Isolated *KRAS* and *NRAS* mutations in adults with monocytosis and/or cytopenia(s)

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Abstract

NRAS and *KRAS* mutations, commonly identified alongside ancestral co-mutations, are generally regarded as pathogenic in adults presenting with monocytosis and/or cytopenia(s). However, their significance in isolation is not well defined. We studied a multi-institutional cohort of 52 patients with isolated RAS mutations and found that 26 (50%) did not meet diagnostic criteria for a myeloid neoplasm. Compared to patients with typical chronic myelomonocytic leukemia/myelodysplastic syndrome, these patients exhibited distinctive clinical features, including a younger age (65 years; range, 29-92 years), female predominance (60%), frequent immune-related disorders (39%), and splenomegaly (65%). Mutations predominantly involved *KRAS* (92%), with 87% affecting codons G12 or G13, and typically occurred at high variant allele frequency (39.0%; range, 2.6-53.0%). In three flow-sorted samples, *KRAS/NRAS* mutations were detected not only in granulocytes and monocytes but also in lymphocytes, reminiscent of pediatric RASopathies. A subset of patients (7/26, 27%) progressed to develop a myeloid malignancy, with acquisition of additional genetic alterations or the development of dysplasia. These findings challenge the assumption that isolated RAS mutations are sufficient to diagnose myeloid neoplasms. Instead, some cases may reflect adult-onset RASopathies or early clonal proliferations with distinct biological behavior. Recognition of such cases warrants refinement of diagnostic criteria and may influence therapeutic decision-making.

Introduction

RAS proteins are small GTPases that play a central role in regulating normal cellular proliferation, differentiation, and survival by transmitting signals from membrane-bound receptors to downstream effectors. RAS proteins are encoded by *KRAS*, *NRAS* and *HRAS* genes. RAS activating point mutations occur in 10-30% of myeloid neoplasms, with *NRAS* mutations being the most frequent, followed by *KRAS* mutations, whereas *HRAS* mutations are rare. The prevalence of *NRAS/KRAS* mutations varies across different types of myeloid malignancies and they are more common in pediatric than adult diseases,[1,2] with notably higher incidences in chronic myelomonocytic leukemia (CMML) and juvenile myelomonocytic leukemia (15% to 20% for each gene) as compared to adult myelodysplastic syndromes (MDS) (2-3%).[3] In myeloid malignancies that affect adults, RAS pathway mutations tend to emerge as late events in the context of clonal hematopoiesis, aligning with a stepwise model of leukemogenesis.[4,5] The presence of *NRAS* or

KRAS mutations is typically considered as indicative of a myeloid neoplasm in adult patients, as these mutations are uncommon in age-related clonal hematopoiesis of indeterminate potential (CHIP), which is dominated by *DNMT3A*, *TET2*, and *ASXL1* (DTA) mutations or, less frequently, *TP53*, *JAK2*, *SF3B1*, *CBL*, *SRSF2*, *PPM1D*, and *BCOR* mutations.[6,7] Whole-exome sequencing data from 200,453 United Kingdom Biobank participants reported that 0.02% had *KRAS* and 0.01% had *NRAS* mutations, with approximately half of the mutations occurring at the hotspots of G12/G13[8] in the context of clonal hematopoiesis.

Gain-of-function somatic mutations in *KRAS* or *NRAS* are the underlying cause of RAS-associated autoimmune leukoproliferative disorder (RALD), an intrinsic lymphocyte apoptosis defect that manifests with immune cytopenia(s), hypergammaglobulinemia and monocytosis. The mutations are found in circulating granulocytes, monocytes as well as lymphocytes, indicating origin from a mutated pluripotent hematopoietic stem cell. RALD is considered a disease of children, with a reported median age of 2 years[9] at disease onset. Overall, one-third of patients manifest disease within the first year of life and it is extremely uncommon to see RALD diagnosed in adulthood.

In our practice, we have encountered cases with isolated *KRAS/NRAS* mutations in adult patients with cytopenia(s) and/or persistent monocytosis raising concern about a myeloid malignancy. While some cases were diagnosed as CMML, other myelodysplastic/myeloproliferative neoplasms (MDS/MPN), or MDS, some cases failed to meet the diagnostic criteria for a myeloid neoplasm and would be considered as having clonal cytopenia of uncertain significance (CCUS), or one of the two precursor entities of monocytosis – clonal monocytosis of undetermined significance (CMUS) and clonal cytopenia and monocytosis of undetermined significance (CCMUS) – introduced by the International Consensus Classification (ICC).[10]

To investigate the significance of *KRAS/NRAS* mutations in the absence of ancestral mutations typically associated with age-related clonal hematopoiesis, we assembled a large cohort of adult patients with suspected myeloid neoplasms from several major medical centers. Each case underwent a thorough clinicopathological evaluation, including determination of the clinical presentation, bone marrow (BM) and peripheral blood (PB) pathology, mutational profiles, and disease course including treatment and progression. Additionally, available samples were collected and sorted to assess the presence of RAS mutations in granulocytes, monocytes, and lymphocytes. The primary aim was to determine whether isolated *KRAS* or *NRAS* mutations alone could suffice for a diagnosis of a myeloid neoplasm in adults with cytopenia and/or monocytosis, and to examine disease and mutation characteristics, including any resemblance to pediatric RALD. The findings may have significant implications for the clinical management of affected patients.

Methods

Patients

The participating medical centers are tertiary centers with specialization in hematologic malignancies. Molecular databases were reviewed for *KRAS* or *NRAS* mutations detected in BM and/or PB via myeloid gene-targeted next-generation sequencing (NGS) panels over 5-7 years (2017–2024, periods varying by institution). Cases with co-mutations and clonal cytogenetic abnormalities were excluded, except for low-level (≤5%) *DNMT3A* or -Y, due to their unlikely contribution to pathogenesis. Clinical data were obtained from electronic medical records. The study was approved by the Institutional Review Boards of all participating institutions.

Bone marrow morphology

Hematoxylin and eosin-stained EDTA decalcified BM biopsy sections and Wright–Giemsa-stained aspirate smears were reviewed. A 500-cell differential was performed, and Perls iron staining was done in most cases. Dysplasia was assessed as per the International MDS Working Group guidelines.[11] Myelofibrosis was graded using the European BM Fibrosis Consensus criteria.[12]

Flow cytometry immunophenotyping and sorting

Flow cytometry validated for MDS/CMML was performed on BM samples in a subset of cases as described previously.[13,14] Classic monocytes were defined as $CD11b^{+}CD64^{+}CD14^{+}CD16^{-}$. When available, PB or BM samples were sorted on a CytoFLEX SRT (Beckman Coulter, Brea, CA, USA). Populations were gated by CD45/SSC and further defined by CD15 (granulocytes), CD14 (monocytes), and CD3 (T cells). Sorted cells were stored at –20°C, and DNA was extracted for NGS (see below).

Diagnosis and classifications

Cases were evaluated using the criteria of the ICC[10] and the 5th edition of World Health Organization (WHO) classification.[15] A diagnosis of CMML requires PB monocytes ≥10% and an absolute monocyte count $\geq 0.5\times10^{9}$/L by both sets of criteria. Additionally, the 5th edition of the WHO classification requires evidence of clonality and dysplasia,[15] whereas the ICC requires abnormal BM findings (hypercellularity with myeloid/monocytic proliferation, without features of acute myeloid leukemia [AML], myeloproliferative neoplasm [MPN], or other causes of monocytosis), along with cytopenia.[16] According to the ICC, cases without BM features of CMML were classified as CMUS (no cytopenia) or CCMUS (with cytopenia).[10] Cases with clonality and cytopenia(s) but not meeting MDS criteria were designated as CCUS,[17] and cases with no cytopenia or dysplasia as CHIP.

Cytogenetics

G-banded metaphase cells from unstimulated BM aspirates were analyzed using standard techniques. Twenty

metaphases were examined, and results reported according to the International System for Human Cytogenetic Nomenclature.

Targeted next-generation sequencing

Targeted NGS for myeloid malignancy-associated genes was performed at each institution as described previously.[18] Panels varied but all covered ≥38 genes commonly detected in MDS and CMML across >90% of coding regions. *KRAS/NRAS* coverage differed across panels, ranging from exons 2 to 6. All panels included complete sequencing of exons 2–4, except one panel that, for exon 4, was limited to hotspots. Variants were annotated according to Human Genome Variation Society guidelines.[19] *NRAS* and *KRAS* mutation variant allele frequencies (VAF) and codons were recorded.

Treatment and follow-up

Treatment and outcome data were collected from medical records. Therapies were categorized as hydroxyurea, hypomethylating agents, JAK inhibitors, other small-molecule inhibitors, observation/supportive care, or allogeneic stem cell transplantation (SCT). Cases were assessed for progression to AML from MDS/CMML, or to MDS/CMML from CMUS, CCMUS, CCUS, or CHIP.

Statistical analyses

Categorical and numerical variables were compared using Fisher exact/χ^2 and Mann-Whitney tests, respectively. Overall survival was measured from diagnosis to death or last follow-up, without censoring for SCT. Survival was estimated by the Kaplan–Meier method and compared by a log-rank test. Analyses were done in GraphPad Prism (San Diego, CA, USA), with statistical significance set at $P<0.05$ (two-sided).

Results

Patients

A total of 52 patients were identified from seven medical centers. All were adults with a median age of 65 years (range, 29-92 years), showing a female predominance (female:male ratio 31:21 [1.6:1]). Twenty patients (39%) had a clinical history of immune-related disorders, including systemic lupus erythematosus (SLE) or SLE-like immune disorders, rheumatoid arthritis, immune thrombocytopenic purpura, vasculitis, polyarthritis, Sjögren syndrome, idiopathic pleuritis, sialadenitis, erythema nodosum, inflammatory bowel diseases, and ill-defined autoimmune disorders. Two patients had concurrent Rosai-Destombes-Dorfman disease. Thirty-three of 51 (65%) had splenomegaly, and four also had hepatomegaly; one patient had had a splenectomy at a young age. Lactate dehydrogenase was elevated in three of 39 (8%) patients. One patient had monoclonal gammopathy of undetermined significance and two patients had smoldering myeloma; in these three cases, the VAF of the RAS mutations markedly exceeded what would be expected if derived from neoplastic plasma cells. In five of the 52 patients, the clinical constellation of symptoms had raised the possibility of RALD, including some with symptoms reminiscent of autoimmune-lymphoproliferative disorders in the medical record, despite disease onset in adulthood.

The clinical and pathological features of the patients are summarized in Table 1.

Cytogenetics and mutations

Karyotype information was available for all 52 patients at

Table 1. Clinical features, peripheral blood data, and mutation information of the 52 patients.

Characteristics	Values
Age, years, median (range) Age ≤50 years, N (%)	65 (29-92) 11 (21)
Male:female, N:N	21:31
Autoimmune and/or immune dysregulation, N (%) RALD, N	20 (39) 5
Histiocytic proliferation, N	2 (Rosai-Dorfman)
Splenomegaly, N (%)	33/51 (65) (1 splenectomy)
Hepatomegaly, N (%)	4/52 (8) (all 4 also had splenomegaly)
WBC count, x10^9/L, median (range) WBC ≥13x10^9/L, N (%)	4.5 (1.1-38.8) 10 (19)
PB monocytes, %, median (range) PB monocytes ≥10%, N (%)	24.0 (4.8-70.0) 48 (92)
AMC, x10^9/L, median (range) AMC ≥1x10^9/L, N (%) AMC 0.5-1x10^9/L, N (%) AMC<0.5x10^9/L, N (%)	1.43 (0.15-6.98) 34 (65) 11 (21) 7 (14)
Hemoglobin, g/dL, median (range)	12.1 (7.0-16.6)
Platelets, x10^9/L, median (range)	145 (23-652)
BM cellularity, %, median (range)	80 (5-100)
BM monocytes, %, median (range)	5 (0-21)
BM blasts/promonocytes, %, median (range) BM blasts/promonocytes ≥5%, N (%)	2 (0-16) 6 (12)
Gene involved, N (%) *KRAS* *NRAS*	 48 (92) 4 (8)
VAF, %, median (range) VAF ≥30%, N (%) VAF 10-30%, N (%) VAF <10%, N (%)	39.0 (2.6-53.0) 33 (63) 12 (23) 7 (14)

RALD: RAS-associated autoimmune leukoproliferative disorder; WBC: white blood cell count; PB: peripheral blood; AMC: absolute monocyte count; BM: bone marrow; VAF: variant allele frequency.

the time of the initial BM examination. A normal karyotype was reported in 50, with the remaining two patients exhibiting loss of Y as the sole finding (in 18 and 19 out of 20 metaphases).

NGS revealed *KRAS* mutations in 48 (92%) patients, and *NRAS* in four, with no patients demonstrating more than one concurrent *RAS* mutation. The median VAF was 39.0% (range, 2.6-53.0%), with 33 (64%) patients having a VAF ≥30%, 12 with a VAF of 10-30% and seven with a VAF <10%. Three patients had low levels (≤5%) of *DNMT3A* co-mutations. Of the five patients with clinical features of RALD, all harbored *KRAS* mutations with a VAF >30%.

Of 48 patients with a *KRAS* mutation, 29 carried mutations at codon G12, 13 at G13, four at A146 and two at other loci (Q61K, L19F). For four patients with *NRAS* mutations, three occurred at codon G12 and one at G60E. In total, 45 of the 52 (87%) patients had *NRAS* or *KRAS* mutations involving codon G12 or G13. The mutation patterns are illustrated in Figure 1A.

Morphological features and disease classifications

After a review of the morphology and laboratory data, 26 patients (18 females and 8 males) were diagnosed with a myeloid neoplasm (Table 2). Of these, 17 (65%) presented with anemia, 12 (46%) with thrombocytopenia, and 11 (42%) with neutropenia; 17 (65%) patients had two or more cytopenia(s), and 24 (92%) had relative (≥10%) and absolute monocytosis (absolute monocyte count [AMC] ≥0.5x10^9/L). All patients showed age-adjusted BM hypercellularity (median 80%; range, 50-100%). Dysplasia involving two or more lineages was seen in 15 patients, unilineage dysplasia (mostly dysmegakaryopoiesis) in eight, and borderline dysplasia (around 10% of each lineage) in three. Three had increased BM blasts (including myeloblasts, monoblasts and promonocytes) of 5-9% and two had blasts ≥10%, 12

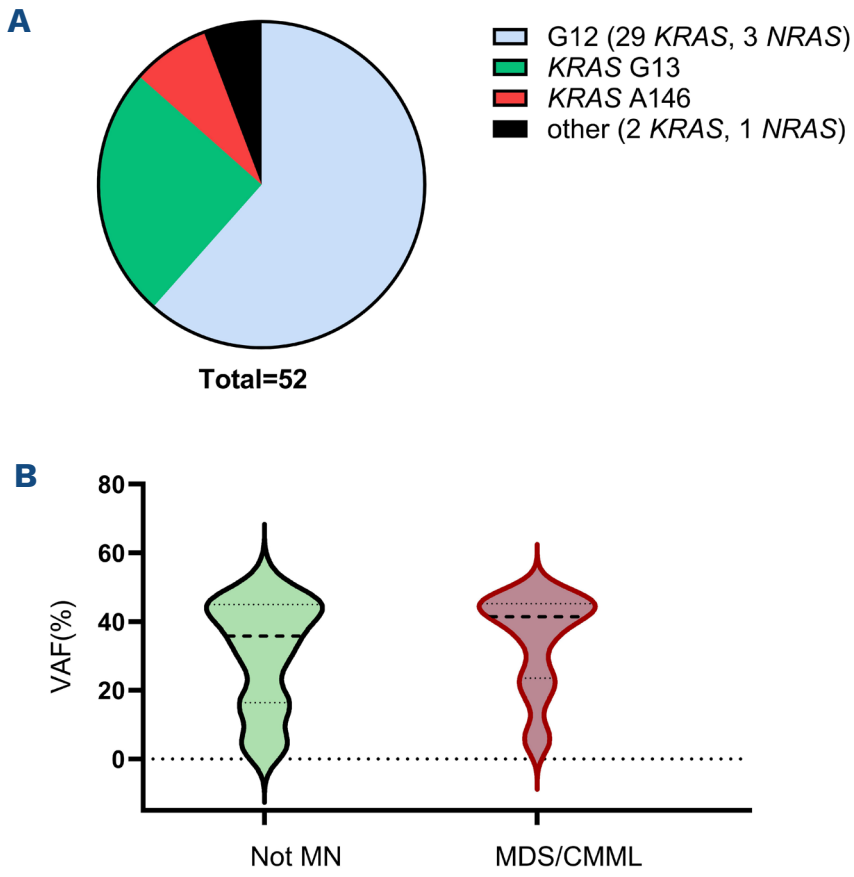

Figure 1. Characteristics of the *KRAS/NRAS* mutations. (A) *KRAS* (N=48) and *NRAS* (N=4) by mutation codons. (B) The median variant allele frequency was not significantly different between patients with or without a diagnosis of myeloid neo-plasm. VAF: variant allele frequency; MN: myeloid neoplasm; MDS: myelodysplastic syndrome; CMML: chronic myelomono-cytic leukemia.

Table 2. Patients' diagnosis, classifications, treatment and follow-up.

Diagnosis and classification	Patients, N	Treatment and progression
Chronic myelomonocytic leukemia (CMML) CMML1-MD CMML2-MD CMML1-MP	24 18 2 4	Nine were treated with HMA, two with ruxolitinib, one with a small molecule inhibitor, one with hydroxyurea only; four received supportive care; eight did not require treatment and information was not available for one patient. Treatment was followed by SCT in four cases No AML progression. Three died of CMML, two of SCT-related complications, and one of an unrelated cause
Myelodysplastic syndrome with low blasts and unilineage dysplasia	1	
Myelodysplastic/myeloproliferative neoplasm, not otherwise specified	1	
Clonal cytopenia and monocytosis of undetermined significance (CCMUS)	15	One was treated with HMA and two with hydroxyurea; 12 did not require treatment Four progressed to CMML at 1.5, 2, 5 and 9 years
Clonal monocytosis of undetermined significance (CMUS)	5	One progressed to CMML after 1 year; the other four did not require treatment
Clonal cytopenia of undetermined significance (CCUS)	5	Two were diagnosed with MDS in <1 year and at 1.5 years and were treated with HMA. Three did not require treatment
Clonal hematopoiesis of indeterminate potential (CHIP)	1	No treatment or progression

MD: myelodysplastic subtype; MP: myeloproliferative subtype; HMA: hypomethylating agent; SCT: stem cell transplantation; AML: acute myeloid leukemia; MDS: myelodysplastic syndrome.

of 24 (50%) had grade 1 myelofibrosis and one of 24 (4%) had grade 2 myelofibrosis. The median percentage of BM monocytes was 5% (range, 1-21%). Twenty-four patients (46%) met the diagnostic criteria for CMML, of whom 20 had the myelodysplastic subtype, including five with an AMC between 0.5-1.0x10^9/L, and four had the myeloproliferative subtype (*Online Supplementary Table S1*). One was classified as having MDS/MPN, not otherwise specified (NOS), with leukocytosis, neutrophilia and anemia, and another was classified as having MDS with low blasts/NOS and unilineage dysplasia. Among the patients diagnosed with myeloid neoplasms, the median VAF of *KRAS* or *NRAS* was 41.4% (range, 4.7-48.9%), and all three cases with a low level of *DNMT3A* mutations belonged to this group of patients. Flow cytometry immunophenotype data were available for three patients, all of whom had abnormal $CD34^+$ myeloblasts (bright CD117, with or without decreased CD38, increased CD123, altered CD4) with two showing classic monocytes >94%.

Criteria for a myeloid neoplasm were not met in the other 26 patients. Based on the presence or absence of cytopenia and/or monocytosis, these cases were designated as CCMUS (N=15), CMUS (N=5), CCUS (N=5), and CHIP (N=1, cytopenia normalized at follow-up) (Table 2). Examples of BM findings and associated clinical features that led to the diagnoses are illustrated in Figure 2. Compared to patients who met the diagnostic criteria for a myeloid neoplasm (CMML, MDS, MDS/MPN-NOS), the patients without a myeloid neoplasm were younger (60 *vs*. 67 years; *P*=0.007), but showed no difference in gender distribution (male: female 13:13 *vs*. 8:18; *P*=0.258), splenomegaly (15/25 *vs*. 18/26; *P*=0.565), or autoimmune/immune-dysregulation conditions (11/26 *vs*. 9/26; *P*=1.0). Complete blood count data, including hemoglobin, white blood cell count, absolute neutrophil count, the percentage of monocytes or AMC, were not statistically different between the two groups (*Online Supplementary Table S1*). The BM cellularity in patients not meeting diagnostic criteria for a myeloid neoplasm showed a trend for lower cellularity, but the difference did not reach statistical significance (80% [range, 5-100%] *vs*. 80% [range, 50-100%]; *P*=0.113). There was no significant difference in BM monocyte percentage, frequency of grade 1 or 2 myelofibrosis, or percentage of blasts/promonocytes. However, these non-neoplastic cases differed from cases of myeloid neoplasms by the absence of significant dysplasia (borderline dysplasia in 3 of the cases that were not myeloid neoplasms). Flow cytometry study was performed in three CCMUS, three CMUS and one CCUS. The $CD34^+$ myeloid precursors were completely normal in six cases and abnormal in one case of CMUS (bright CD117 and decreased CD38). Classic monocytes were >94% in two patients including the case of CMUS with abnormal $CD34^+$ myeloblasts.

The median *KRAS/NRAS* VAF did not differ between patients with or without a diagnosis of a myeloid neoplasm, being 35.8% (range, 2.6-53.0%) and 41.4% (range, 4.7-48.9%), respectively (*P*=0.376) (Figure 1B). The mutation patterns of *KRAS* were also not significantly different between the two groups (*P*=0.835). Of the four patients with *NRAS* mutations, two were diagnosed with CMML, and two were considered to have CMUS. Of the seven patients with a VAF <10%, all involving *KRAS*, three had CMML, two had CCMUS, one had CCUS, and one had CHIP. The diagnostic distribution was not significantly different from that of cases with higher VAF. Details of the *KRAS/NRAS* mutations, including involved codons, VAF, and corresponding clinicopathological diagnoses, are provided in *Online Supplementary Table S2*.

KRAS or *NRAS* mutations detected in granulocytes, monocytes and lymphocytes

Cell sorting was conducted on PB samples from three patients, with BM cells also sorted in one of the patients. NGS was carried out on granulocytes or a combined granulocyte/monocyte fraction when cell counts were low, as well as on lymphocytes. Among these patients, one patient had a BM diagnosis of CMUS, and two CCMUS. One patient received decitabine/cedazuridine based on a working diagnosis of CMML, while the other two were untreated for monocytosis at the time of sample collection. *KRAS/NRAS* mutations were detected in granulocytes, monocytes, and lymphocytes in all three patients, with the mutational burden generally lower in lymphocytes than in granulocytes and monocytes. Notably, in one patient with multiple autoimmune diseases, the *KRAS* VAF was significantly higher in PB lymphocytes (41.9%) than in BM $CD3^+$ T cells (14.4%). The clinical information and mutation details are summarized in Table 3.

Treatment and follow-up

The median follow-up duration was 29.7 months (range, 0-180 months). Of the 26 patients with a diagnosis of a myeloid neoplasm, nine were treated with hypomethylating agents, two with ruxolitinib, one with a BET (bromodomain and extraterminal domain) inhibitor, one with hydroxyurea only, and four with supportive care only including erythroid-stimulating agents (aranesp and luspatercept); eight patients did not require treatment for their myeloid neoplasm. Four patients underwent SCT. One patient was lost to follow-up. At last follow-up, none of these patients had progressed to AML. Six patients had died, two of SCT complications, one of unrelated causes, and three of CMML. Of the 15 patients with a diagnosis of CCMUS, one received a hypomethylating agent, two received cytoreduction with hydroxyurea, and the remaining 12 required no treatment for monocytosis or cytopenia. During the follow-up period, one patient progressed to CMML at 2 years, with a newly acquired *ASXL1* mutation and an expansion of the *KRAS* mutation VAF from 2.6% to 32.6%; another three patients progressed to CMML at 18 months, 5 years and 9 years, with *KRAS* remaining as the sole genetic abnormality (2 had persistent high VAF, and one persistent low VAF <10%).

Interestingly, two of these four patients with a CMML diagnosis at follow-up without additional genetic abnormalities did not require treatment.

Of five patients with a diagnosis of CMUS, one progressed to CMML after 1 year, with acquisition of clonal cytogenetic abnormalities involving chromosomes 7 and 21, but NGS showed no additional mutations other than *KRAS*. The patient underwent SCT. The remaining four patients did not require treatment.

Of five CCUS patients, two were diagnosed with MDS at follow-up BM examination (at 3 and 18 months) due to the subsequent demonstration of diagnostic dysplasia, despite no additional molecular genetic abnormalities being detected. Both patients received hypomethylating agent treatment after the MDS diagnosis. The remaining three patients with CCUS who did not progress to MDS or another myeloid neoplasm did not require treatment.

The follow-up information is summarized in Table 2.

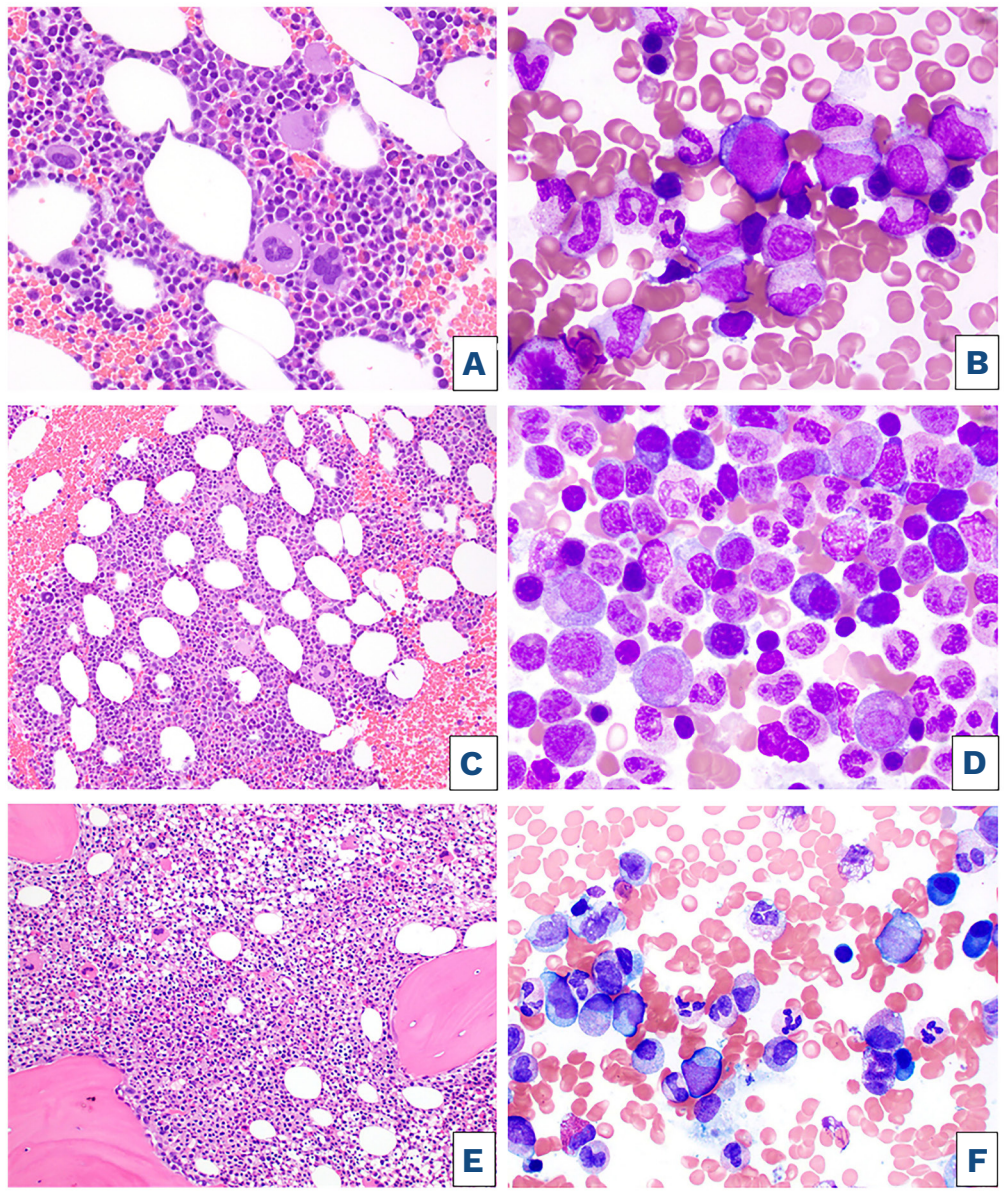

Figure 2. Representative illustrations of bone marrow from patients not meeting diagnostic criteria of a myeloid neoplasm. (A, B) A 49-year-old man had incidental monocytosis for 6 months, a white blood cell count (WBC) of $7.7x10^9$/L with 17% monocytes, normal hemoglobin and platelet levels, and no organomegaly. A *KRAS* mutation was detected at a variant allele frequency (VAF) of 29.5%. Bone marrow biopsy (A), and aspirate (B) showed normal cellularity with unremarkable trilineage hematopoiesis. Follow-up 1 year later showed a similar level of monocytosis and the bone marrow remained unremarkable. This case is classified as clonal monocytosis of undetermined significance (CMUS). (C, D) A 76-year-old female was diagnosed with colon cancer and found to have leukocytosis (WBC, $15.9x10^9$/L) and monocytosis (25%), anemia and thrombocytosis. Bone marrow showed hypercellularity, slight myeloid hyperplasia, and increased polyclonal plasma cells. A *KRAS* mutation was detected at 43.9% VAF. Over a follow-up of 26 months, hemoglobin and platelet levels normalized, but WBC and monocytes (15-17%) remained elevated at a similar level. Because the patient's initial cytopenia was confounded by colon cancer, this case was initially diagnosed as chronic myelomonocytic leukemia, but later reclassified as CMUS. (E, F) A 34-year-old female, with a medical history significant for Rosai-Dorfman disease diagnosed at the age of 18 years, splenectomy at a young age and rheumatoid arthritis, was found to have leukocytosis (WBC, $21.4x10^9$/L) and monocytosis (12%), normal hemoglobin concentration and platelet counts. A *KRAS* mutation was detected with a VAF of 36.5%. Bone marrow biopsy (E) was hypercellular with normal appearing megakaryocytes and the bone marrow aspirate (F), showed no significant dysplasia, with a mild increase in plasma cells. The case was classified as CMUS and likely RAS-associated leukoproliferative disorder in an adult. One year later, she progressed to chronic myelomonocytic leukemia with newly acquired cytogenetic abnormalities involving chromosomes 7 and 21 and a persistent isolated *KRAS* mutation. The patient underwent stem cell transplant, and was alive at last follow-up. (A, C, E) Hematoxylin and eosin, original magnification x400 (A), x200 (C), and x100 (E). (B, D, F) Wright-Giemsa, original magnification x1,000.

Table 3. *KRAS* and *NRAS* mutations in sorted granulocytes, monocytes and lymphocytes.

Patients	Clinical history	BM diagnosis	Mutations	Sample type*	VAF in granulocytes/ monocytes, %	VAF in lymphocytes, %
Patient 1: 79 years/F	Colon cancer and incidental leukocytosis	CCMUS	*KRAS* G12S	PB	43.3	13.2
Patient 2: 42 years/F	Multiple autoimmune diseases**	CCMUS	*KRAS* G13D	PB	47.8	41.9
				BM	50.1/48.5	14.4 ($CD3^+$ T cells)
Patient 3: 48 years/F	Rheumatoid arthritis	CMUS	*NRAS* G60E	PB	21.1/20.0	9.4

*When the samples were collected, patient 2 had received hypomethylating agents, while patients 1 and 3 remained untreated. **Including multiple sclerosis, atypical systemic lupus erythematosus, immune thrombocytopenic purpura, pulmonary fibrosis, and hypothyroidism. BM: bone marrow; VAF: variant allele frequency; F: female; CCMUS: clonal cytopenia and monocytosis of undetermined significance; PB: peripheral blood; CMUS: clonal monocytosis of undetermined significance.

Patients' outcomes

The median overall survival for patients with a diagnosis of a myeloid neoplasm and for those not meeting a diagnosis of myeloid neoplasms were not reached. Kaplan-Meier survival comparison showed a trend toward a shorter median overall survival in patients with a diagnosis of a myeloid neoplasm, but statistical significance was not reached (P=0.087) (Figure 3).

Discussion

CMML is a hematologic malignancy characterized by ineffective hematopoiesis, proliferation of monocytes and increased risk of progression to AML. The median age at diagnosis of CMML is approximately 73 years, and the disease shows a male preponderance.[20,21] CMML is subcategorized into myeloproliferative and myelodysplastic subtypes based on a white blood cell count cutoff of 13x10^9/L. Clonal cytogenetic abnormalities are seen in around 30% of cases,[14,21] and somatic mutations are seen in more than 90% of CMML patients.[22-24] The latter most commonly involve genes implicated in epigenetic/splicing dysregulation, such as *TET2* (~60%), *ASXL1* (~40%), and *SRSF2* (~50%). RAS pathway mutations (*NRAS*, *KRAS*, *CBL*, *PTPN11*, and *NF1*) occur in about 30% of CMML, usually as late events, and the vast majority occur in the context of ancestral mutations.[24-26] They are more commonly observed in myeloproliferative CMML,[2,27-29] linked to a more aggressive clinical course. Isolated RAS mutation(s) in the absence of other co-mutations are rare, and have been reported in only 2% of CMML.[24] It remains unclear whether RAS mutations alone can drive clonal expansion or whether they require preceding mutations to manifest.[25,26]

CCUS is a pre-malignant clonal cytopenia condition defined as persistent cytopenia(s) accompanied by mutations in one or more myeloid disorder-associated genes, which is distinguished from MDS by the absence of morphological dysplasia. In cases of monocytosis, the detection of myeloid disorder-associated mutations in the absence of a morphological diagnosis remains controversial. One study[30] proposed that the presence of mutations in patients with persistent monocytosis (AMC >1x10^9/L) could support a diagnosis of CMML, even in the absence of full diagnostic criteria. However, an unbiased prospective study[31] of community-dwelling individuals showed that monocytosis increased with age, predominantly affecting males, with over 50% exhibiting clonal hematopoiesis, yet only a minority developed *bona fide* CMML. Similarly, a second cohort study[32] reported a 10-year cumulative progression to a myeloid neoplasm of 2.4%, 9.1% and 18.6% for patients with AMC 0.5-1 x10^9/L, AMC ≥1 x10^9/L and monocytosis associated with cytopenia(s), respectively. Both studies[31,32] observed enrichment of age-related clonal hematopoiesis genes and spliceosome mutations, while RAS mutations were rare. To address these precursor lesions, the ICC introduced two new entities, CMUS and CCMUS.

The clinicopathological features of patients in our series appear distinct from the spectrum of CCUS/CCMUS/CMML. These patients had *KRAS* or *NRAS* mutations detected in the absence of ancestral mutations involving epigenetic/splicing pathway mutations, which is considered crucial to initiate CMML pathogenesis biased to myelomonocytic lineage proliferation.[22,23,33] Half of these patients failed to meet the diagnostic criteria of CMML or MDS, and were considered as having the precursor lesions of CMUS, CCMUS, CCUS or CHIP. *DNMT3A* is the most prevalent age-related clonal hematopoiesis mutation and is reported at a low frequency (around 5%) in CMML.[22,23,33] We retained three patients with a low level of *DNMT3A* (VAF ≤5%) in our cohort, based on the assumption that *DNMT3A* mutations at such low levels are unlikely to contribute to CMML leukemogenesis. Surprisingly, all three of these patients fulfilled the diagnostic criteria of CMML. In contrast to typical CMML or CMUS/

CCMUS, which usually affect older males with a median age of 73 years, our cohort showed a female predominance and a younger median age at onset of 65 years. Furthermore, unlike the typical proliferative phenotype associated with RAS mutations in CMML,[24] less than 20% of our patients had a white blood cell count ≥13x10^9/L. Among those diagnosed with CMML, the majority had the myelodysplastic subtype of CMML rather than myeloproliferative subtype (83% *vs*. 17%), despite most RAS mutations being detected at high VAF. This finding contrasts with the data from a large cohort of 832 CMML patients, in whom RAS mutations were more frequently observed in myeloproliferative CMML (61%) than in myelodysplastic CMML (39%).[24] Despite the infrequency of leukocytosis, splenomegaly was present in nearly two-thirds of our patients. Strikingly, nearly 40% of the patients presented with immune-related disorders – an incidence notably higher than the 10-20% reported in MDS or CMML.[34,35] In fact, several of our patients had clinical manifestations reminiscent of RALD, but with symptom onset in adulthood.

In adult CMML and MDS, *NRAS* mutations are more common than *KRAS*, with G12 codon alterations accounting for 50-70% of all *NRAS*-mutated cases. In contrast, *KRAS* mutations are less frequent and more heterogeneous, with codon G12 involved in only 25-40% of cases.[2] The RAS mutation profiles observed in our patients were strikingly atypical for CMML or MDS; instead, they resembled those seen in RALD. Consistent with RALD,[36] our cohort of patients showed a preponderance of *KRAS* mutations (92%), most with a high VAF, and 87% affecting codons of G12 or G13. In three patients diagnosed with CMUS or CCMUS, PB and/or BM samples revealed *KRAS/NRAS* mutations not only in granulocytes and monocytes but also in lymphocytes. This multilineage involvement, together with the mutational patterns, supports the possibility of an adult-onset acquired RASopathy, at least in a subset of these patients. While we cannot rule out the possibility of germline mutations, especially in patients with *KRAS/NRAS* mutations detected with a VAF close to 50%, none of our patients exhibited clinical features typically associated with congenital RASopathies characterized by multisystem developmental disorders. Furthermore, it is important to note that germline *NRAS/KRAS* mutations,[37] which may contribute to a small subset of RASopathies, rarely affect G12/G13 of *NRAS* and *KRAS*.

It is acknowledged that the distinction of CMML/MDS from their precursor entities can be extremely challenging. BM cytomorphology might be altered due to other comorbidities, especially underlying immune-related disorders. In real-world clinical practice, the interpretation of BM findings could be biased by the detection of RAS mutations. A diagnosis of CMML in this setting requires other criteria, including supportive findings of BM morphology. Flow cytometry immunophenotyping assessing CD34$^+$ myeloid precursors[13] and monocyte partition[38,39] have been shown to have great value in the diagnosis of CMML. Although flow cytometry was only performed on a minor subset of cases, CD34$^+$ myeloid precursors were immunophenotypically normal in six of seven and classic monocytes <94% in five of seven cases of CMUS/CCMUS, highlighting the potential utility of this ancillary test in this setting. Progression to CMML was documented in several patients with CMUS/CCMUS/CCUS, in some cases coinciding with the acquisition of additional molecular genetic abnormalities and in some cases due to the development of dysplastic morphology in the BM. Notably, the mutation profile and other clinicopathological features were not significantly different between patients with a diagnosis of CMML/MDS and CMUS/CCMUS/CCUS/CHIP, except for a younger age in the latter group. Furthermore, more than half of the patients, including a significant subset with a diagnosis of CMML and some deemed to have CMML progression based on BM dysplasia, did not require treatment. Remarkably, none of our patients progressed to AML during the follow-up period. This is highly atypical for CMML, in which patients usually have a median overall survival of 30-40 months, a leukemia-free survival of 28-36 months, and an AML progression rate of 15-30%.[20,21,24,40] In a large cohort of CMML patients,[24] RAS pathway mutations were shown to be associated with an inferior overall survival (35.5 months) and leukemia-free survival (28.7 months), compared to the outcomes of CMML patients without these mutations.

The clinical features and mutation profiles strongly suggest that these entities form a continuum in the context of isolated RAS mutations. In the VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome, BM speciments[41] typically show hypercellularity, with granu-

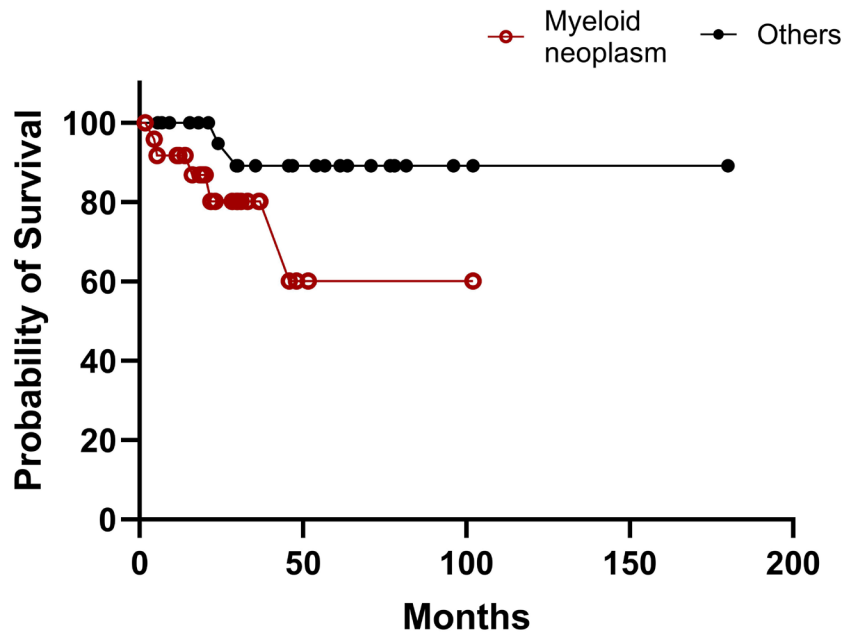

Figure 3. Kaplan-Meier overall survival comparison of patients with a diagnosis of a myeloid neoplasm *versus* patients who did not meet the diagnostic criteria for a myeloid neoplasm. The median overall survival was not reached for either group. The patients with a diagnosis of a myeloid neoplasm showed a trend toward inferior survival but this did not reach statistical significance (*P*=0.087).

locytic hyperplasia, and vacuolated myeloid and erythroid precursors. Varying degrees of dysplasia can be observed across hematopoietic lineages, and some cases may exhibit increased reticulin fibrosis, likely driven by inflammation. However, overt dysplasia, increases in blasts and acquisition of genetic abnormalities, are features of MDS in the setting of VEXAS syndrome. We speculate that disease progression in patients with isolated RAS mutations may follow a similar model, akin to the somatic *UBA1* mutation that serves as a foundational event in VEXAS syndrome, with subsequent genetic alterations contributing to the development of MDS.[42]

In summary, isolated somatic RAS mutations are rarely detected in adult patients with monocytosis, and/or cytopenia(s). Notably, at least half of these patients do not meet the diagnostic criteria for a myeloid neoplasm. The clinical features, including a high incidence of immune-related disorders, splenomegaly, young age, and female predominance, alongside the distinct *KRAS/NRAS* mutation profile, detected not only in granulocytes and monocytes but also in lymphocytes, strongly suggest that, at least in some cases, these patients may have an adult-onset RASopathy. The accumulation of further genetic changes likely drives the progression from monocytosis or cytopenia to more overt myeloid neoplasms such as CMML. These findings challenge current diagnostic paradigms, emphasizing the need for more defined criteria, similar to the VEXAS disease model, before the initiation of disease-modifying therapies. Such cases should be considered separately in future clinical guidelines and clinical trial design.

Disclosures

No conflicts of interest to disclose.

Contributions

This is a Bone Marrow Pathology Group (BMPG) study. SAW, CYO, RPH, AO, GM-B, MMP and KKR conceived the study. CYO, HKT, PDB, NS, MC, AB, WT, OW, JTG, RCL, DAA, RPH and KKR collected and reviewed the cases. WT, SAW and NL performed experiments. SAW, AO, DAA, RPH, KKR, KF, JTG, OW, WT, EDH and CEB-R carried out the microscopy reviews. SAW wrote the original draft. All authors reviewed and edited the manuscript.

Data-sharing statement

Data are available from the corresponding author upon reasonable request.

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