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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 *NFE2*[1] 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

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Data sharing requests should be sent to Philipp W. Raess (raess@ohsu.edu).

The full-text version of this article contains a data supplement.

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	Retroperitoneum	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/65	Mediastinum	Plasma cell myeloma	iMS	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	Retroperitoneum	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/59	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/69	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/65	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/67	Axillary lymph node	AML	NA	Wild-type

Clinicopathologic findings of cases A through M, as described in Werstein et al[3]; 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.

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

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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