TO THE EDITOR:

In a multi-institutional cohort of myeloid sarcomas, NFE2 mutation prevalence is lower than previously reported

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Myeloid sarcomas are extramedullary accumulations of blasts that share many morphologic, immunophenotypic, and molecular features with intramedullary acute myeloid leukemia (AML). However, factors contributing to the extramedullary localization of leukemic blasts in myeloid sarcomas remain incompletely understood.

Recent reports have suggested that isolated myeloid sarcomas are often characterized by mutations in the transcription factor NFE21 and that altered NFE2 activity predisposes to myeloid sarcoma in murine models.2 However, these reports are based on relatively limited case numbers, with NFE2 mutations collectively identified in 5 of 19 human myeloid sarcomas. We previously performed targeted sequencing on a large cohort of myeloid sarcomas (n = 24) and showed discordant mutational profiles with concurrent bone marrow biopsies; however, the mutational status of NFE2 was not investigated.3 Here, we characterize the NFE2 locus in 38 myeloid sarcomas, including a subset of the previously reported cases, as well as additional cases.

Sequencing of 38 myeloid sarcomas, including 9 isolated myeloid sarcomas (without a history of antecedent or concomitant myeloid neoplasia) did not reveal any somatic variants in NFE2. The true prevalence of NFE2 mutations in myeloid sarcoma is difficult to precisely quantify because of the limited number of cases evaluated in this and the prior studies, but our data indicate that it is lower than previously suggested. Clinicopathologic characteristics and sequencing details are shown in Table 1. Sequencing of all coding regions of NFE2 was performed via a targeted next-generation sequencing panel, whole-exome sequencing, and/or Sanger sequencing (Table 1; supplemental Methods). The limit of detection (LOD) for variants in NFE2 is 2% to 5% in most cases; a small number of cases have a higher LOD because of sample quality and technical limitations. All samples had high tumor fraction (>50% of cellularity in all cases), negating the effect of higher LOD in these selected cases.

We considered potential reasons for the discrepancy between our results and the previously published myeloid sarcomas. There are no definitive genetic, demographic, or anatomical differences between the cases in our series and the previously described cases, although the relatively small sample size and case heterogeneity prevent a definitive statistical analysis. NPM1 and DNMT3A were comutated with NFE2 in 2 of 7 of the previously described myeloid sarcomas; the rates of NPM1 and DNMT3A mutations in our series were not significantly different (Fisher’s exact test, supplemental Table 1). Three of 6 previously described myeloid sarcomas with available clinicopathologic data occurred in the gynecologic tract; this rate is higher than seen in our series (0 cases in the gynecologic tract), but this comparison suffers from selection bias. From a purely statistical perspective, the probability of not identifying an NFE2 mutation in this series is ≤0.001% if the previously reported rate of NFE2 mutations is the true mutational rate (binomial probability). A
A coding variant in *NFE2* was identified in 1 of 38 patients in our cohort (NM_001136023.3: c.1094G>C, p.Arg365Pro). This variant is observed in 0.04% of the general population (gnomAD v2.2.1, Broad Institute) and was confirmed to be a germline heterozygous variant by Sanger sequencing of a separate nonneoplastic esophageal biopsy. It was classified as a variant of unknown significance by American College of Medical Genetics and Genomics criteria for inherited disease genetic analysis and as a tier 4 variant by Association for Molecular Pathology criteria for tumor-based mutational analysis.4,5 Most somatic pathogenic variants in *NFE2* in myeloid sarcomas are truncating.

### Table 1. Clinicopathologic characteristics and NFE2 sequencing results

| Case ID | Sex/age, y | Myeloid sarcoma site | Bone marrow pathology | Clinical scenario | NFE2 coding region |
|---------|------------|----------------------|-----------------------|------------------|-------------------|
| A       | F/68       | Skin, abdomen        | MPN                  | MPN with AML transformation | Wild-type         |
| B       | M/54       | Gingiva              | Negative             | iMS              | Wild-type         |
| C       | M/37       | Parotid gland        | Negative             | iMS              | Wild-type         |
| D       | M/61       | Testis               | AML                  | Systemic AML     | Wild-type         |
| E       | M/73       | Perirenal soft tissue| 4% Blasts            | iMS, t-AML       | Wild-type         |
| F       | M/48       | Supraclavicular lymph node | AML                  | Systemic AML     | Wild-type         |
| G       | F/51       | Soft tissue, arm     | Negative             | iMS, relapse     | Wild-type         |
| H       | M/28       | Lymph node           | Negative             | iMS, relapse     | Wild-type         |
| I       | M/65       | Lymph node           | MDS-EB2              | MDS-EB2          | Wild-type         |
| J       | F/38       | Retropitoneum        | Negative             | iMS              | Wild-type         |
| K       | F/63       | Soft tissue, leg     | AML-MRC              | History of CMML  | Wild-type         |
| L       | F/60       | Skin, scalp          | Negative             | iMS, relapse     | Wild-type         |
| M       | M/73       | Skin, chest          | Negative             | iMS, concurrent metastatic melanoma | Wild-type |
| N       | F/85       | Mediastinum          | Plasma cell myeloma  |                  | Wild-type         |
| O       | F/70       | Breast               | Negative             | iMS, relapse t-AML | Arg365Pro Germline (heterozygous) |
| P       | M/68       | Sacrum               | NA                   | Preceding MDS, post-HSCT | Wild-type         |
| Q       | F/53       | Retropitoneum        | Negative             | iMS, monocytic differentiation | Wild-type         |
| R       | F/39       | Skin                 | AML                  | Systemic AML     | Wild-type         |
| S       | M/4 mo     | Skin                 | AML                  | Systemic AML     | Wild-type         |
| T       | M/16       | Soft tissue, scalp   | AML                  | Systemic AML     | Wild-type         |
| U       | M/55       | Ethmoid sinus/orbit  | AML                  | Relapse with AML  | Wild-type         |
| V       | F/89       | Parotid              | AML                  | Relapse with AML  | Wild-type         |
| W       | F/24       | Tonsil               | AML                  | Synchronous AML  | Wild-type         |
| X       | M/7 mo     | Groin                | Negative             | iMS, de novo     | Wild-type         |
| Y       | F/64       | Nasopharynx          | Negative             | iMS, de novo     | Wild-type         |
| Z       | F/75       | Cervical lymph node  | AML                  | Synchronous AML  | Wild-type         |
| AA      | F/38       | Paraspinal mass      | AML                  | iMS initially, relapsed with AML | Wild-type |
| AB      | M/57       | Chest wall           | AML                  | NA               | Wild-type         |
| AC      | F/57       | Femur                | ET                   | MPN-ET           | Wild-type         |
| AD      | F/89       | Buttock              | aCML                 | Synchronous MDS/MPN | Wild-type         |
| AE      | M/82       | Testis/skin          | AML                  | Synchronous AML  | Wild-type         |
| AF      | F/61       | Nasopharynx          | MDS-EB2              | MDS-EB2          | Wild-type         |
| AG      | M/27       | Tonsil/neck mass     | AML-MRC              | iMS, relapse     | Wild-type         |
| AH      | F/85       | Paraspinal mass      | t-AML                | iMS, relapse     | Wild-type         |
| AI      | F/55       | Epidural             | NA                   | NA               | Wild-type         |
| AJ      | F/41       | Breast               | CML                  | NA               | Wild-type         |
| AK      | M/61       | Nasopharynx          | NA                   | NA               | Wild-type         |
| AL      | F/87       | Axillary lymph node  | AML                  | NA               | Wild-type         |

Clinicopathologic findings of cases A through M, as described in Werstein et al; all other cases are newly reported. aCML, atypical chronic myeloid leukemia, *BCR-ABL1* negative; AML-MRC, acute myeloid leukemia with myelodysplastic-related change; CML, chronic myeloid leukemia, *BCR-ABL1* positive; CMML, chronic myelomonocytic leukemia; ET, essential thrombocythemia; F, female; HSCT, hematopoietic stem cell transplantation; iMS, isolated myeloid sarcoma; M, male; MDS, myelodysplastic syndrome; MDS-EB2, myelodysplastic syndrome with excess blasts-2; mo, month; MPN, myeloproliferative neoplasm; NA, not available; t-AML, therapy-related acute myeloid leukemia.
frameshift or nonsense mutations. Therefore, this germline variant is likely not associated with myeloid sarcoma.

Pathogenic NFE2 mutations have been reported in a small subset of myeloid neoplasms (2.1% in polycythemia vera, 2.6% in primary myelofibrosis, and 3.2% in AML).2,6,7 NFE2 mutations have been hypothesized to promote leukemic stem cell homing to nonhematopoietic tissues, leading to the development of myeloid sarcomas.2 However, the absence of any pathogenic somatic NFE2 mutations in the largest cohort of myeloid sarcomas sequenced to date suggests that other factors are more commonly responsible for extramedullary blast localization.

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