catenion, First Manhattan Co., FORMA Therapeutics, Ghost Tree Capital, Keros Therapeutics, Noble insight, Protagonist Therapeutics, Sanofi Aventis U.S., Slingshot Insight, Techspert.io and BVF Partners L.P., Rallybio LLC and venBio Select LLC. A.T.T. has been or is consultant for Novartis, Celgene Corp (Bristol Myers Squibb), Vifor Pharma, Silence Therapeutics and Ionis Pharmaceuticals; and received research funding from Novartis, Celgene Corp (Bristol Myers Squibb), La Jolla Pharmaceutical Company, Roche, Protagonist Therapeutics and Agios Pharmaceuticals.

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Khaled M. Musallam[1], Maria D. Cappellini[2], Vip Viprakasit[3], Antonis Kattamis[4], Stefano Rivella[5], Ali T. Taher[6]

[1]*International Network of Hematology, London, UK*

[2]*Department of Clinical Sciences and Community, University of Milan, Milan, Italy*

[3]*Department of Pediatrics & Thalassemia Center, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand*

[4]*First Department of Pediatrics, National and Kapodistrian University of Athens, Athens, Greece*

[5]*Department of Pediatrics, Division of Hematology, Raymond G. Perelman Center for Cellular and Molecular Therapeutics; Abramson Research Center at The Children's Hospital of Philadelphia (CHOP); Cell and Molecular Biology Affinity Group (CAMB), Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania*

[6]*Department of Internal Medicine, American University of Beirut Medical Center, Beirut, Lebanon*

Correspondence

Ali T. Taher, Professor of Medicine, Hematology & Oncology; Director – Naef K. Basile Cancer Institute; Vice Chair – Research, Department of Internal Medicine; American University of Beirut Medical Center; P.O. Box 11-0236, Beirut 11072020, Lebanon.

Email: ataher@aub.edu.lb

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ORCID

Maria D. Cappellini https://orcid.org/0000-0001-8676-6864

Vip Viprakasit https://orcid.org/0000-0003-3162-1849

Ali T. Taher https://orcid.org/0000-0001-8515-2238

REFERENCES

1. Musallam KM, Rivella S, Vichinsky E, Rachmilewitz EA. Non-transfusion-dependent thalassemias. *Haematologica*. 2013;98:833-844.
2. Taher AT, Cappellini MD, Musallam KM. Recent advances and treatment challenges in patients with non-transfusion-dependent thalassemia. *Blood Rev*. 2012;26(Suppl 1):S1-S2.
3. Cappellini MD, Cohen A, Porter J, Taher A, Viprakasit V. *Guidelines for the Management of Transfusion Dependent Thalassaemia (TDT)*. 3rd ed. Nicosia, Cyprus: Thalassaemia International Federation; 2014.
4. Taher A, Musallam K, Cappellini MD. *Guidelines for the Management of non Transfusion Dependent Thalassaemia (NTDT)*. Nicosia, Cyprus: Thalassaemia International Federation; 2017.
5. Cazzola M, De Stefano P, Ponchio L, et al. Relationship between transfusion regimen and suppression of erythropoiesis in beta-thalassaemia major. *Br J Haematol*. 1995;89:473-478.