Acute myeloid leukemia with mast cell differentiation is characterized by interstitial mast cells, complex karyotype, *TP53* alterations and poor prognosis

Among 2,167 acute myeloid leukemia (AML) cases at our institution, we identified 21 (approx. 1%) cases of AML with mast cell (MC) differentiation (AML-MC), defined as: (1) increased immature MC (>0.3%; >3 Standard Deviation [SD] above normal/reactive bone marrow (BM) MC by flow cytometric immunophenotyping (FCI) that are: $CD117^{+bright}$, $HLA\text{-}DR^{+low/negative}$, $CD45^{+dim}$ with low side scatter (SSC), $CD38^{+}$, $CD123^{+}$, $CD34^{+partial/dim}$ (Figure 1A); (2) cells with metachromatic granules observed on PB and/or BM aspirate smears; and (3) ≥1% MC shown by tryptase immunohistochemistry in BM biopsy specimens. This study was approved by the Institutional Review Board. Our cohort included 11 men and 10 women, median age 68 years (range, 28-83 years). Twelve patients had a history of malignancy: 9 (43%) had myeloid neoplasms (including 6 myelodysplastic syndrome, 2 chronic myelomonocytic leukemia, and one chronic myeloid leukemia [CML]), 3 (14%) with lymphoid neoplasms, and 3 (14%) with solid tumors. Among them, 2 patients had both lymphoid and myeloid neoplasms, and one patient had both solid tumor and myeloid neoplasm. The classification of these AML cases is listed in *Online Supplementary Table S1*. The myeloblasts (without MC differentiation) were mostly large, with small amounts of agranular cytoplasm (Figure 1B-D). The blasts with MC differentiation were usually small- to medium-sized, with a few metachromatic cytoplasmic granules, consistent with metachromatic blasts (Figure 1B-D). Atypical immature or mature MC that were round to oval, mostly mononuclear or occasionally bi-lobed or segmented nucleated, and often with hypo-granular cytoplasm, were also present. The median count of cells with metachromatic granules was 6% (range, 1-41%). Five of 18 (28%) cases had ≥10% cells with metachromatic granules, consistent with myelomastocytic leukemia (MML). Background dysplasia was present in 18 of 20 (90%) cases (Figure 1D). Dysplasia was observed in granulocytic (N=15; 75%), erythroid (N=14; 70%), and megakaryocytic (N=10; 50%) lineages, and involved multiple lineages in 16 (80%) and a single lineage in 2 (10%) cases. Immunohistochemistry for CD117 highlighted myeloblasts (dim) and MC (bright) in the BM (Figure 1F). The median MC percentage by tryptase was 5% of the BM cellularity (range, 1-40%) and the MC were distributed in an interstitial pattern without forming aggregates. The intensity of tryptase expression in MC was weak to moderate in most cases (Figure 1G).

Next-generation sequencing (NGS) analysis using panels designed to target genes commonly mutated in myeloid neoplasms was performed on all cases: 20 cases using an 81-gene panel, 1 case using a 28-gene panel. *TP53* was the most frequently mutated, detected in 11 of 21 (52%) cases, followed by *NRAS* (N=7; 33%), *ASXL1* (N=4; 19%), and *RUNX1* (N=4; 19%) (Figure 2A). No other gene mutations including *KIT* were identified. *TP53* mutations identified in the AML-MC cases included nonsense (N=8), missense (N=3), and splice site mutations (N=2) (Figure 2B). Eleven of 13 (85%) *TP53* mutations were present within the DNA binding domain; 2 (15%) mutations occurred in splice sites. In the 10 *TP53* wild-type cases, *NRAS* was most often mutated (N=4; 40%). There was no significant difference in the percentage of MC between the *TP53* mutant and wild-type cases (*Online Supplementary Figure S1A*). Thirteen of 19 (68%) cases had a complex karyotype (Figure 2A). Nine of 21 (43%) cases showed *TP53* deletion by fluorescence *in situ* hybridization (FISH) (Figure 2A). Seven of 21 (33%) cases had both *TP53* deletion and mutation (biallelic *TP53* alterations) (*Online Supplementary Figure S1B*).

Ten (48%) patients were treated with intensive chemotherapy, with or without targeted therapy. Seven (33%) patients received hypomethylating agents with or without venetoclax. Two (10%) patients were treated with immunomodulator therapy alone and one (5%) was treated with targeted therapy alone. Three (14%) patients received allogeneic stem cell transplant (SCT). After a median follow-up of 7.4 months (range, 0.2-41.9 months), 13 of 21 (62%) patients died, with a median overall survival (OS) of 9.6 months (Figure 3A). Patients aged 65 years or older had a significant shorter OS than those younger than 65 years (Figure 3B). The percentage of MC did not affect OS, using a cut-off value of 5% (Figure 3C), 2% or 10% (*data not shown*). The OS of AML-MC patients showed no difference after stratifying patients by *TP53* mutation status (mutated *vs.* wild-type) (Figure 3D). Patients with a non-complex karyotype or those who received SCT tended to show a better OS than patients with a complex karyotype or without SCT, but these differences did not reach statistical significance (Figure 3E, F).

Our definition of AML-MC is very similar to the “pre-MML” condition” described by Panda *et al.*[11] MML requires that MC comprise ≥10% of BM cells, an arbitrary and stringent cut-off that is likely set too high; as a result only about 10 cases have been reported in the literature.[1-7] Similar to MML, the MC in AML-MC were interstitially distributed in the background of dysplasia. These MC are immunopheno-

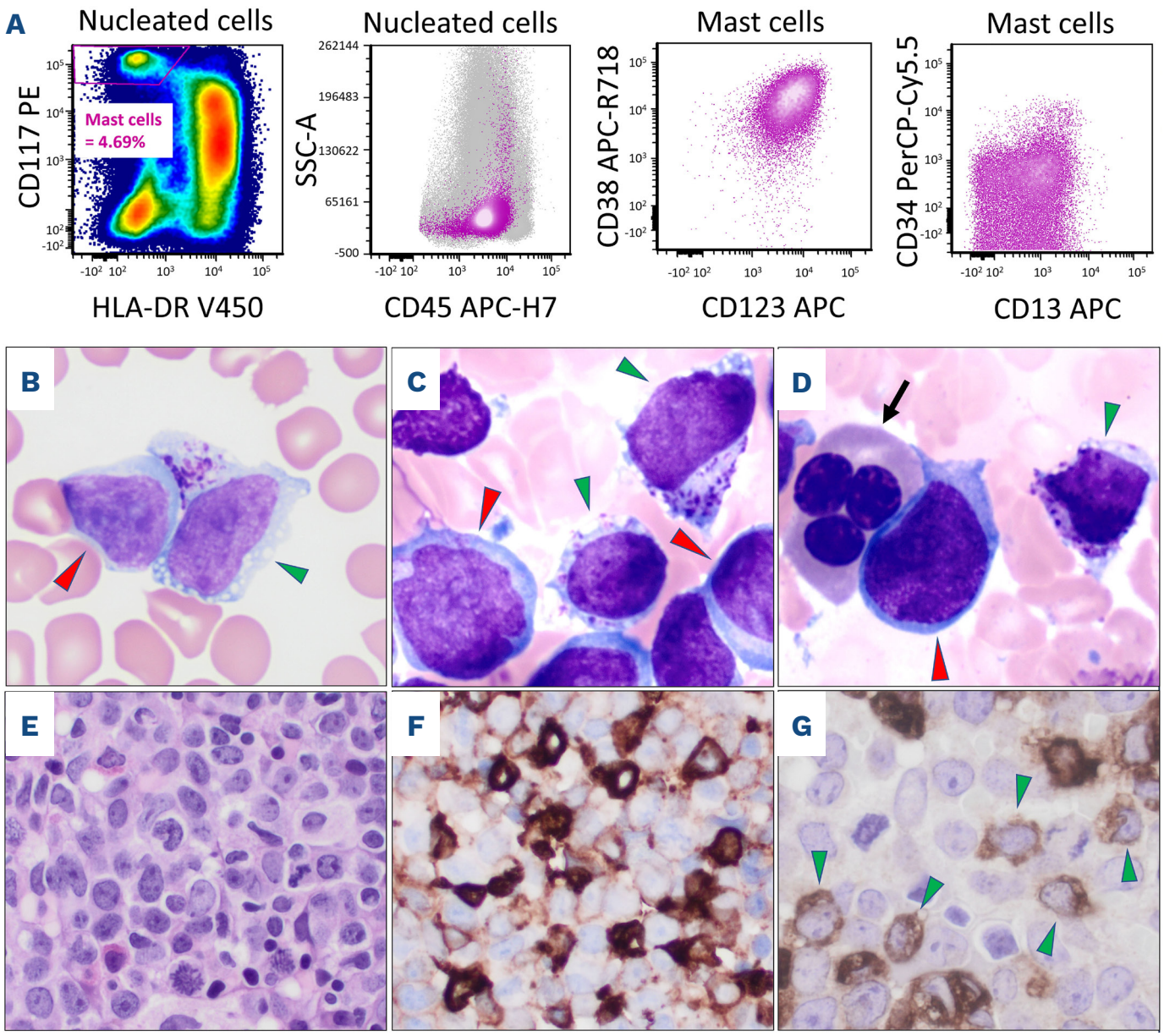

Figure 1. A representative case of acute myeloid leukemia with mast cell differentiation. (A) Mast cells (MC) are positive for CD117 (bright), CD45 (dim), CD38, CD123, CD34 (partial/dim), and CD13 (partial), and are negative for HLA-DR, with low side scatter (SSC), consistent with immature MC. (B) Peripheral blood and (C and D) bone marrow (BM) aspirate smears reveal myeloblasts (red arrow heads) which are large, with irregular nuclear contour, fine chromatin, inconspicuous nucleoli, and a small amount of agranular cytoplasm. Metachromatic blasts (green arrow heads) have a small to moderate amount of cytoplasm containing a few metachromatic granules. Erythroid dysplasia is present in the background (black arrow). (E) BM biopsy shows a hypercellular marrow (approx. 100% cellularity) with markedly increased blasts. Immunohistochemical stains for (F) CD117 (strong) and (G) tryptase (weak) highlight scattered MC. (F) CD117 also stains the myeloblasts (dim). (B-D) Wright-Giemsa stain, x1000. (E) Hematoxylin-eosin stain, x400. Immunohistochemistry: (F) x400 and (G) x600. AML-MC: acute myeloid leukemia with mast cell differentiation.

typically immature, in contrast to the mature MC (negative for CD34, brighter CD45, higher SSC), as can be seen in chronic lymphocytic leukemia and reactive conditions (*data not shown*). Among the 9 AML-MC patients with a history of myelodysplastic and/or myeloproliferative neoplasms, 5 cases of the earlier neoplasms were assessed by FCI, and none showed increased immature MC. Tryptase is often strongly expressed on mature MC, but can be decreased or lost in MML and mast cell leukemia,[8,12] thought to be attributable to the MC immaturity. Low tryptase expression on immature MC is also seen in AML-MC cases, supported by their immunophenotypic signature and decreased tryptase staining. Interestingly, elevated serum tryptase levels were reported in MML cases, and some patients had symptoms due to inappropriate release of MC mediators.[1,6-8,13] None of the AML-MC patients we report were tested for serum tryptase or had mediator-related symptoms.

Although little is known about the genetic and molecular pathogenesis underlying MML, a complex karyotype is found in 75% of cases.[1-4,8-10,13,14] In this cohort of AML-MC cases, about 70% showed a complex karyotype and 50% had *TP53* deletion. One case (5%) had t(8;21), which has been reported in MML.[1,5,6] We also had 2 (10%) AML-MC cases with inv(16), which has not been previously reported in MML. Another recurrent genetic abnormality was t(9;22), seen in 2 (10%) BP-CML cases. CML in accelerated/blast phase has been reported to develop MML or to have increased immature MC.[2,11] *KIT* mutations are observed in approximately 20-40% of core binding factor (CBF) AML cases, but they are typically not detectable in MML as was observed in this cohort.[4,8,13,14] *TP53* was the most frequently mutated gene, occurring in about 50% of AML-MC cases, and 64% of the

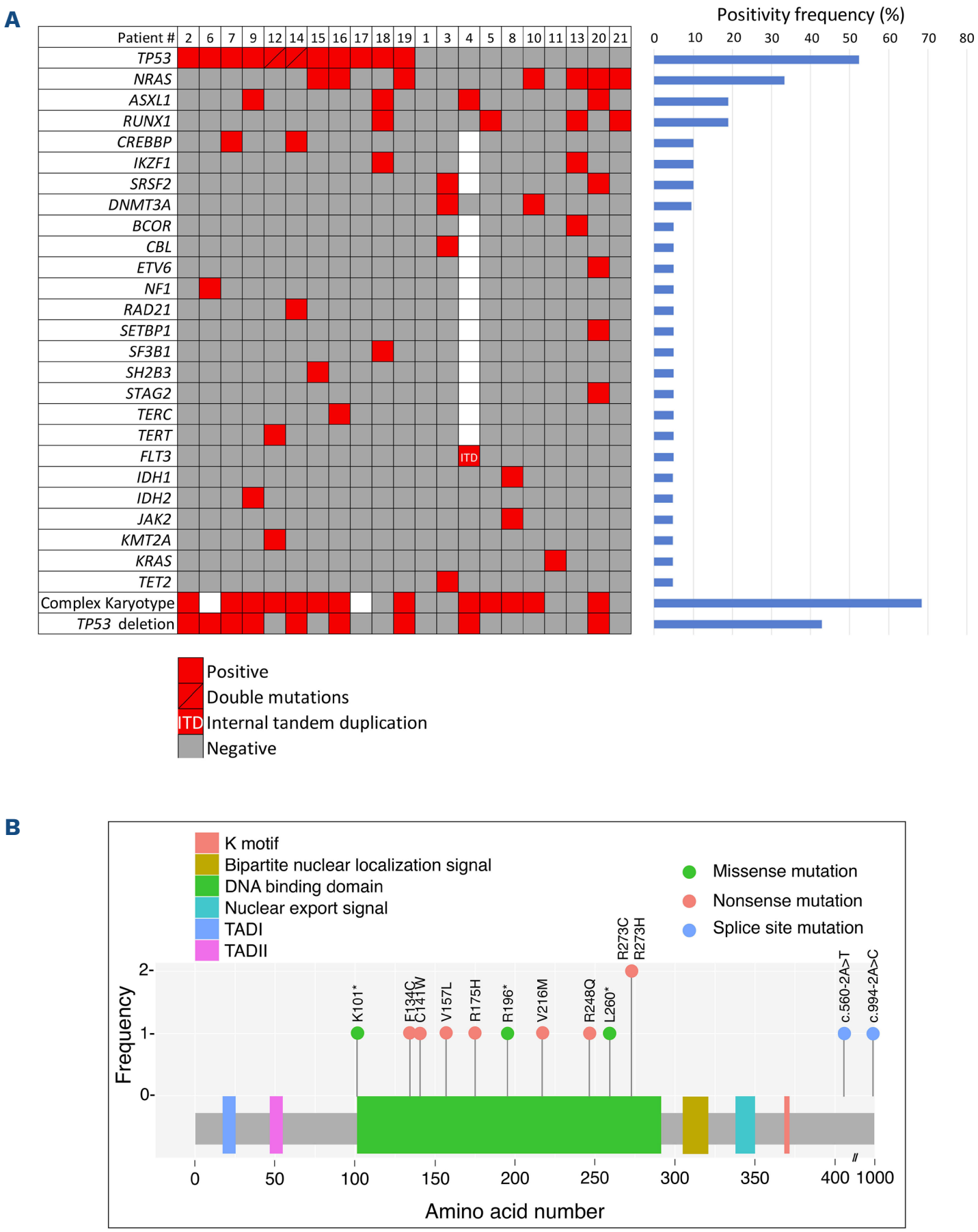

Figure 2. The molecular and cytogenetic findings of acute myeloid leukemia with mast cell differentiation. (A) Oncoplot of gene mutations and status of karyotype and *TP53* deletion. (B) Lollipop graph of *TP53* mutations in acute myeloid leukemia with mast cell (AML-MC) differentiation. (Template from Uniprot).

TP53-mutated cases had biallelic inactivation (one copy mutated, the other copy lost). However, there was no significant difference in the percentage of MC between *TP53* mutant and wild-type cases, suggesting that *TP53* is not a driver for MC differentiation.

The prognosis of AML-MC patients was poor, with a median OS of 9.6 months, similar to OS reported in MML patients.[1-3,9,10] Patient outcome was even worse if they were 65 years or older. Generally, AML with t(8;21) and inv(16) are associated with a favorable prognosis.[15] In the current study, after initial diagnosis, the patient with t(8;21) died in 17 months and one of 2 patients with inv(16) died in four months, both due to infection and respiratory failure; the sample size of CBF AML-MC cases in this study is too small to assess their behavior. The patients with a non-complex karyotype, or if they received SCT, tended to show a better

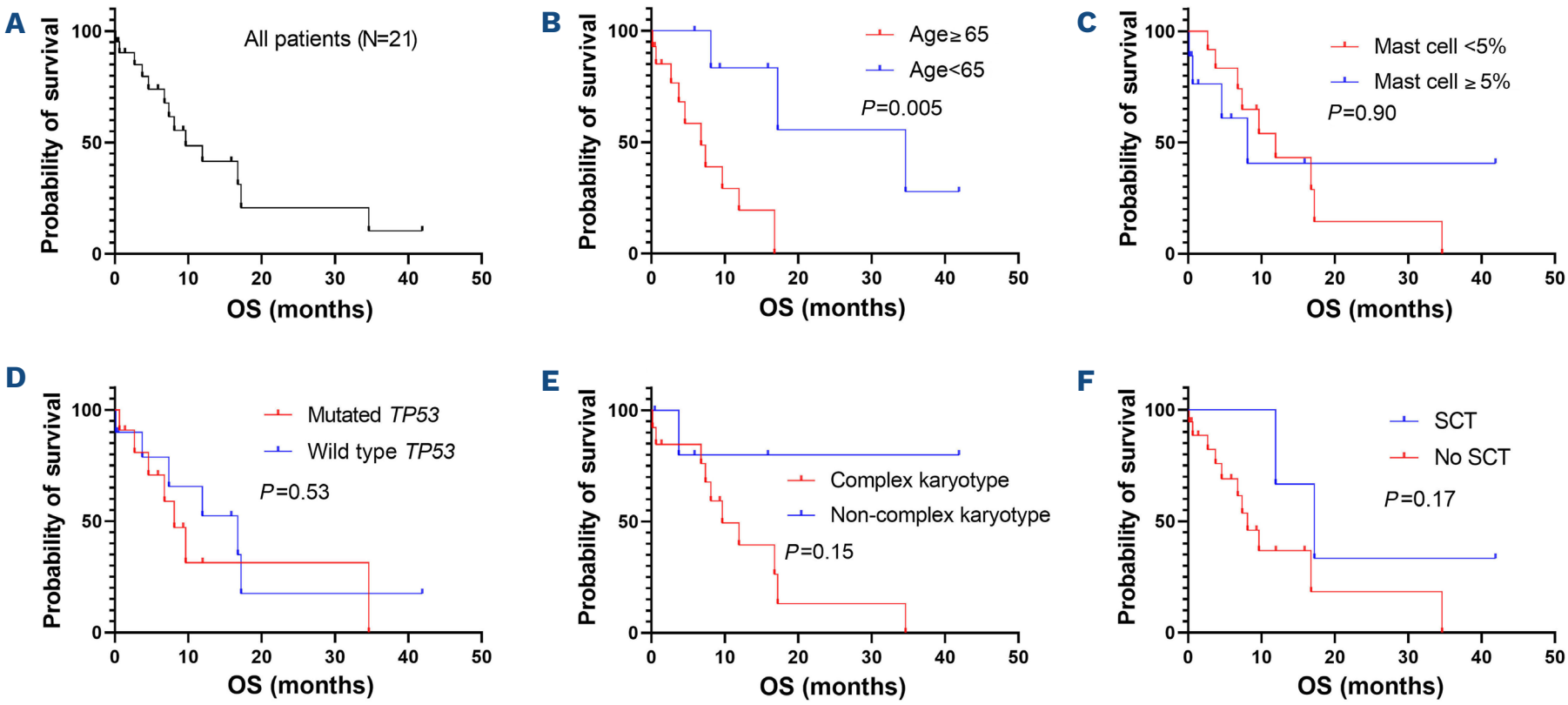

Figure 3. The overall survival of patients with acute myeloid leukemia with mast cell differentiation. Overall survival (OS) in (A) all 21 patients with acute myeloid leukemia with mast cell (AML-MC) differentiation, (B) patients aged ≥65 years and <65 years, (C) patients with ≥5% MC and <5% MC, (D) patients with mutated and wild-type *TP53*, (E) patients with a complex karyotype and non-complex karyotype, and (F) patients with and without allogeneic stem cell transplant (SCT).

OS than patients with a complex karyotype or without SCT, but these differences did not reach statistical significance, possibly also due to the small sample size. SCT was reported to achieve prolonged survival in 2 MML with t(8;21) patients.[1,6]

The percentage of MC in AML was not associated with OS, using a cut-off value of 2%, 5%, or 10%, suggesting that once the immature MC component is increased, arbitrarily setting a MC% cut-off may not be relevant.

In summary, patients with AML-MC are characterized by interstitial MC, multilineage dysplasia, complex karyotype, *TP53* alterations, and poor prognosis. The results of this study support recognition of this rare subset of aggressive AML cases, which are not adequately captured by current prognostic systems.

Authors

Do Hwan Kim,[1] Sa A Wang,[1] Wei Wang,[1] Guilin Tang,[1] Shaoying Li,[1] C. Cameron Yin,[1] Pei Lin,[1] Marina Konopleva,[2] M. James You,[1] Roberto N. Miranda,[1] Xiaoqiong Wang,[1] Qing Wei,[1] L. Jeffrey Medeiros[1] and Jie Xu[1]

[1]Department of Hematopathology and [2]Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Correspondence:
J. XU - jxu9@mdanderson.org

https://doi.org/10.3324/haematol.2024.284976

Received: January 4, 2024.
Accepted: March 20, 2024.
Early view: March 28, 2024.

Disclosures

No conflicts of interest to disclose.

Contributions

DHK collected and analyzed the data and wrote the manuscript. SAW, WW, SL, PL, MJY and RNM contributed data and edited the manuscript. GT and QW analyzed the cytogenetic data. CCY analyzed the molecular data. MK treated the patients. XW collected data. LJM analyzed data and wrote the manuscript. JX designed the study, collected and analyzed the data, supervised the study and wrote the manuscript. All authors reviewed and approved the manuscript.

Acknowledgments

The study was partially supported by Faculty Startup Fund (to JX) and Research Grant (to DHK and JX) from the Division of Pathology and Laboratory Medicine, The University of Texas MD Anderson Cancer Center.

Data-sharing statement

Data are available for sharing upon request to the corresponding author.

References

1. Sperr WR, Drach J, Hauswirth AW, et al. Myelomastocytic leukemia: evidence for the origin of mast cells from the leukemic clone and eradication by allogeneic stem cell transplantation. Clin Cancer Res. 2005;11(19 Pt 1):6787-6792.
2. Valent P, Spanblochl E, Bankl HC, et al. Kit ligand/mast cell growth factor-independent differentiation of mast cells in myelodysplasia and chronic myeloid leukemic blast crisis. Blood. 1994;84(12):4322-4332.
3. Wimazal F, Sperr WR, Horny HP, et al. Hyperfibrinolysis in a case of myelodysplastic syndrome with leukemic spread of mast cells. Am J Hematol. 1999;61(1):66-77.
4. Rich A, Sun J, Aldayel AS, et al. Myelomastocytic leukemia with aberrant CD25 expression: case report and review of the literature. Clin Lymphoma Myeloma Leuk. 2014;14(5):e173-177.
5. Johnson RC, Savage NM, Chiang T, et al. Hidden mastocytosis in acute myeloid leukemia with t(8;21)(q22;q22). Am J Clin Pathol. 2013;140(4):525-535.
6. Intzes S, Wiersma S, Meyerson HJ. Myelomastocytic leukemia with t(8;21) in a 3-year-old child. J Pediatr Hematol Oncol. 2011;33(8):e372-375.
7. Arredondo AR, Gotlib J, Shier L, et al. Myelomastocytic leukemia versus mast cell leukemia versus systemic mastocytosis associated with acute myeloid leukemia: a diagnostic challenge. Am J Hematol. 2010;85(8):600-606.
8. Valent P, Sotlar K, Sperr WR, et al. Refined diagnostic criteria and classification of mast cell leukemia (MCL) and myelomastocytic leukemia (MML): a consensus proposal. Ann Oncol. 2014;25(9):1691-1700.
9. Valent P, Sperr WR, Samorapoompichit P, et al. Myelomastocytic overlap syndromes: biology, criteria, and relationship to mastocytosis. Leuk Res. 2001;25(7):595-602.
10. Valent P, Samorapoompichit P, Sperr WR, Horny HP, Lechner K. Myelomastocytic leukemia: myeloid neoplasm characterized by partial differentiation of mast cell-lineage cells. Hematol J. 2002;3(2):90-94.
11. Panda D, Chatterjee G, Khanka T, et al. Mast cell differentiation of leukemic blasts in diverse myeloid neoplasms: a potential pre-myelomastocytic leukemia condition. Cytometry B Clin Cytom. 2021;100(3):331-344.
12. Sanchez-Munoz L, Teodosio C, Morgado JM, Escribano L. Immunophenotypic characterization of bone marrow mast cells in mastocytosis and other mast cell disorders. Methods Cell Biol. 2011;103:333-359.
13. Leguit R, Hebeda K, Kremer M, et al. The spectrum of aggressive mastocytosis: a workshop report and literature review. Pathobiology. 2020;87(1):2-19.
14. Horny HP, Sotlar K, Reiter A, Valent P. Myelomastocytic leukemia: histopathological features, diagnostic criteria and differential diagnosis. Expert Rev Hematol. 2014;7(4):431-437.
15. Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022;36(7):1703-1719.