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

Running title: Isolated RAS mutations in adults

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This is a bone marrow pathology group (BMPG) study

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Case collection and review: CYO, HKT, PDB, NS, MC, AB, WT, OW, JTG, RCL, DA, RPH, KKR

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Conflict of interest

All authors have no relevant conflict of interest to disclose. This is a Bone Marrow Pathology Group study

Data sharing

Data are available from the corresponding author upon reasonable request

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 typical chronic myelomonocytic leukemia (CMML)/myelodysplastic syndrome (MDS), these patients exhibited distinctive clinical features, including a younger age (65 years; range, 29–92), 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 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.

Keywords: KRAS, NRAS, monocytosis, cytopenia, CMUS, CCMUS, CCUS, CHIP, RASopathy, RALD

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* the most frequent, followed by *KRAS*, whereas *HRAS* mutations are rare. The prevalence of *N/KRAS* mutations varies across different types of myeloid malignancies, and is more common in pediatric than adult diseases[1,2], and with a notably higher incidence in chronic myelomonocytic leukemia (CMML) and juvenile myelomonocytic leukemia (JMML)(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 (CH), 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 DTA (*DNMT3A, TET2, ASXL1)* mutations or, less frequently,*TP53, JAK2, SF3B1, CBL, SRSF2, PPM1D,* and *BCOR* mutations[6,7]. Whole-exome sequencing (WES) data from 200,453 United Kingdom Biobank participants reported 0.02% *KRAS* and 0.01% *NRAS* mutations and, 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 median reported 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 *K/NRAS* mutations in adult patients with cytopenia(s) and/or persistent monocytosis raising concern for 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 either clonal cytopenia of uncertain significance (CCUS), or within 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 *K/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 clinicopathologic evaluation, including determination of the clinical presentation, bone marrow and peripheral blood 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 *K/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. These 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 bone marrow (BM) and/or peripheral blood (PB) via myeloid gene-targeted NGS panels over 5-7 years (2017–2024, 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 per the International MDS Working Group (IWGM-MDS) 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 BMs 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). 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 ICC[10] and The 5th edition of World Health Organization (WHO 5th) [15]criteria. A CMML diagnosis requires PB monocytes ≥10%; and absolute monocyte count ≥0.5×10□/L by both. Additionally, WHO 5th requires evidence of clonality and dysplasia[15],whereas ICC requires abnormal BM findings (hypercellularity with myeloid/monocytic proliferation, without features of AML, myeloproliferative neoplasm (MPN), or other causes of monocytosis), along with cytopenia[16]. Per 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 CCUS[17], and cases no cytopenia or dysplasia as clonal hematopoiesis of indeterminate potential (CHIP).

Cytogenetics

G-banded metaphase cells from unstimulated BM aspirates were analyzed using standard techniques. Twenty metaphases were examined, and results reported per the International System for Human Cytogenetic Nomenclature.

Targeted next-generation sequencing (NGS)

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 per Human Genome Variation Society guidelines[19]. *NRAS* and *KRAS* mutation VAFs and codons were recorded.

Treatment and follow-up

Treatment and outcome data were collected from medical records. Therapies were categorized as hydroxyurea, hypomethylating agents (HMA), JAK inhibitors, other small-molecule inhibitors, observation/supportive care, or allogeneic 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's exact/Chi-square 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 Kaplan–Meier and compared by log-rank test. Analyses were done in GraphPad Prism (San Diego, CA), with significance at $p < 0.05$ (two-sided).

RESULTS

Patients

A total of 52 patients were identified from 7 medical centers. All were adult patients with a median age of 65 years (29-92), showing a female predominance (female: male ratio 31:21, 1.6). 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 (ITP), 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. 33/51 (65%) had splenomegaly, and 4 also had hepatomegaly; one patient had had a splenectomy at a young age. Lactate dehydrogenase (LDH) was elevated in 3/39 (8%) patients. One patient had monoclonal gammopathy of undetermined significance (MGUS), and two patients had smoldering myeloma; in these three cases, the variant allele frequencies (VAFs) of the *RAS*

mutations markedly exceeded what would be expected if derived from neoplastic plasma cells. In 5/52 patients, the clinical constellation of symptoms had raised the possibility of RALD, including some with symptoms reminiscent of autoimmune-lymphoproliferative disorders (ALPs) in the medical record, despite disease onset in adulthood.

The clinical and pathological features are summarized in Table 1.

Cytogenetics and Mutations

Karyotype information was available in all 52 patients at the time of initial BM examination. A normal karyotype was reported in 50, with the remaining 2 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 4, with no patients demonstrating >1 concurrent *RAS* mutation. The median VAF was 39.0% (2.6-53.0%), with 33 (64%) patients having a VAF ≥30%, 12 with VAF 10-30% and 7 with VAF <10%. Three patients had low levels of *DNMT3A* co-mutations, ≤5%. Of the 5 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, 4 at A146 and 2 at other loci (Q61K, L19F). For 4 patients with *NRAS* mutations, 3 occurred at codon G12 and one at G60E. In total, 45/52 (87%) patients had *N/KRAS* mutations involving codon G12 or G13. The mutation patterns are illustrated in Figure 1A.

Morphologic 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%) with relative (≥10%) and absolute monocytosis (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 (mostly dysmegakaryopoiesis) in 8, and borderline dysplasia (around 10% of each lineage) in 3. Three had increased BM blasts (including myeloblasts, monoblasts and promonocytes) of 5-9% and 2 had blasts ≥10%, 12/24 (50%) had myelofibrosis grade MF1 and 1/24 (4%) had MF2. The median percentage of BM monocytes was 5% (range 1-21%). Twenty-four (46%) met the diagnostic criteria for CMML, of which 20 were the myelodysplastic subtype (MD-CMML) including 5 with AMC between 0.5 to 1.0 x10^9/L, and 4 were the myeloproliferative subtype (MP-CMML) (Supplementary Table 1). One was classified as MDS/MPN, NOS, with leukocytosis, neutrophilia and anemia; and one MDS with low blasts/NOS and unilineage dysplasia. Among the patients diagnosed with myeloid neoplasms, the median VAF of *K/NRAS* was 41.4% (4.7 to 48.9%), and all 3 cases with a low-level of *DNMT3A* belonged to this group of patients. Flow cytometry immunophenotype data was available in three patients, all demonstrating abnormal CD34+ myeloblasts (bright CD117, with or without decreased CD38, increased CD123, altered CD4) and 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 bone marrow 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 versus 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 (CBC) data including hemoglobin (Hb), white blood cell count (WBC), absolute neutrophil count (ANC), the percentage of monocytes or AMC were not statistically different between the two groups (Supplemental Table 1). 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 MF-1 or MF-2 fibrosis, 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 non-MN cases). Flow cytometry study was performed in 3 CCMUS, 3 CMUS and one CCUS. The CD34+ myeloid precursors were completely normal in 6 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 *K/NRAS* VAF did not differ between patients with or without a diagnosis of a myeloid neoplasm, 35.8% (2.6-53.0%) vs 41.4% (4.7 to 48.9%) (p=0.376) (Figure 1B). The mutation patterns of *KRAS* were also not significantly different between the two groups (p=0.835). Of the 4 patients with *NRAS* mutations, two patients were diagnosed with CMML, and two patients were considered to have CMUS. Of the 7 patients with a VAF <10%, all involving *KRAS*, 3 were CMML, 2 CCMUS, 1 CCUS, and 1 CHIP. The diagnostic distribution was not significantly different from cases with higher VAFs. Details of *KRAS/NRAS* mutations, including involved codons, VAFs, and corresponding clinicopathological diagnoses, are provided in Supplemental Table 2.

K/NRAS **Mutations detected in Granulocytes, Monocytes and Lymphocytes**

Cell sorting was conducted on peripheral blood samples from three patients, with bone marrow 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. *K/NRAS* mutations were detected in granulocytes, monocytes, and lymphocytes in all three patients, with mutational burden generally lower in lymphocytes compared to 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 (0-180 months). Of the 26 patients with a diagnosis of a myeloid neoplasm, 9 were treated with HMA, 2 with ruxolitinib, 1 with BET (bromodomain and extraterminal domain) inhibitor, 1 with hydroxyurea only, and 4 with supportive care only including erythroid-stimulating agents (aranesp and luspatercept); 8 patients did not require treatment for their myeloid neoplasm. 4 patients received stem cell transplant (SCT). 1 patient was lost to follow-up. At last follow-up, none of these patients had progressed to AML. Six patients had died, 2 of SCT complications, 1 of unrelated causes, and 3 of CMML.

Of the 15 patients with a diagnosis of CCMUS, one received HMA, 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 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 4 patients with a CMML diagnosis at follow-up without additional genetic abnormalities did not require treatment.

Of 5 patients with a diagnosis of CMUS, one progressed to CMML after one year, with acquisition of clonal cytogenetic abnormalities involving chromosome 7 and 21, but NGS showed no additional mutations other than *KRAS*. The patient underwent SCT. The remaining 4 patients did not require treatment.

Of 5 CCUS patients, two patients were diagnosed with MDS at follow-up bone marrow 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 HMA treatment after the MDS diagnosis. The remaining 3 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.

Patient outcome

The median overall survival (OS) 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 OS in patients with a diagnosis of a myeloid neoplasm, but statistical significance was not reached (p=0.087). (Figure 3)

DISCUSSION

Chronic myelomonocytic leukemia (CMML) is a hematological malignancy characterized by ineffective hematopoiesis, proliferation of monocytes and increased risk for acute myeloid leukemia (AML) progression. The median age at CMML diagnosis is approximately 73 years, and the disease shows a male preponderance[20,21]. CMML is further subcategorized as

“myeloproliferative” (MP-CMML) and “myelodysplastic” (MD-CMML) subtypes based on a white blood cell count cutoff of 13 x 10^9/L. Clonal cytogenetic abnormalities are seen in around 30% of cases[14,21], and somatic mutations are seen in >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 vast majority occur in the context of ancestral mutations[24-26]. They are more commonly observed in MP-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 reported in only 2% of CMML[24]. It remains unclear whether *RAS* mutations alone can drive clonal expansion or require preceding mutations to manifest[25,26].

Clonal cytopenia of undetermined significance (CCUS) is a pre-malignant clonal cytopenia(s) 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 morphologic dysplasia. In cases of monocytosis, the detection of myeloid disorder-associated mutations in the absence of morphological diagnosis remains controversial. One study[30] proposed that the presence of mutations in patients with persistent monocytosis (absolute monocyte count-AMC >1 x10□/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, and over 50% exhibiting clonal hematopoiesis (CH), 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 CH genes and spliceosome mutations, while RAS mutations were rare. To address these precursor lesions, the ICC introduced two new entities: clonal monocytosis of

undetermined significance (CMUS) and clonal cytopenia and monocytosis of undetermined significance (CCMUS).

The clinicopathologic features of patients in this series appear distinct from the spectrum of CCUS/CCMUS/CMML. These patients had *K/NRAS* mutations detected in the absence of ancestral mutations involving in epigenetic/splicing pathway mutations, which is considered crucial to initiate CMML pathogenesis biased to myelomonocytic lineage proliferation[33,22,23]. Half of these patients failed to meet the diagnostic criteria of CMML or MDS, and were considered as the precursor lesions of CMUS, CCMUS, CCUS or CHIP. *DNMT3A* is the most prevalent age-related clonal hematopoiesis (CH) mutation and is reported at a low frequency (around 5%) in CMML.[33,22,23] We retained 3 patients with a low level of *DNMT3A* (VAF≤ 5%) in the cohort, based on the assumption that *DNMT3A* mutation at such low levels is 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 of 65 years. Furthermore, unlike the typical proliferative phenotype associated with *RAS* mutations in CMML[24], less than 20% of our patients had a WBC ≥ $13x10^9$/L. Among those diagnosed with CMML, the majority had MD-CMML rather than MP-CMML (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, where RAS mutations were more frequently observed in MP-CMML (61%) compared to MD-CMML (39%)[24]. Despite the infrequency of a leukocytosis, splenomegaly was present in nearly two thirds of the patients. Strikingly, nearly 40% of 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 RAS-associated autoimmune leukoproliferative disorder (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, resembled those seen in RAS-associated autoimmune leukoproliferative disorder (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, peripheral blood and/or bone marrow 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, at the genetic level. While we cannot rule out the possibility of germline mutations, especially in patients with *K/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 the 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 finding of bone marrow 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 6/7 and classic monocytes <94% in 5/7 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, some coincided with the acquisition of additional molecular genetic abnormalities, while some were due to the development of dysplastic morphology in the bone marrow. Notably, the mutation profile and other clinicopathologic 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 bone marrow dysplasia, did not require treatment. Remarkably, none of our patients progressed to AML during the follow-up period. This is highly atypical for CMML, where patients usually have a median overall survival (OS) of 30–40 months, a leukemia-free survival (LFS) of 28–36 months, and an AML progression rate of 15–30%[20,21,24,40]. In the large cohort of CMML patients[24], RAS pathway mutations have been shown to associate with an inferior OS (35.5 months) and LFS (28.7 months), compared to CMML patients without these mutations.

The clinical features and mutation profile are highly suggesting that these entities form a continuum in the context of isolated *RAS* mutations. In VEXAS syndrome, bone marrows[41] typically show hypercellularity, with granulocytic 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, increase in blasts and acquisition of genetic abnormalities, are features of MDS in the setting of VEXAS. 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, 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 *K/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 like 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.

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Table 1, Patient clinical features, peripheral blood data, and mutation information

Patients (n=52)	
Age, years, median (range)	65 years (29-92)
• ≤50 years	11 (21%)
Gender (Male:Female)	21:31
Autoimmune/or immune dysregulation	20 (39%)
• RALD	5
Histiocytic proliferation	2 (Rosai-Dorfman)
Splenomegaly	33/51 (65%) (1 splenectomy)
Hepatomegaly	4/52 (8%) (all 4 also had splenomegaly)
White blood cell count (WBC) ($x10^9$/L)	4.5 (1.1-38.8)
➢ WBC≥13 $x10^9$/L	10 (19%)
Peripheral blood Monocyte %	24.0% (4.8-70.0)
➢ ≥10%	48 (92%)
➢ Absolute monocyte count (AMC)	1.43 (0.15-6.98)
○ AMC ≥1 $x10^9$/L	34 (65%)
○ AMC 0.5-1 $x10^9$/L	11 (21%)
○ AMC<0.5 $x10^9$/L	7 (14%)
Hemoglobin (g/dL)	12.1(7.0-16.6)
Platelets ($x10^9$/L)	145 (23-652)
Bone marrow cellularity	80% (5-100)
Bone marrow monocyte count	5% (0-21)
Bone marrow blasts/promonocytes	2% (0-16)
○ ≥5%	6 (12%)
Gene involved	48 *KRAS* (92%), 4 *NRAS* (8%)
VAF	39.0% (2.6-53.0)
• VAF >=30	33 (63%)
• VAF 10-30	12 (23%)
• VAF <10	7 (14%)

Abbreviations: RALD: ras-associated autoimmune leukoproliferative disorder; VAF: Variant Allele Frequency.

Table 2. Patients: Diagnosis, Classifications, Treatment and Follow-up

Diagnosis and Classification	Patients (n)	Treatment and Progression
Chronic myelomonocytic leukemia (CMML)	24	9 treated with hypomethylating agent (HMA); 2 Ruxolitinib; 1 small molecule inhibitor; 1 with hydroxyurea only; 4 supportive care; 8 no requirement for treatment, 1 no information. 4 followed by stem cell transplant (SCT) No AML progression. 3 died of CMML, 2 SCT-related complications, and 1 unrelated cause
o CMML1-MD o CMML2-MD	18 2	
o CMML1-MP	4	
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	1 HMA, 2 hydroxyurea, 12 no treatment requirement. 4 progressed to CMML at 1.5, 2, 5 and 9 years, respectively
Clonal monocytosis of undetermined significance (CMUS)	5	One progressed to CMML after 1 year, and other 4 did not require treatment
Clonal cytopenia of undetermined significance (CCUS)	5	2 were diagnosed with MDS in <1 year and at 1.5 years, respectively, treated with HMA. 3 did not require treatment
Clonal hematopoiesis of indeterminate potential	1	No treatment or progression

MD: myelodysplastic subtype; MP: myeloproliferative subtype

Table 3. *KRAS* and *NRAS* mutations in Sorted Granulocytes, Monocytes and Lymphocytes

Patients	**Clinical History**	**BM Diagnosis**	**Mutations**	**Sample type***	**VAF of Granulocytes/ Monocytes**	**VAF of Lymphocytes**
Patient 1: 79 y/F	Colon cancer and incidental leukocytosis	CCMUS	*KRAS* G12S	PB	43.30%	13.20%
Patient 2: 42y/F	Multiple autoimmune diseases**	CCMUS	*KRAS* G13D	PB	47.80%	41.90%
				BM	50.1%/48.5%	14.40% (CD3+ T cells)
Patient 3: 48 y/F	Rheumatoid arthritis	CMUS	*NRAS* G60E	PB	21.1%/20%	9.40%

*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 (SLE), immune thrombocytopenic purpura (ITP), pulmonary fibrosis, hypothyroidism. CMUS: Clonal Monocytosis of Undetermined Significance; CCMUS: Clonal Cytopenia and Monocytosis of Undetermined Significance. PB: peripheral blood; BM: bone marrow

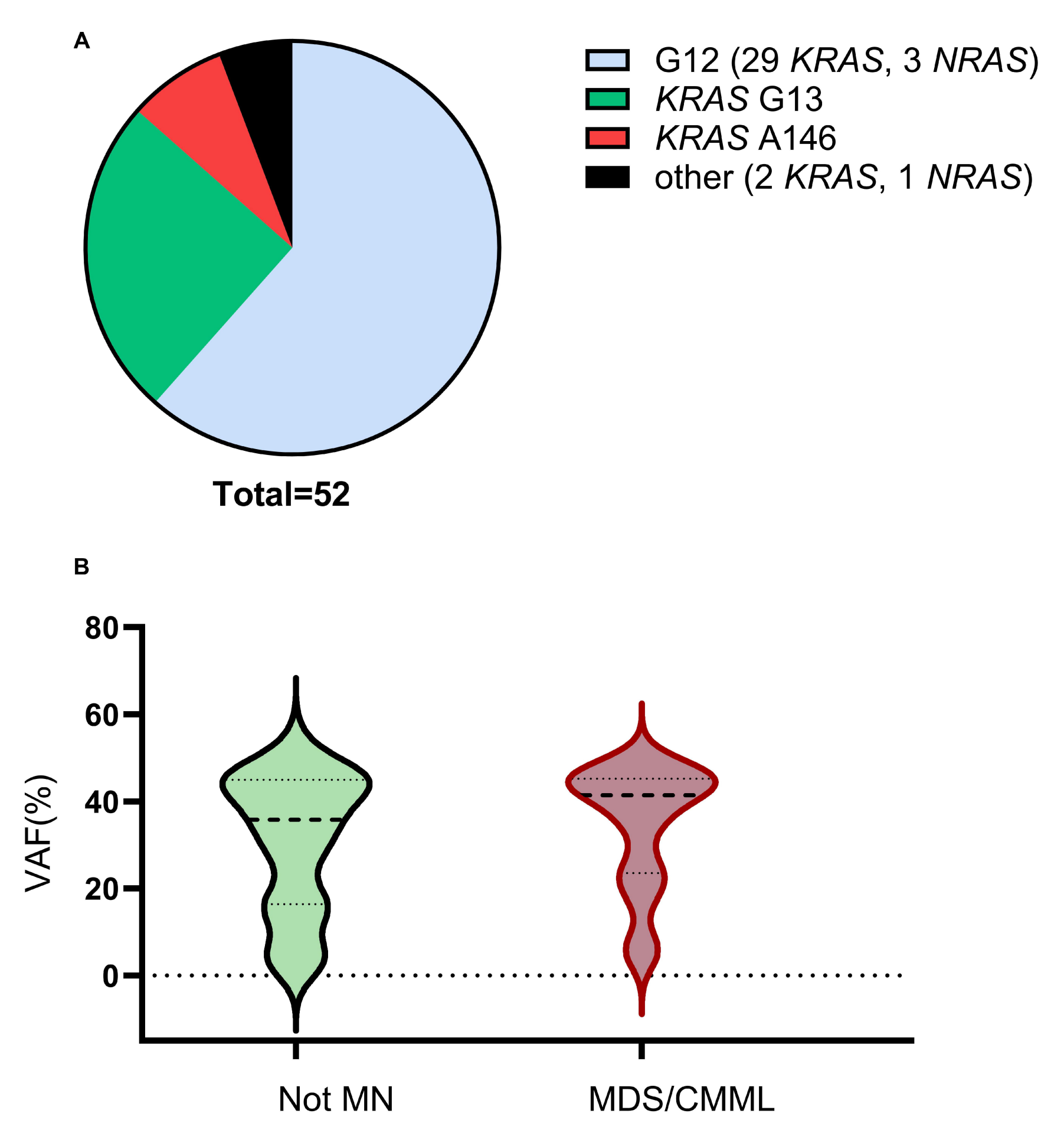
FIGURES AND LEGENDS:

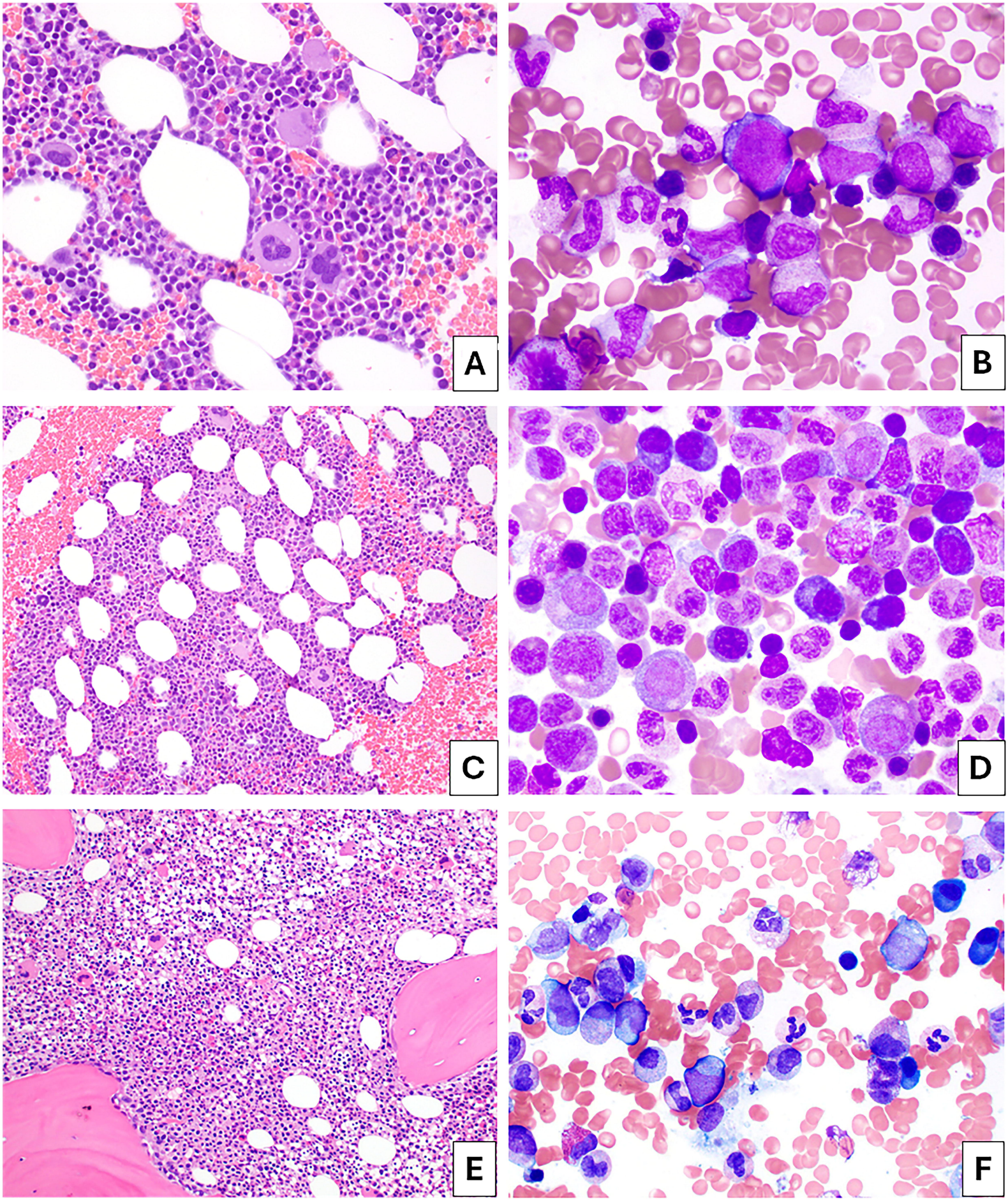
Figure 1. *KRAS/NRAS* mutation characteristics: A. *KRAS* (n=48) and *NRAS* (n=4) by mutation codons. B. The median variant allele frequency (VAF) was not significantly different between patients with or without a diagnosis of myeloid neoplasm (MN)

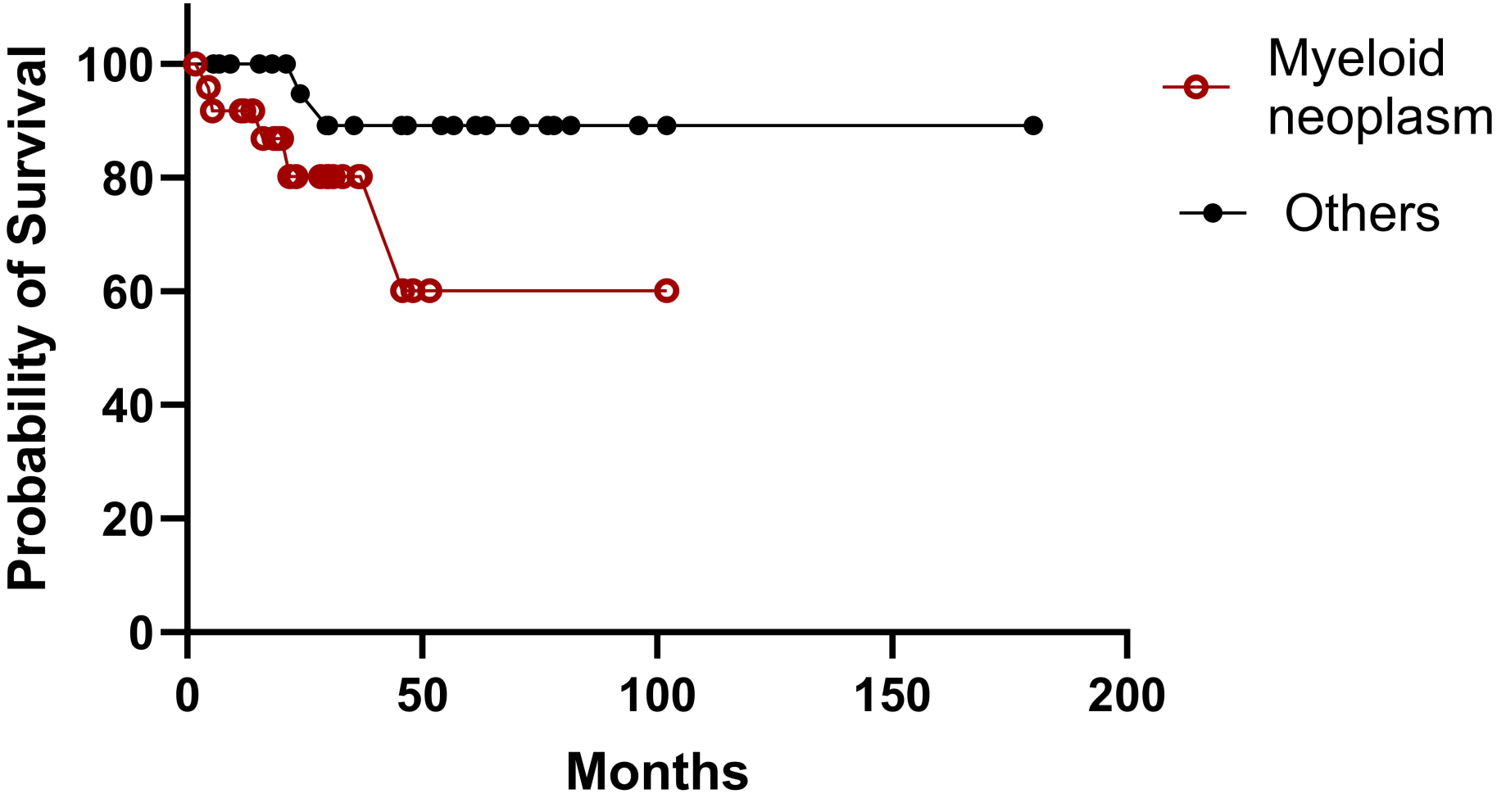
Figure 2. Representative bone marrow illustrations of patients not meeting diagnostic criteria of a myeloid neoplasm. A and B: a 49-year-old man with incidental monocytosis for 6 months, WBC 7.7x10^9/L with 17% monocytes, normal hemoglobin and platelets, no organomegaly. *KRAS* mutation was detected 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 one year later showed similar level of monocytosis, and bone marrow remained unremarkable. This case is classified as clonal monocytosis of undetermined significance (CMUS). C and D. a 76-year-old female was diagnosed with colon cancer and found to have leukocytosis (15.9x10^9/L) and monocytosis (25%), anemia and thrombocytosis. Bone marrow biopsy showed a hypercellularity, slight myeloid hyperplasia, and increased polyclonal plasma cells. *KRAS* mutation was detected at 43.9% VAF. Over a follow-up of 26 months, hemoglobin and platelets 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 with CMML, but reclassified as CMUS. E and F, a 34-year-old female, past medical history was significant for Rosai-Dorfman disease diagnosed at age 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 and platelets. *KRAS* mutation was detected with a VAF of 36.5%. Bone marrow biopsy was hypercellular with normal appearing megakaryocytes (E), BM aspirate (F) showed no significant dysplasia, with mild increase in plasma cells. The case was classified as CMUS and likely RAS-associated leukoproliferative disorder (RALD) in an adult. One year later, she progressed to chronic myelomonocytic leukemia with newly acquired cytogenetic abnormalities involving chromosomes 7 and 21 and persistent isolated *KRAS* mutation. The patient underwent stem cell transplant, and was alive at last follow-up.

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)


A
G12 (29 KRAS, 3 NRAS)
KRAS G13
KRAS A146
other (2 KRAS, 1 NRAS)
Total=52
B
80
60
40
20
0
VAF(%)
Not MN
MDS/CMML


A
B
C
D
E
F


Probability of Survival
100
80
60
40
20
0
0
50
100
150
200
Months
Myeloid neoplasm
Others

Supplemental material

NGS was performed at the respective institutions, with panels comprising 68 to 648 genes. A total of 38 genes were shared across all panels, as shown below.

ASXL1, BCOR, BCORL1, BRAF, CALR, CBL, CEBPA, CSF3R, DNMT3A, ETV6, EZH2, FLT3, GATA1, GATA2, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NF1, NPM1, NRAS, PHF6, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SH2B3, SRSF2, STAG2, STAT3, TET2, TP53, U2AF1, WT1, and ZRSR2

Supplemental Table 1: Comparison of patients diagnosed with a myeloid neoplasm and patients with a diagnosis of precursor lesions

	Myeloid Neoplasm (n=26)	Precursor entities (CCUS, CMUS, CCMUS) (n=26)	p
Age (median, range)	67 (25-92)	60(29-82)	0.007
Gender (Male:Female)	8:18	13:13	0.258
White blood cell count x10^9/L (median, range)	5.25(2.2-38.8)	4.35(1.1-21.40)	0.323
Absolute monocyte count x10^9/L (median, range)	1.55(0.21-6.98)	1.28(0.15-3.98)	0.106
• AMC<0.5 and or <10%	• 2	• 6	
• AMC 0.5-1.0 and >10%	• 5	• 5	
• AMC≥1.0	• 19	• 15	
Hemoglobin g/dL, (median, range)	11.3(7.0-15.9)	12.3(7.7-16.6)	0.131
Platelets x10^9/L (median, range)	151 (23-564)	120(26-652)	0.979
Organomegaly	18/26	15/25	0.565
Immune-related disorders	9/26	11/26	0.776
Bone marrow cellularity (5%)	80(50-100)	80(5-100)	0.113
Bone marrow monocytes (5%)	5.0(1-21)	5.5(0-16)	0.735
Bone Marrow blasts/promonocytes	2.0 (0-16)	1.5(0-5.0)	0.109
Dysplasia	Multiplineage dysplasia in 15, unilineage in 8, and no significant dysplasia in 3	Borderline/mild in one lineage in 3 cases	
Bone Marrow fibrosis MF1 and MF2	12/24 MF1, 1/24, MF2	6/12 MF1, and 1/12 MF2	
RAS VAF (%) (median, range)	41.4(4.7-48.9)	35.8(2.6-53)	0.376

Supplemental Table 2. NRAS/KRAS Mutation information and Corresponding Diagnoses

		KRAS/NRAS	Mutation codons	VAF (%)
1	CCMUS. clinical RALD	KRAS	G13D	46.9
2	CMUS	KRAS	G12R	29.5
3	CMUS, clinical RALD	KRAS	G13C	36.5
4	CCUS	KRAS	G13C	15.3
5	CCMUS	KRAS	G12V	37.9
6	CMUS	NRAS	G60E	14.1
7	CCMUS	KRAS	A146P	46.6
8	CCMUS	KRAS	G12S	43.9
9	CMML-1 MP	NRAS	G12D	48.9
10	CMML-1 MD	KRAS	G12D	42.2
11	CCMUS	KRAS	G12D	2.6
12	CCMUS (0.5-1 $x10^9$/L)	KRAS	A146T	17.7
13	CCMUS	KRAS	G12D	7.8
14	CCUS	KRAS	G13C	53.0
15	CMML-1 MD	KRAS	G12A	4.7
16	CMML-1 MD-oligomonocytic	NRAS	G12D	43.7
17	CMML-1 MD	KRAS	G13C	8.4
18	CMUS	NRAS	G12D	29.6
19	CMML-1 MP	KRAS	G12R	20.3
20	CMML-1 MD	KRAS	G12R	4.8
21	CMML-1 MD-oligomonocytic	KRAS	G12D	26.0
22	CMML-1 MD	KRAS	G13D	47.0
23	CMML-1 MD	KRAS	G12D	35.7
24	CMML-oligomonocytic	KRAS	A146T	22.3
25	CHIP	KRAS	G13D	4.1
26	CCMUS (0.5-1 $x10^9$/L)	KRAS	G12D	16.7
27	CMML-2 MD	KRAS	G13D	45.2
28	MDS	KRAS	A146V	17.2
29	CMML-1 MD	KRAS	G13D	46.6
30	CMML-1 MD	KRAS	G12D	40.8
31	CMML-oligomonocytic	KRAS	G13C	45.2
32	MDS/MPN-NOS	KRAS	G12R	44.0

33	CMML-1 MD	KRAS	G12D	33.1
34	CMML-1 MD	KRAS	G12D	40
35	CMML-1 MD	KRAS	G12D	45.0
36	CMML-2 MD	KRAS	G12D	47.4
37	CMML-1 MP	KRAS	G12D	23.9
38	CCMUS	KRAS	G12D	40
39	CMML-1 MP	KRAS	G12D	47
40	CCMUS (0.5-1 x10^9/L)	KRAS	G13D	33
41	CCMUS	KRAS	G12D	47
42	CCMUS (0.5-1 x10^9/L AMC)	KRAS	G12D	45
43	CCMUS, clinical RALD,	KRAS	Q61K	41
44	CMML-1 MD	KRAS	L19F	42
45	CMML-1 MD	KRAS	G12C	43
46	CCUS, clinical RALD	KRAS	G12D	43
47	CCUS	KRAS	G13D	25
48	CMML-1 oligo	KRAS	G12V	37
49	CCUS	KRAS	G12S	3
50	CCMUS	KRAS	G12D	46
51	CCMUS (0.5-1 x10^9/L)	KRAS	G13D	35
52	CCMUS, clinical RALD	KRAS	G12D	45

Abbreviation: CMUS: clonal monocytosis of undetermined significance; CCMUS: clonal cytopenia and monocytosis of undetermined significance; CCUS: clonal cytopenia of uncertain significance; MDS/MPN: myelodysplastic/myeloproliferative neoplasms; RALD: RAS-associated autoimmune leukoproliferative disorder; CHIP: clonal hematopoiesis of indeterminate potential; CMML: chronic myelomonocytic leukemia; MD: myelodysplastic subtype, MP: myeloproliferative subtype.