To the editor:

Accumulation of classical monocytes defines a subgroup of MDS that frequently evolves into CMML

Dorothée Selimoglu-Buet,[1] Bouchra Badaoui,[2,3] Emmanuel Benayoun,[2] Andréa Toma,[4] Pierre Fenaux,[5] Bruno Quesnel,[6] Gabriel Etienne,[7] Thorsten Braun,[8] Nassera Abermil,[9,10] Margot Morabito,[1] Nathalie Droin,[1] Eric Solary,[1] and Orianne Wagner-Ballon,[2,3] on behalf of the Groupe Francophone des Myélodysplasies

[1]INSERM Unité Mixte de Recherche (UMR) 1170, Faculté de Médecine, Université Paris-Sud, Gustave Roussy, Villejuif, France; [2]Département d'Hématologie et Immunologie Biologiques, Hôpitaux Universitaires Henri Mondor APHP, Créteil, France; [3]INSERM Unité 955, Université Paris Est Créteil, France; [4]Service d'Hématologie Clinique, Hôpitaux Universitaires Henri Mondor Assistance Publique–Hôpitaux de Paris (AP–HP), Créteil, France; [5]Service d'Hématologie Clinique, Hôpital Saint-Louis AP–HP, Paris, France; [6]Service d'Hématologie Clinique, Centre Hospitalier Régional Universitaire de Lille, Lille, France; [7]Service d'Hématologie Clinique, Institut Bergonié, Bordeaux, France; [8]Service d'Hématologie Clinique, Hôpital Avicenne AP–HP, Bobigny, France; [9]Sorbonne Universités, Université Pierre et Marie Curie (UPMC) Université Paris 06, UMR_S 938, CDR Saint-Antoine, Paris, France; and [10]Service d'Hématologie Biologique, Hôpital Saint-Antoine AP–HP, Paris, France

Even though the diagnosis criteria of chronic myelomonocytic leukemia (CMML) have been recently revised by the World Health Organization (WHO),[1] recognition of this disease can be challenging. We demonstrated recently that a percentage of classical monocytes $CD14^{++}CD16^{-}$ (MO1) ≥94% of total monocytes, as measured by flow cytometry, could rapidly and efficiently distinguish a CMML from a reactive monocytosis with a specificity of 95.1% and a sensitivity of 91.9%.[2] The association between MO1 accumulation and CMML was subsequently validated[3] and proposed as an additional diagnostic modality in CMML.[4,5] A relative monocytosis, defined as a percentage of peripheral blood monocytosis ≥10%, has been described in a subgroup of myelodysplastic syndrome (MDS) likely to evolve into genuine CMML.[6-9] Here, we compare the monocyte subset repartition in CMML and in MDS.

Between January 2015 and March 2017, we analyzed 158 CMML patients and 84 MDS patients for whom the diagnosis was made according to the 2008 WHO classification,[10] following local ethical committee's rules. Of the 158 patients with CMML, 152 (96%) fulfilled the latest 2016 WHO criteria that include both a persistent peripheral blood monocytosis $\geq 1 \times 10^9$/L and monocytes accounting for ≥10% of the white blood cell (WBC) differential count.[1] These patients were subdivided into CMML-0 (62), CMML-1 (66), and CMML-2 (24) (Figure 1A; Table 1).[1,11] All peripheral blood parameters but platelet count were similar between these 3 groups (Figure 1B-E). CMML-2 patients displayed a significantly deeper thrombocytopenia ($104 \pm 90 \times 10^9$/L) in comparison with CMML-1 ($132 \pm 93 \times 10^9$/L; $P < .05$) and CMML-0 ($204 \pm 143 \times 10^9$/L; $P < .05$). In the 124 cases in which the CMML Prognostic Scoring System (CPSS)[12] could be tested, we did not notice any difference between CMML-0 and CMML-1, both mainly in the low and intermediate-1 categories, whereas 67% of CMML-2 were in the intermediate-2 and high groups. A small fraction of CMML-0 (19%) and CMML-1 (30%) were proliferative,[1] leading us to test also the Revised International Prognostic Scoring System (IPSS-R),[13,14] with most CMML-0 in the very low and low-risk categories, whereas most CMML-1 were in the low and intermediate groups (Table 1).

In the peripheral blood, the absolute count as well as the percentage of monocytes was similar in the 3 CMML groups. In the bone marrow, the percentage of mature and immature monocytes,[15] excluding monoblasts and promonocytes,[1,10] was ≥5% in 89% of CMML patients and significantly higher in CMML-1 compared with CMML-0 (9% ± 5% vs 12% ± 7%; $P < .05$; Figure 1F). These results support the categorization of CMML into 3 groups, including CMML-0 with <2% peripheral blood and <5% medullary blasts.

MO1 accumulation ≥94% was identified in 141 of the 152 CMML (Figure 1A,G), indicating a sensitivity of 92.8% in accordance with our previous results.[2] This sensitivity increased with CMML subtype, reaching 100% in CMML-2. The 11 CMML cases, according to the 2016 WHO criteria, displaying MO1 < 94%, included 7 CMML-0 and 4 CMML-1. Six of them, all with CMML-0, showed a monocyte subset repartition similar to that observed in healthy donors, notably including the presence of MO3 ($CD14^{low}CD16^{+}$) cells (Figure 1H). Molecular analyses and follow-up are then necessary to support or exclude CMML diagnosis in these cases. In the 5 other patients, including the 4 CMML-1 cases, a characteristic, easily recognized, "bulbous" aspect of monocyte subset repartition was observed (Figure 1I) due to the disappearance of MO3 subset combined with the increase of intermediate monocytes MO2 ($CD14^{++}CD16^{+}$) subset. Each of these patients demonstrated an associated inflammatory state (eg, one of them had a typical ankylosis spondylitis), whereas the others had an elevated C-reactive protein (23.3 ± 21.0 mg/L). Accordingly, inflammation was previously shown to provoke an accumulation of MO2 subset.[16-18] For example, in a woman with a typical monocyte subset repartition at CMML diagnosis (Figure 1J), the occurrence of an auricular chondritis was associated with an increase in MO2 subset, generating a "bulbous" aspect of the flow image (Figure 1K), which disappeared with the resolution of inflammation after corticotherapy (Figure 1L). Therefore, interpretation of monocyte subset repartition at CMML diagnosis must remain cautious in case of a "bulbous" profile and should take into account an associated inflammatory situation.

In 6 of the 158 patients with a CMML according to 2008 WHO criteria, WBC differential count did not display ≥10% of monocytes. All these patients had a proliferative disease with hyperleukocytosis ($35.6 \pm 25.6 \times 10^9$/L), fulfilled the other WHO 2016 criteria, displayed a marrow monocytosis ≥5% (8% ± 6%), showed an MO1 accumulation (97.4% ± 1.5%) by flow analysis, and demonstrated a molecular profile compatible with a CMML diagnosis. These observations suggest that a WBC count showing ≥10% of monocytes may not be an absolute criterion for CMML diagnosis.

Among the 152 patients with a CMML diagnosis according to WHO 2016 criteria, 15 had evolved from a preexisting MDS with excess of blasts (MDS-EB, N = 7), multilineage lineage dysplasia (MDS-MLD, N = 4), single lineage dysplasia (N = 2), ring sideroblasts and multilineage dysplasia (N = 1), and isolated del(5q) (N = 1). At MDS diagnosis, their peripheral blood monocyte count was

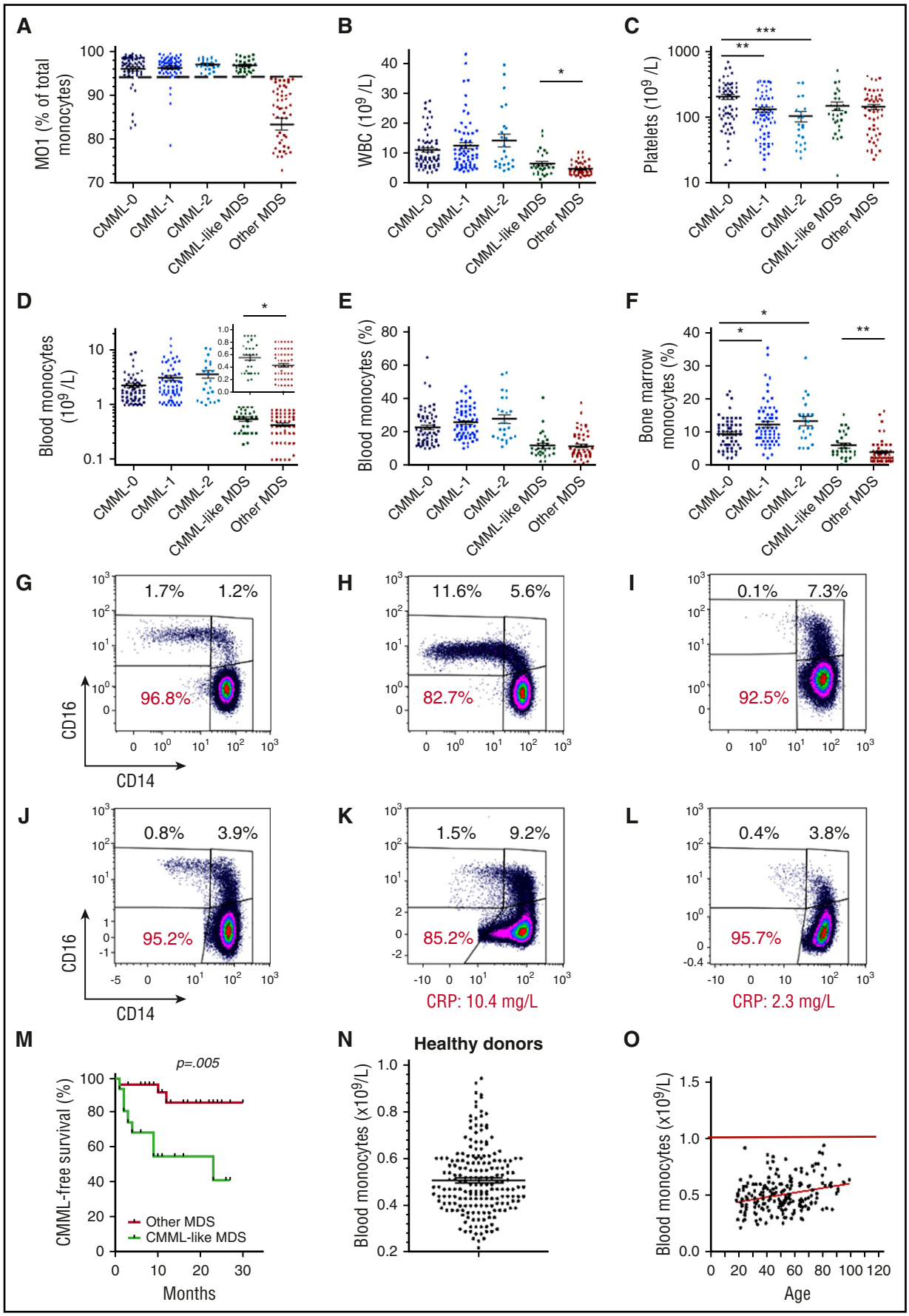

Figure 1.

Table 1. Clinical and biological parameters in CMML and MDS patients diagnosed according to 2016 revised WHO criteria

	Total CMML	CMML-0	CMML-1	CMML-2	Total MDS	CMML-like MDS	Other MDS
Patients, n (%)	152 (100)	62 (41)	66 (43)	24 (16)	84 (100)	29 (35)	55 (65)
Age, y	74 ± 10	72 ± 11	75 ± 9	74 ± 9	72 ± 10	70 ± 11	73 ± 10
Male, n (%)	101 (66)	42 (68)	46 (70)	13 (54)	49 (58)	14 (48)	35 (64)
Hb, g/dL	11.7 ± 2.5	12.1 ± 2.4	11.6 ± 2.6	10.8 ± 2.2	10.8 ± 2.1	10.0 ± 1.7	11.2 ± 2.1
Platelets, $\times 10^9$/L	157 ± 122	204 ± 143	132 ± 93	104 ± 90	145 ± 104	148 ± 110	143 ± 102
WBC, $\times 10^9$/L	11.9 ± 8.1	10.7 ± 6.1	12.2 ± 8.7	14.0 ± 10.5	5.0 ± 2.9	6.1 ± 3.9	4.4 ± 2.0
Neutrophils, $\times 10^9$/L	6.4 ± 5.2	6.1 ± 4.3	6.5 ± 5.9	6.8 ± 5.6	3.0 ± 2.6	4.0 ± 3.5	2.5 ± 1.7
Monocytes, $\times 10^9$/L	2.8 ± 2.4	2.2 ± 1.6	3.1 ± 2.7	3.6 ± 2.8	0.5 ± 0.2	0.6 ± 0.2	0.4 ± 0.2
Blood monocytes, %	24 ± 10	22 ± 11	25 ± 9	27 ± 13	11 ± 8	11 ± 8	11 ± 8
Marrow monocytes, %	11 ± 6	9 ± 5	12 ± 7	13 ± 7	4 ± 4	6 ± 4	4 ± 3
Marrow blasts, %	6 ± 4	2 ± 1	7 ± 2	14 ± 3	5 ± 4	6 ± 4	5 ± 4
Myelodysplasic form, n (%)	104 (68)	44 (71)	46 (70)	14 (58)	83 (99)	28 (97)	55 (100)
Mean MO1 fraction, %	96.2 ± 3.1	96.0 ± 3.7	96.2 ± 3.0	96.9 ± 1.3	88.0 ± 10.3	96.7 ± 1.4	83.3 ± 10.1
Patients with MO1 ≥94%, n (%)	141 (93)	55 (89)	62 (94)	24 (100)	29 (35)	29 (100)	0 (0)
De novo diagnosis, n (%)	112 (74)	54 (87)	45 (68)	13 (54)	—	—	—
IPSS-R, n (%)	123 (81)	47 (76)	55 (83)	21 (88)	74 (88)	28 (97)	46 (84)
Very low IPSS-R, n (%)	22 (18)	22 (47)	0 (0)	0 (0)	14 (19)	3 (11)	11 (24)
Low IPSS-R, n (%)	46 (37)	20 (42)	25 (45)	1 (5)	20 (27)	8 (29)	12 (26)
Intermediate IPSS-R, n (%)	35 (29)	5 (11)	22 (40)	8 (38)	30 (40)	14 (50)	16 (35)
High IPSS-R, n (%)	17 (14)	0 (0)	6 (11)	11 (52)	5 (7)	1 (3)	4 (9)
Very high IPSS-R, n (%)	3 (2)	0 (0)	2 (4)	1 (5)	5 (7)	2 (7)	3 (6)
CPSS, n (%)	124 (82)	48 (77)	55 (83)	21 (88)	—	—	—
Low CPSS, n (%)	54 (44)	24 (50)	30 (54)	0 (0)	—	—	—
Intermediate-1 CPSS, n (%)	36 (29)	16 (33)	13 (24)	7 (33)	—	—	—
Intermediate-2 CPSS, n (%)	30 (24)	8 (17)	12 (22)	10 (48)	—	—	—
High CPSS, n (%)	4 (3)	0 (0)	0 (0)	4 (19)	—	—	—

CMML patients were subdivided into 3 groups, CMML-0, CMML-1, and CMML-2, according to the WHO classification, and MDS patients into 2 groups according to presence or absence of MO1 accumulation ≥94% in the peripheral blood at diagnosis. All parameters are mean ± standard deviation, unless otherwise specified. We indicate the mean MO1 fraction in each subgroup of patients and the percentage of patients whose MO1 fraction was equal or higher than 94%, defined as "Patients with MO1 ≥94%."

$0.7 \pm 0.1 \times 10^9$/L. A marrow monocytosis ≥5% was detected in 12 out of 14 cases in which bone marrow could be reevaluated. In one of these MDS patients, monocyte subset analysis was available at MDS diagnosis and showed an MO1 accumulation. These observations suggested that MDS with marrow monocytosis, peripheral blood monocyte count neighboring the threshold of monocytosis, and MO1 accumulation could evolve in genuine CMML. To explore this hypothesis, we prospectively analyzed monocyte subset repartition in the blood of 84 MDS patients at diagnosis, including 26 MDS-MLD, 6 MDS–single lineage dysplasia, 12 MDS–ring sideroblasts, 28 MDS-EB1, 10 MDS-EB2, 1 MDS with isolated del(5q), and 1 MDS unclassifiable. MO1 accumulation ≥94% was detected in 29 of them (35%) (Figure 1A), validating a recently reported observation.[3] Compared with other MDS, these "CMML-like" MDS displayed a higher WBC number (6.1 ± 3.9 vs $4.4 \pm 2.0 \times 10^9$/L; $P = .05$), a higher absolute monocyte count (0.6 ± 0.2 vs $0.4 \pm 0.2 \times 10^9$/L; $P < .05$), and a higher fraction of monocytes in the bone marrow (5.8 ± 3.8 vs $3.7 \pm 3.5 \times 10^9$/L; $P < .05$). Unlike the recent report of Lee Moffitt's group,[3] MDS without MO1 accumulation was not associated with poor cytogenetic risk or higher R-IPSS in this series.

Follow-up of 44 MDS patients with 2 distinct complete blood counts (CBCs) available showed that a monocyte count $\geq 1 \times 10^9$/L with a monocyte fraction ≥10% of WBC was detected significantly more often in those with a CMML-like phenotype at diagnosis than in other MDS patients ($P < .01$; Figure 1M). In less than 1 year, 7 out of 16 MDS patients with a "CMML-like" phenotype at diagnosis evolved into overt CMML.

We also evaluated the relevance of the 1×10^9/L threshold for monocytosis[1,10,19] by analyzing CBC from 192 healthy adults (median age: 51; range: 19-99). Median value of blood monocytes in this healthy population was 0.49×10^9/L (Figure 1N). Mathematical extrapolation in relation to age showed an increase of monocytosis with years, reaching 0.6×10^9/L at 100 years of age, suggesting that the French-American-British[19]/WHO[1,10] threshold is probably overestimated (Figure 1O). Further studies with larger sample size would be useful to redefine accurately the monocytosis threshold. Of note, a category of "oligomonocytic" CMML with a monocyte fraction ≥10% but absolute monocyte count between 0.5 and 1×10^9/L was recently described, with a subset of these patients eventually developing genuine CMML.[20]

Overall, we suggest that "CMML-like" MDS could be an entity that is likely to evolve into genuine CMML and that an MO1 accumulation ≥94% should be considered to be included as a major criterion for both CMML and CMML-like MDS diagnosis.

Figure 1. MO1 accumulation characterized CMML and a CMML-like subgroup of MDS. MDS and CMML, including CMML-0, CMML-1, and CMML-2, are defined according to the WHO classification. "CMML-like" MDS indicates MDS with a fraction of classical $CD14^{++}CD16^{-}$ monocytes (called MO1) ≥94% of total monocytes. (A-F) Comparison of the fraction of MO1 among peripheral blood monocytes (A), WBC count (B), platelet count (C), absolute monocyte count (D), monocyte fraction in WBC differential count (E), and bone marrow monocytes (F) in the 5 studied groups. *$P < .05$; **$P < .01$; ***$P < .001$; Student *t* test. (G-L) CD14 vs CD16 dot plots showing the monocyte subset repartition in peripheral blood (MO1: $CD14^{++}CD16^{-}$; MO2: $CD14^{+}CD16^{+}$; MO3: $CD14^{low}CD16^{+}$). Characteristic profile observed in CMML patients (G), CMML with normal subset repartition (H), CMML patient in inflammatory condition showing the "bulbous" profile with an increased MO2 population (I), example of a CMML patient before (J), during (K), and after (L) occurrence of a chondritis (C-reactive protein [CRP] values are indicated below the dot plots). (M) CMML-free evolution of MDS patients with ("CMML-like MDS", n = 16) or without ("other MDS", n = 28) classical monocyte accumulation at diagnosis (MO1 ≥ 94%). Monocytosis up to 1×10^9/L and monocyte percentage ≥10% were assessed by 2 consecutive CBC; $P = .005$, log-rank test. (N) Absolute monocyte count repartition in 192 healthy blood donors (median of age: 51; range: 19-99) with median value of 0.49×10^9/L (lower 95 confidence interval [CI]: 0.49; upper 95 CI: 0.53×10^9/L). (O) Linear regression of absolute monocyte count related to age in 192 controls (monocyte count = 0.002038*age + 0.4037).

Acknowledgments: The authors are indebted to Jeffie Lafosse (Institut Gustave Roussy) for collecting the clinical and biological annotations and the Groupe Francophone des Myélodysplasies for constant support.

Our group is certified as an Equipe Labellisée by the Ligue Nationale Contre le Cancer and receives grants from the French National Cancer Institute and the Association Laurette Fugain.

Contribution: D.S.-B., O.W.-B., and E.S. designed the study, analyzed the data, and wrote the manuscript; B.B. and E.B. collected the data; A.T., P.F., G.E., B.Q., and T.B. provided patient samples; N.A. performed cytogenetic analysis; and M.M. and N.D. performed the sample collection and collected data.

Conflict-of-interest disclosure: D.S.-B., N.D., E.S., and O.W.-B. have a patent issued relevant to the work. The remaining authors declare no competing financial interests.

A complete list of the members of the Groupe Francophone des Myélodysplasies appears in "Appendix."

ORCID profiles: N.A., 0000-0002-0066-5884.

Correspondence: Orianne Wagner-Ballon, Département d'Hématologie et Immunologie Biologiques, Hôpitaux Universitaires Henri Mondor APHP, Inserm U955, Université Paris-Est Créteil, 51 Avenue du Maréchal de Lattre de Tassigny, F-94100 Créteil, France; e-mail: orianne.wagnerballon@aphp.fr.

Appendix: study group members

The members of the Groupe Francophone des Myélodysplasies are: Pierre Fenaux, Norbert Vey, Lionel Adès, Agnès Guerci, François Dreyfus, Michaela Fontenay, Sophie Raynaud, Claude Preudhomme, Eric Solary, Odile Beyne-rauzy, Raphael Itzykson, Sophie Park, Olivier Kosmider, Thomas Cluzeau, Andréa Toma, Bruno Quesnel, Gabriel Etienne, Thorsten Braun, and Orianne Wagner-Ballon.

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. Selimoglu-Buet D, Wagner-Ballon O, Saada V, et al; Francophone Myelodysplasia Group. Characteristic repartition of monocyte subsets as a diagnostic signature of chronic myelomonocytic leukemia. *Blood*. 2015;125(23):3618-3626.
3. Talati C, Zhang L, Shaheen G, et al. Monocyte subset analysis accurately distinguishes CMML from MDS and is associated with a favorable MDS prognosis [letter]. *Blood*. 2017;129(13):1881-1883.
4. Ball M, List AF, Padron E. When clinical heterogeneity exceeds genetic heterogeneity: thinking outside the genomic box in chronic myelomonocytic leukemia. *Blood*. 2016;128(20):2381-2387.
5. Greenberg PL. The classical nature of distinctive CMML monocytes [commentary]. *Blood*. 2017;129(13):1745-1746.
6. Rigolin GM, Cuneo A, Roberti MG, Bardi A, Castoldi G. Myelodysplastic syndromes with monocytic component: hematologic and cytogenetic characterization. *Haematologica*. 1997;82(1):25-30.
7. Bowen DT. Chronic myelomonocytic leukemia: lost in classification? *Hematol Oncol*. 2005;23(1):26-33.
8. Singh ZN, Post GR, Kiwan E, Maddox A-M. Cytopenia, dysplasia, and monocytosis: a precursor to chronic myelomonocytic leukemia or a distinct subgroup? Case reports and review of literature. *Clin Lymphoma Myeloma Leuk*. 2011;11(3):293-297.
9. Wang SA, Galili N, Cerny J, et al. Chronic myelomonocytic leukemia evolving from preexisting myelodysplasia shares many features with de novo disease. *Am J Clin Pathol*. 2006;126(5):789-797.
10. Orazi A, Bennett OF, Germing U, et al. Chronic myelomonocytic leukemia. In: Swerdlow SH, Campo E, Harris NL, et al, eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC;2008:76-79.
11. Schuler E, Schroeder M, Neukirchen J, et al. Refined medullary blast and white blood cell count based classification of chronic myelomonocytic leukemias. *Leuk Res*. 2014;38(12):1413-1419.
12. 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.
13. Greenberg PL, Tuechler H, Schanz J, et al. Revised international prognostic scoring system for myelodysplastic syndromes. *Blood*. 2012;120(12):2454-2465.
14. Padron E, Garcia-Manero G, Patnaik MM, et al. An international data set for CMML validates prognostic scoring systems and demonstrates a need for novel prognostication strategies. *Blood Cancer J*. 2015;5:e333.
15. Goasguen JE, Bennett JM, Bain BJ, Vallespi T, Brunning R, Mufti GJ; International Working Group on Morphology of Myelodysplastic Syndrome. Morphological evaluation of monocytes and their precursors. *Haematologica*. 2009;94(7):994-997.
16. Ziegler-Heitbrock L, Ancuta P, Crowe S, et al. Nomenclature of monocytes and dendritic cells in blood. *Blood*. 2010;116(16):e74-e80.
17. Wong KL, Tai JJ-Y, Wong W-C, et al. Gene expression profiling reveals the defining features of the classical, intermediate, and nonclassical human monocyte subsets. *Blood*. 2011;118(5):e16-e31.
18. Wong KL, Yeap WH, Tai JJY, Ong SM, Dang TM, Wong SC. The three human monocyte subsets: implications for health and disease. *Immunol Res*. 2012;53(1-3):41-57.
19. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of the myelodysplastic syndromes. *Br J Haematol*. 1982;51(2):189-199.
20. 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 [published online ahead of print 26 May 2017]. *Mod Pathol*.

DOI 10.1182/blood-2017-04-779579