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ARTICLE

Clinicopathologic spectrum of myeloid neoplasms with concurrent myeloproliferative neoplasm driver mutations and *SRSF2* mutations

Mehrnoosh Tashakori[1], Joseph D. Khoury[1], Mark J. Routbort[1], Keyur P. Patel[1], Sa A. Wang[1], Chi Young OK[1], Siba El-Hussein[1], Rashmi Kanagal-Shamanna[1], Rajyalakshmi Luthra[1], Shimin Hu[1], Pei Lin[1], Naveen Pemmaraju[2], Prithviraj Bose[2], Srdan Verstovsek[2], Carlos E. Bueso-Ramos[1], L. Jeffrey Medeiros[1] and Sanam Loghavi[1]✉

Myeloproliferative neoplasms (MPNs) are frequently associated with classic driver mutations involving *JAK2*, *MPL* or *CALR*. *SRSF2* is among the most frequently mutated splicing genes in myeloid neoplasms and *SRSF2* mutations are known to confer a poor prognosis in patients with MPNs. In this study, we sought to evaluate the clinicopathologic spectrum of myeloid neoplasms harboring concurrent MPN-driver mutations and *SRSF2* mutations. The study cohort included 27 patients, 22 (82%) men and five (19%) women, with a median age of 71 years (range, 51–84). These patients presented commonly with organomegaly ($n = 15$; 56%), monocytosis ($n = 13$; 48%), morphologic dysplasia ($n = 11$; 41%), megakaryocytic hyperplasia and/or clustering ($n = 10$; 37%) and bone marrow fibrosis >MF-1 (17/22; 77%). About one third of patients either initially presented with acute myeloid leukemia (AML) or eventually progressed to AML. Eighteen (68%) patients had a dominant clone with *SRSF2* mutation and nine (33%) patients had a dominant clone with a classic MPN-associated driver mutation. Our data suggest that the presence of an *SRSF2* mutation preceding the acquisition of a MPN driver mutations is not a disease-defining alteration nor is it restricted to any specific disease entity within the spectrum of myeloid neoplasms. In summary, patients with myeloid neoplasms associated with concurrent *SRSF2* and classic MPN driver mutations have clinical and morphologic features close to that of classic MPNs often with frequent dysplasia and monocytosis.

Modern Pathology (2022) 35:1677–1683; https://doi.org/10.1038/s41379-022-01118-3

INTRODUCTION

Myeloproliferative neoplasms (MPNs) are a heterogeneous group of hematopoietic stem cell disorders characterized by a clonal proliferation of erythroid, myeloid and/or megakaryocytic cells. Philadelphia (Ph)-negative MPNs are further subclassified into polycythemia vera, characterized by overproduction of red blood cells; essential thrombocythemia, defined by overproduction of platelets; and primary myelofibrosis, characterized by proliferation of abnormal megakaryocytes and granulocytes and stepwise evolution of bone marrow fibrosis depending on the stage of disease. However, distinguishing between these entities based solely on clinical and morphologic findings can be challenging; a subset of cases have overlapping features between the aforementioned entities or with myelodysplastic syndromes and are therefore classified as MPN-unclassifiable or myelodysplastic/myeloproliferative neoplasm (MDS/MPN)-unclassifiable, respectively[1,2].

The advent of massive parallel sequencing and identification of recurrent somatic gene mutations has had an impact on the classification and prognostication of myeloid neoplasms including MPNs. Mutations of *JAK2*, *MPL*, and *CALR* have been recognized as driver mutations in MPNs, and these mutations have been incorporated into the diagnostic criteria for MPNs. Yet, these mutations do not occur in isolation and are often seen in concert with other mutations and chromosomal alterations, many of which also influence disease phenotype and prognosis[3]. Mutations of spliceosome regulator genes can occur in MPNs and have been shown to be enriched in cases classified as primary myelofibrosis and MDS/MPN; these mutations are associated with an increased risk for progression to myelofibrosis[3].

SRSF2 (*Serine/arginine-rich Splicing Factor 2*) is located on chromosome 17q25.2 and is among the most frequently mutated spliceosome regulator genes in myeloid neoplasms[4,5]. The most common *SRSF2* mutations involve the proline 95 (P95) residue and alter the RNA binding specificity of SRSF2. These mutations lead to aberrant splicing of hematopoietic regulators, eventually resulting in impaired hematopoiesis. Murine models have shown that *Srsf2* P95H results in myeloid dysplasia in an EZH2-dependent manner[6]. Numerous clinical studies have shown that *SRSF2* mutations occur in various myeloid neoplasms including MDS, MDS/MPN (with particular enrichment in chronic myelomonocytic leukemia), MPNs, and acute myeloid leukemia (AML)[7–10]. Moreover, *SRSF2* mutations confer a poorer prognosis in patients with myeloid neoplasms[3,4,11,12].

[1]Department of Hematopathology, UT MD Anderson Cancer Center, Houston, TX, USA. [2]Department of Leukemia, UT MD Anderson Cancer Center, Houston, TX, USA. ✉email: SLoghavi@mdanderson.org

Received: 24 March 2022 Revised: 23 May 2022 Accepted: 24 May 2022
Published online: 11 June 2022

Concurrent classic MPN driver mutations (*JAK2, MPL, CALR*) with *SRSF2* mutations can give rise to myeloid neoplasms with features overlapping between classic Ph-negative MPNs and those with a phenotype more akin to MDS/MPNs or MDS. Recognition of the clinicopathologic spectrum of these entities is helpful for the most appropriate classification of these entities, given that patient management and clinical trial enrollment criteria may differ based on pathologic classification[13–15]. Although *SRSF2* mutations play a substantial role in the phenotypic manifestations of myeloid neoplasms as well as patient outcomes, these mutations are thus far considered to be non-driver mutations.

In this study, our aim was to focus on *SRSF2* mutations in the context of myeloid neoplasms with MPN-associated driver mutations. We correlated the presence of *SRSF2* mutations with disease classification and sought to explore the phenotypic features of myeloid neoplasms harboring classic MPN-associated driver mutations and concurrent *SRSF2* mutation.

MATERIALS AND METHODS

We searched our sequencing archives for myeloid neoplasms harboring concurrent classic MPN driver mutations (*JAK2, MPL, CALR*) and *SRSF2* mutations resulted between 09/28/2016 (assay go-live) and 07/01/2019. In order to capture the entire spectrum of disease presentation, we included de novo and untreated myeloid neoplasms as well as cases that were previously treated with this combination of mutations. Using this selection criteria, we identified a total of 27 patients among 4740 patients (0.5%) that had undergone sequencing for suspected myeloid neoplasms during the time interval. Clinical and laboratory data were obtained from the electronic medical records. This study was approved by the Institutional Review Board (IRB) at MD Anderson Cancer Center (MDACC) and conducted in accord with the Declaration of Helsinki.

Bone marrow morphology

Hematoxylin and eosin-stained sections of bone marrow (BM) trephine biopsy specimens and Wright–Giemsa-stained BM aspirate smears were used for morphologic classification according to current World Health Organization diagnostic criteria[16]. A 200- or 500-nucleated cell differential cell count was performed when possible. Special attention was paid to megakaryocytic morphology (number, loose vs tight clustering, atypia vs dysplasia including small forms or nuclear hypolobation). Furthermore, erythroid and/or granulocytic lineages were also examined for dysplasia. Dysplasia was assessed using the criteria proposed by Della Porta et al.[17]. Reticulin and Masson trichrome stains were performed using tissue sections prepared from paraffin-embedded trephine biopsy specimens and an automated stainer (Leica Biosystems, Buffalo Grove, IL). We evaluated the degree of reticulin and collagen fibrosis and BM fibrosis was graded according to the criteria proposed by the European Bone Marrow Fibrosis Consensus Group[18].

Conventional karyotyping

Bone marrow aspirate cells were cultured and harvested after 24 and 48 h, and chromosome slides were prepared according to standard protocol for G banding as described previously[19]. A minimum of 20 metaphases were analyzed when possible and karyotypes were reported using the 2016 International System for Human Cytogenetic Nomenclature[20].

Molecular analysis

Next-generation sequencing (NGS) analysis was performed interrogating all exons or hotspot regions of 81 genes mutated frequently in myeloid malignancies (Supplementary Table 1) validated at the CLIA-certified molecular diagnostic laboratory at MDACC as described previously[21]. A sequencing library was prepared using 250 ng of genomic DNA and respective sequencing libraries were subjected to the Illumina MiSeq (Illumina, Inc., San Diego, CA, USA) sequencer. Variant calling was performed using the SureCall application (Agilent HaloPlex Target Enrichment System). The Integrative Genomics Viewer (IGV, Broad Institute) was used for data visualization. A minimum sequencing coverage of x250 (bidirectional true paired-end sequencing) was required. The analytical sensitivity was established at 1–2% mutant reads in a background of wild type reads.

RESULTS

Patients

The study group included 22 (82%) men and five (19%) women with a median age of 71 years (range, 51–84). At the time of molecular characterization the cases were classified as follows: primary myelofibrosis (PMF, 14 cases, 52%), acute myeloid leukemia (AML, 4 cases, 15%), chronic myelomonocytic leukemia (CMML, three cases, 11%), polycythemia vera (PV, 2 cases, 7.4%), post-ET/PV myelofibrosis (two cases, 7.4%), MPN-unclassifiable (MPN-U, one case, 3.6%), and MDS/MPN-unclassifiable (MDS/MPN-U, one case, 3.6%) (Fig. 1A).

All patients except two with the diagnosis of PV had molecular characterization months after the initial diagnosis. The median interval between the time of initial diagnosis and molecular characterization was 18.9 months (range, 0–293) and the breakdown for each category was as follows: PMF, 18.7 months (range, 0.6–81.6); post-ET/PV myelofibrosis, 37.2 months (range, 18.8–55.5); CMML, 36.2 months (range, 12.6–66.4); AML, 34.9 months (range, 7.1–292.6), MPN-U, 1.4 months, and MDS/MPN-U, 14.7 months. Seventeen of 27 (63%) patients had received therapy for their myeloid neoplasm (other than cytoreductive agents alone) prior to molecular characterization(Table 1). Therapeutic agents included ruxolitinib ($n = 15$); azacitidine ($n = 3$); thalidomide ($n = 2$); cladribine+ low dose cytarabine ($n = 1$). These agents were administered alone or in combination with one another.

Peripheral blood indices at the time of molecular characterization were variable among these patients (Table 1). The median absolute monocyte count (AMC) was as follows: PMF, 0.945×10^9/L (range, 0–7.59); PV, 0.90×10^9/L (range, 0.31–1.48); post-ET/PV myelofibrosis, 0.06×10^9/L (range, 0–0.12); CMML, 1.58×10^9/L (range, 0.97–17.56); AML, 0.26×10^9/L (range, 0.02–7.4); MPN-U, 6.48×10^9/L; and MDS/

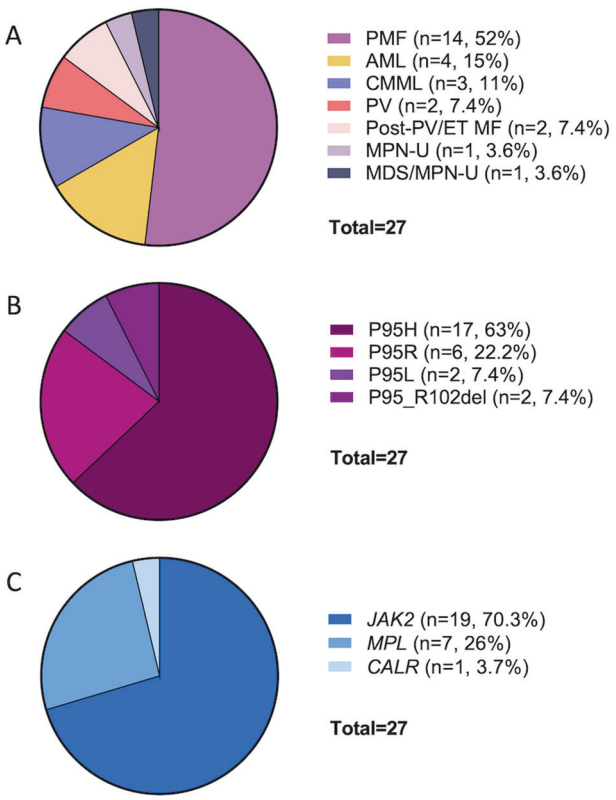


Fig. 1 Detailed spectrum of disease classification and molecular characterization of study group. A Spectrum of diseases in the study group, (**B**) Spectrum of mutations in *SRSF2*, (**C**) Spectrum of MPN-related mutations.

Table 1. Clinicopathologic features of the study group.

	Primary myelofibrosis (*N* = 14)	Polycythemia vera (*N* = 2)	Post-ET/PV myelofibrosis (*N* = 2)	Chronic myelomonocytic leukemia (*N* = 3)	Acute myeloid leukemia (*N* = 4)	MPN-U (*N* = 1)	MDS/MPN-U (*N* = 1)
Male: female	12:2	2:0	2:0	2:1	3:1	0:1	1:0
Median age (range)	73.8 (56.3–85.2)	72.65 (71 and 74.4)	76.34 (74.2 and 78.5)	76.36 (66.8–79.4)	70.87 (64.6–77)	72.8	79.7
Splenomegaly *n* (%)	8/14 (57%)	0/2 (0%)	1/2 (50%)	2/3 (66.6%)	3/4 (75%)	1/1 (100%)	0/1 (0%)
Hb (g/dL), median (range)	9.4 (5.5–14.6)	11.7 (10 and 13.4)	8.85 (8.4 and 9.3)	9.1 (8.7–10.5)	10.55 (9.5–12.1)	13.2	7.4
Transfusion dependence *n* (%)	4/14 (28.6%)	0/2 (0%)	1/2 (50%)	0/3 (0%)	0/4 (0%)	0/1 (0%)	1/1 (100%)
ANC ($\times 10^9$/L), median (range)	9.71 (3–22.4)	11.88 (4.8 and 19)	1.7 (0.3 and 3.1)	17.56 (10.1–20.1)	0.65 (0.1–11.3)	29.7	0.88
AMC ($\times 10^9$/L), median (range)	0.945 (0–7.6)	0.90 (0.3 and 1.5)	0.06 (0 and 0.1)	1.58 (1–17.6)	0.26 (0.02–7.4)	6.5	0.2
Platelets ($\times 10^9$/L), median (range)	107.5 (3–448)	903 (676 and 1,130)	500 (279 and 721)	127 (82–256)	46.50 (15–241)	124	42
Ring sideroblasts *n* (RS %)	1/14 (35%)	0/2 (0%)	0/2 (0%)	0/3 (0%)	0/4 (0%)	0/1 (0%)	1/1 (48%)
Erythroid dysplasia *n* (%)	3/14 (21.4%)	0/2 (0%)	0/2 (0%)	1/3 (33.3%)	1/4 (25%)	0/1 (0%)	1/1 (100%)
Granulocytic dysplasia *n* (%)	5/14 (35.7%)	0/2 (0%)	0/2 (0%)	2/3 (66.6%)	0/4 (0%)	0/1 (0%)	1/1 (100%)
Megakaryocytes *n* (%)							
MDS-like	1/14 (7.1%)	0	0	1/3 (33.3%)	1/4 (25%)	0	1/1 (100%)
MPN-like	2/14 (14.3%)	1/2 (50%)	0	0	0/4 (0%)	0	0
Hybrid MDS/MPN-like	9/14 (64.3%)	1/2 (50%)	1/2 (50%)	0	1/4 (25%)	1/1 (100%)	0
Unremarkable	0	0	0	1/3 (33.3%)	0	0	0
Too few to assess	2/14 (14.3%)	0	1/2 (50%)	1/3 (33.3%)	2/4 (50%)	0	0
BM fibrosis MF2–3 *n* (%)	12/14 (85.7%)	1/2 (50%)	2/2 (100%)	2/3 (66.6%)	not available	0/1 (0%)	not available
Abnormal karyotype *n* (%)	6/14 (42.85%)	0/2 (0%)	0/2 (0%)	1/3 (33.3%)	3/4 (75%)	0/1 (0%)	1/1 (100%)
Complex karyotype/−5 *n* (%)	4/14 (28.6%)	0/2 (0%)	0/2 (0%)	0/3 (0%)	1/4 (25%)	0/1 (0%)	0/1 (0%)
MPN-driver mutation, Median VAF (range)	*JAK2* 10/14 38% (6–63) *MPL* 4/14 45% (28–88)	*JAK2* 2/2 31% (20 and 43)	*JAK2* 1/2 23% *CALR* 1/2 58%	*JAK2* 2/3 36% (11 and 60) *MPL* 1/3 32%	*JAK2* 2/4 25 (15 and 35) *MPL* 3/4 11 (6–20)	*JAK2* 1/1 73%	*JAK2* 1/1 2%
SRSF2 mutation, median VAF (range)	44% (2–65)	26% (24 and 47)	27% (5 and 49)	44% (36–48%)	47% (32–52)	51%	42%
AML progression *n* (progression time from initial diagnosis, months)	1/14 (34.67)	1/2 (20.53)	0/2 (0%)	0/3 (0%)	2/4 (36.3 and 296.7)	0/1 (0%)	0/1 (0%)
Median OS months (range)	41.81 (9.9–82.3)	24.27 (23.5 and 25.03)	51.98 (45.33 and 58.63)	47.43 (35.82–47.55)	35.2 (9.73–313.47)	20.4	19.73
NGS performed at the time of initial diagnosis *n* (%)	0/14 (0%)	2/2 (100%)	0/2 (0%)	0/3 (0%)	0/4 (0%)	0/1 (0%)	0/1 (0%)
Months between initial diagnosis and NGS, median (range)	14/14 (100%) 18.69 (0.62–80.61)	N/A	2/2 (100%) 37.17 (18.81 and 55.53)	3/3 (100%) 36.2 (12.62–66.44)	4/4 (100%) 34.88 (7.13–292.6)	1/1(100%) 1.38	1/1 (100%) 14.7
Previous treatment (other than cytoreduction) received prior to NGS	8/14 (57%)	0/2	2/2 (100%)	3/3 (100%)	2/4 (50%)	1/1 (100%)	1/1 (100%)

Hb hemoglobin, *ANC* Absolute neutrophil count, *AMC* absolute monocyte count, *RS* ring sideroblast, *MDS* myelodysplastic syndrome, *MPN* myeloproliferative neoplasm, *MF* myelofibrosis, *VAF* variant allele frequency, *AML* acute myeloid leukemia, *OS* overall survival, *NGS* next-generation sequencing.

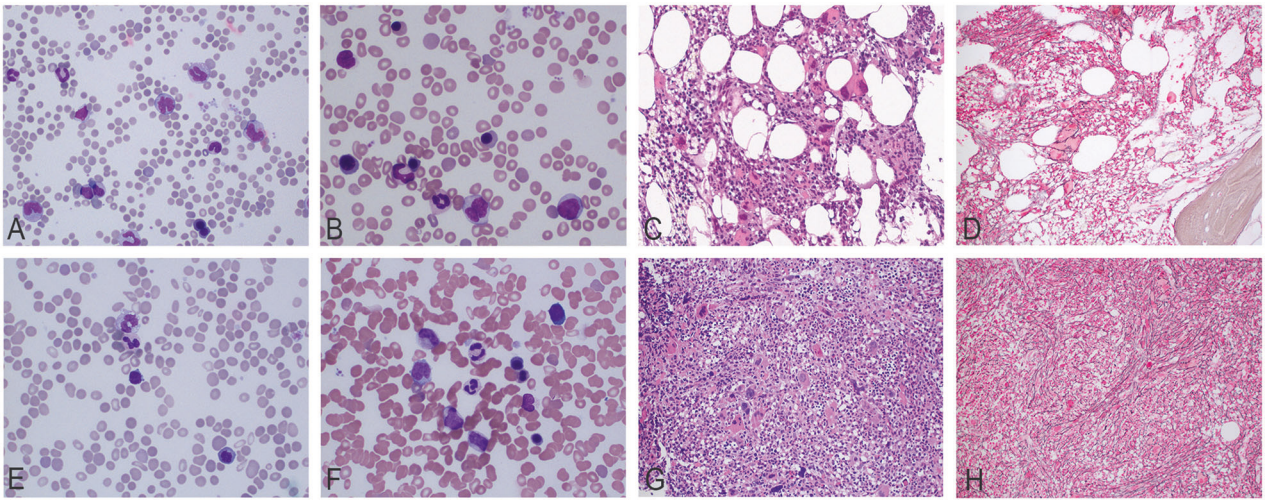


Fig. 2 Examples of spectrum of morphologic features in the study group. PMF with monocytosis, patient 22 (top panel, (**A**) peripheral blood smear, (**B**) bone marrow aspirate smear, (**C**) H&E stained trephine core biopsy section, (**D**) reticulin stained trephine core biopsy section) and CMML with MF-3, patient 29 (bottom panel, (**E**) peripheral blood smear, (**F**) bone marrow aspirate smear, (**G**) H&E stained trephine core biopsy section, (**H**) reticulin stained trephine core biopsy section).

MPN-U, 0.15×10^9/L. Overall, 13 (48%) patients had absolute monocytosis ($>1 \times 10^9$/L), predominantly in patients with PMF and CMML (Fig. 2A, E).

Fifteen of 27 (55.5%) patients had splenomegaly at the time of molecular characterization. (Table 1). Transfusion-dependency was present in four of 14 (28.6%) patients with PMF, one of two (50%) patients with AML patients and one patient with MDS/MPN-U (Table 1).

Morphologic dysplasia was present in 11 (40.7%) patients including 3/8 (38%) previously untreated patients (all three with disease manifesting as PMF); seven had dysplasia in ≥2 lineages (six granulocytic, six erythroid, and four megakaryocytic) and four had single lineage dysplasia in granulocytes ($n = 2$) and megakaryocytes ($n = 2$). The morphologic classification of these 11 neoplasms included six PMF, two CMML, two AML and one MDS/MPN-U. The PMF group included three with dysplasia in granulocytic and erythroid lineages, one with dysplasia in granulocytic and megakaryocytic lineages, one with granulocytic dysplasia, and one with dysplasia in megakaryocytes. One patient with CMML had trilineage dysplasia and the other had granulocytic dysplasia. AML patients included one with dysplasia in the erythroid and megakaryocytic lineages and one with erythroid dysplasia. The patient with MDS/MPN-U had trilineage dysplasia (Table 1).

Megakaryocytic hyperplasia, defined as >6 megakaryocytes per high power field on average, and/or clustering was observed in 10 (37%) cases: six PMF, two CMML, and two PV (Fig. 2C, G). We further evaluated the morphology of megakaryocytes in each group: PMF patients had predominantly hybrid MDS/MPN-like megakaryocytic morphology (nine of 14, 64.3%), followed by MPN-like (two of 14, 14.3%), and MDS-like (one of 14, 7.1%). Most of the remaining groups had at least one case with hybrid morphologic features.

Bone marrow fibrosis was present in all cases assessed ($n = 22$, including 7/7 evaluable untreated cases): five (23%) MF-1, 11 (50%) MF-2, and six (27%) MF-3. The 17 patients with MF-2 and MF-3 had PMF ($n = 12$), post-ET/PV myelofibrosis ($n = 2$), CMML ($n = 2$) and PV ($n = 1$) (Table 1) (Fig. 2D, H). Ten (37%) patients had osteosclerosis; among these, eight had PMF.

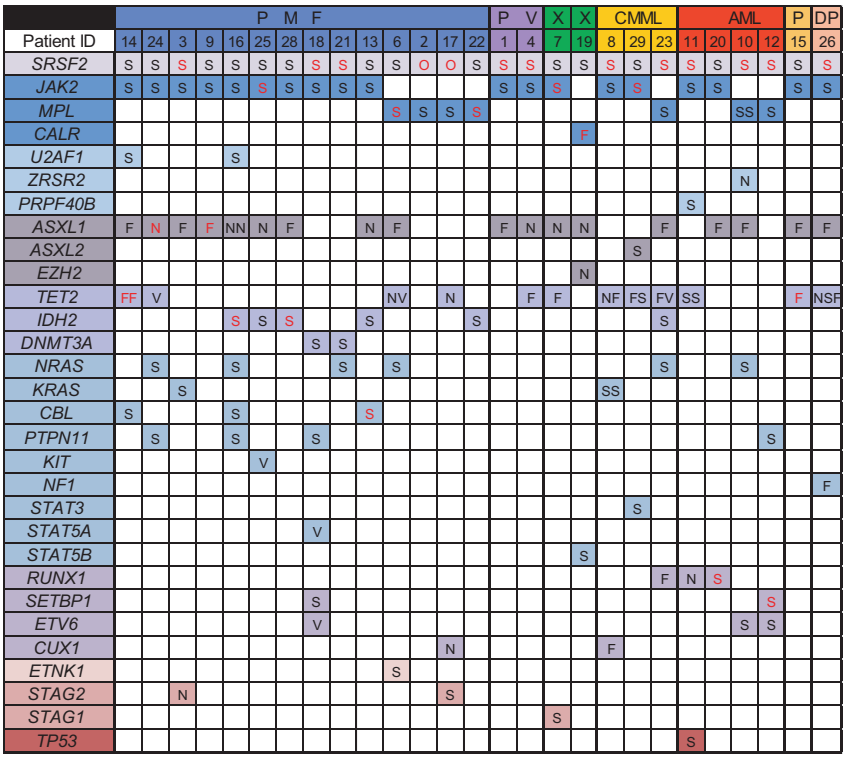
Conventional cytogenetic analysis at the time of molecular studies revealed 16 (59.3%) cases had a diploid karyotype and 11 (40.7%) cases had an abnormal karyotype. Among eight previously untreated cases, seven had a diploid karyotype and one case had del(13)(q12q22). Five of 11 patients had a complex karyotype or monosomy 5: four patients with PMF and one patient with AML-MRC (Table 1). The most prevalent aberrations were del(20q) ($n = 3$) and del(13q) ($n = 3$) and all of these patients had PMF.

Spectrum of genetic changes

SRSF2 mutations included P95H ($n = 17$, 63%), P95R ($n = 6$, 22.2%), P95L ($n = 2$, 7.4%), p.P95_R102del ($n = 2$, 7.4%) with a median variant allelic frequency (VAF) of 44.5% (range, 2–65%) (Fig. 1B). MPN driver mutations involved *JAK2* ($n = 19$, 70.3%), *MPL* ($n = 7$, 26%) and *CALR* ($n = 1$, 3.7%); these mutations were mutually exclusive (Figs. 1C and 3). All patients with *JAK2* mutations had the canonical *JAK2* V617F variant with a median VAF of 36% (range, 2–73%). *MPL* mutations had a median VAF of 24.2% (range, 6–88%) and included the W515L ($n = 5$), L629Q ($n = 1$), V501M ($n = 1$), and S493A ($n = 1$) variants; one patient had two concurrent *MPL* mutations: W515L and S493A. The one patient with *CALR* mutation had a *CALR* K385fs variant with VAF of 58%.

Other recurrent mutations were present. Eighteen (66.6%) patients had *ASXL1* mutations; one patient had two concurrent *ASXL1* mutations (S1028* and G658*). The median VAF for *ASXL1* mutations was 22% (range, 5–46%) and the most frequent mutation was *ASXL1* G646fs ($n = 5$). Twelve (44%) patients had *TET2* mutations; eight had two different *TET2* mutations and one patient had three distinct mutations. The median VAF for *TET2* mutations was 37% (range, 4–94%). Eight (29.6%) patients had mutations involving the RAS pathway: six *NRAS* and two *KRAS*. The median VAF of the *NRAS* mutations was 23% (range, 2–46%) and the mutations included G12D/A/C/S ($n = 5$) and Y64D ($n = 1$). The *KRAS* mutations were G12R with VAF of 15% and A59T with VAF of 45%. Six (22%) patients had *IDH2* R140Q mutations with a median VAF of 45% (range, 3–48%); of note these patients had chronic myeloid neoplasms, mostly PMF. Other mutations were present with a lower frequency (Fig. 3).

We inferred clonal dominance based on VAF with the mutation with the highest VAF considered as being present in a dominant clone and found that when all mutations were considered, *SRSF2* was present in the dominant clone in 13 (48.1%) patients with a median VAF of 47% (range, 24–65%). Other co-dominant mutations included *JAK2* [$n = 3$; median VAF of 60% (range, 23–63 %), *IDH2* ($n = 2$; VAFs: 44 and 43%), *TET2* ($n = 2$; one case with two mutations with VAFs of 48 and 49% and the other case with VAF of 95%), *MPL* ($n = 2$; VAFs: 88 and 32%), *ASXL1* ($n = 2$; VAFs: 45% each), *CBL* ($n = 1$, VAF: 82%), *RUNX1* ($n = 1$, VAF: 88%), *SETBP1* ($n = 1$, VAF: 49%) and *CALR* (n = 1, VAF: 58%).



X	Post-PV-ET MF
P	MPN-U
DP	MDS/MPN-U

S	Missense
F	Frameshift
N	Nonsense
V	Variant of Unknown Significance
O	Other

Number of letters = number of mutation
Red letter = dominant clone based on VAF
Patient 12 with 2 dominant clones

Fig. 3 Mutational landscape in the study group, subclassified per disease group. Patients 1, 4, 6, 11, 14, 17, 22, 25 were previously untreated (de novo).

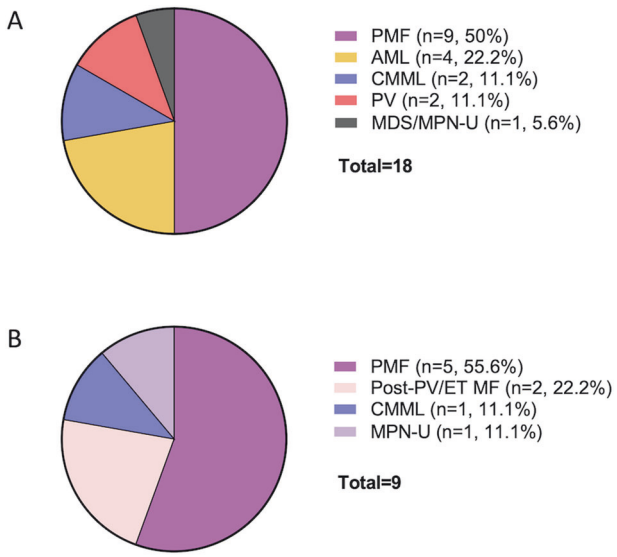


Fig. 4 Clonal dominance in relation to disease subclassification. A Spectrum of diseases with *SRSF2* dominant clones, (**B**) Spectrum of diseases with MPN-related dominant clones.

We then subclassified the patients based on the dominance of the *SRSF2* mutant clone or classic MPN-associated mutations, only considering these two mutation groups. Eighteen (66.7%) patients had a dominant *SRSF2* clone and these neoplasms were PMF ($n = 9$, 50%), AML ($n = 4$, 22.2%), CMML ($n = 2$, 11.1%), PV ($n = 2$, 11.1%) and MDS/MPN-U ($n = 1$, 5.6%) (Fig. 4A). Nine (33.3%) patients had classic dominant MPN-associated mutations and these neoplasms were PMF ($n = 5$, 55.6%), post-PV/ET MF ($n = 2$, 22.2%), CMML ($n = 1$, 11.1%), and MPN-U ($n = 1$, 11.1%) (Fig. 4B). We further examined the clonal dominance of *SRSF2* and MPN-associated mutations in each disease group (Fig. 5).

Although concurrent splicing factor mutations are commonly mutually exclusive in myeloid neoplasms[5], in this cohort three patients had 2 splicing factor mutations. Two patients had PMF with concurrent *SRSF2* and *U2AF1* mutations; one with *SRSF2* P95H (VAF: 33.5%) and *U2AF1* Q157P (VAF: 15%) and the other with *SRSF2* P95H (VAF: 2%) and *U2AF1* Q157P (VAF: 37%). One patient had AML-MRC with a *SRSF2* P95R (VAF: 52%) and *ZRSR2* R169* (VAF: 37%) (Fig. 3). Notably, no splicing factor gene had more than one mutation and, expectedly, no patients had *SF3B1* mutations[22].

Clinical outcomes

Four patients with chronic neoplasms progressed to AML during the course of their disease. The median time to AML transformation was 35.5 months (range, 20.5–297.6). The initial diagnosis in these patients was PMF ($n = 2$), PV ($n = 1$), and CMML ($n = 1$) (Table 1). Among the four patients who progressed to AML, two were diagnosed with AML at the time of molecular characterization (index case in this cohort) and two cases (initially PV and PMF) progressed later. Both of the two patients that progressed later had *SRSF2* mutations in their chronic phase of disease. One patient initially diagnosed with PV, retained their *SRSF2* mutation but lost their *JAK2* mutation with acquisition of additional mutations involving *BRAF*, *KRAS* and *RAD21* at the time of AML transformation; the other patient, initially diagnosed with PMF, transformed with extramedullary disease (myeloid sarcoma of skin); the skin sample was not sequenced but the concurrent bone marrow had lost the *SRSF2* mutation and retained the *JAK2* mutation at this time, along with additional mutations involving *NRAS* and *NF1*. Among 27 patients, 24 patients were treated with agents other than supportive therapy as follows: 10 received a JAK2 inhibitor, five were treated with hypomethylating agents, and seven received both a JAK2 inhibitor and a hypomethylating agent. Four patients underwent allogeneic hematopoietic stem cell transplant (HSCT). The median overall survival of patients in this study was 41 months (range, 9.7–313.5).

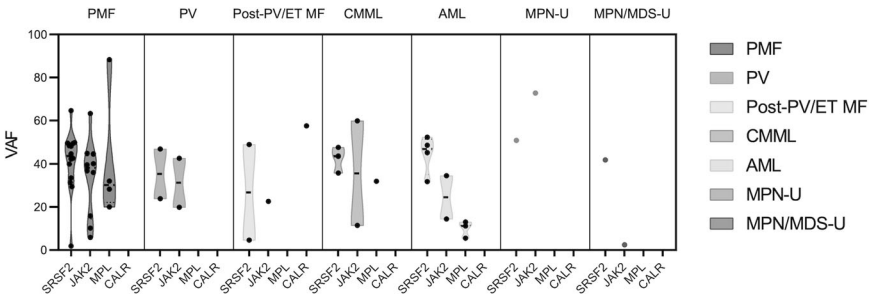


Fig. 5 Violin plot of VAF for *SRSF2* and MPN-related mutations in each disease group.

Among different disease groups, the median overall survival was relatively comparable, with patients in the post-PV/ET MF subgroup having the longest median overall survival [52 months, (45.3 and 58.6)] (Table 1). Six (20.7%) patients had died by the time we conducted this study. All patients who received an allogeneic HSCT were alive with no disease recurrence at last follow up with a median survival of 42.7 months (range, 17.3–49.3).

DISCUSSION

In this study, we evaluated the clinicopathologic spectrum of myeloid neoplasms harboring concurrent MPN-driver mutations and *SRSF2* mutations. We show that the co-occurrence of these mutations is seen predominantly in elderly men who present commonly with organomegaly, monocytosis, morphologic dysplasia, megakaryocytic hyperplasia and/or clustering and bone marrow fibrosis. Mostly, these patients have classic MPN-like phenotypes. About one third of these patients either presented with AML or eventually progressed to AML. A limitation of our study is the inclusion of patients that had undergone therapy prior to molecular characterization (17/27), however, most of these patients were treated with ruxolitinib or hypomethylating agents or a combination of the two, which typically do not substantially alter disease morphology or phenotype; only one patient had received cytotoxic chemotherapy prior to molecular characterization.

Given the different management and therapeutic options for patients with MPN versus those with MDS/MPN cases, more specifically PMF versus CMML, the definitive diagnosis of myeloid neoplasms with intermediate features between these two entities is of clinical interest[13–15] (Fig. 2). Monocytosis is frequently seen in myeloid neoplasms with *SRSF2* mutations which was also common in our study cohort[13]. In our cohort, eight of 19 MPN cases had absolute and relative monocytosis. Moreover, all three CMML cases had fibrosis to a variable degree. In this cohort, 22 patients already had an established diagnosis prior to molecular studies; thus, the monocytosis or fibrosis did not lead to disease reclassification. However, it is important to recognize that monocytosis and fibrosis can occur in the course of disease and lead to manifestations of MDS/MPN-like features.

SRSF2 is a member of the serine/arginine-rich (SR) protein family that plays a role in pre-mRNA splicing through its RNA recognition motif (RRM) domain. *SRSF2* mutations occur predominantly at proline 95. P95H/L/R mutants enhance binding affinity of the RRM domain via conformational changes up to 5 fold greater than wild type *SRSF2*[6,23]. In contrast, the P95A mutant does not influence binding affinity. In our cohort, 27 neoplasms had missense mutations, including P95H in 19 cases, P95R in six, and P95L in two. Two patients had P95_R102del (c.284_307del).

Kim et al.[6] showed in a detailed mouse study that *Srsf2*P95H/wild type mice develop macrocytic anemia and leukopenia with preserved BM cellularity and myeloid and erythroid dysplasia, recapitulating the clinicopathologic features of MDS. Numerous studies also have shown that *SRSF2* mutations are frequent in MDS and MDS/MPN patients[4,9,12]. In our cohort, slightly more than half of patients had dysplasia in single or multiple lineages. Moreover, Papaemmanuil et al.[4] suggested that *SRSF2* mutations occur as early genetic events in MDS pathogenesis and predestine neoplastic clones to acquire specific genetic and genomic alterations. Mutant *SRSF2* was a dominant clone in most cases in our cohort; yet these neoplasms mostly showed MPN-type features. Our observations suggest that *SRSF2* mutations could be early genetic events in a subset of MPN patients.

Lee et al.[22] showed that a minority (~2%) of patients with myeloid neoplasms have >1 splicing factor mutation and that concurrent *SRSF2* and *SF3B1* mutations cannot be tolerated during hematopoiesis due to impaired HSPC self-renewal, differentiation, and survival. In our cohort, co-mutant *SRSF2* and *SF3B1* did not exist. However, we identified four cases with coexistent splicing factor mutations including *ZRSR2* and *U2AF1*. Using bulk and single cell analyses, Tyler and colleagues[23] reported rare myeloid neoplasms with escape of such mutational epistasis demonstrating a selection for less common mutants in the presence of concomitant splicing factor mutations. We also observed the same pattern in two patients: concomitant *SRSF2* P95H and a rare missense mutation in *ZRSR2* with similar VAFs (45 and 50%, respectively) and *SRSF2* P95R with a truncating *ZRSR2* R169* mutation with slightly different VAFs (53 and 37%, respectively). The other two patients had concurrent common mutations, *SRSF2* P95H and *U2AF1* Q157P; however, the VAFs of the mutants were substantially different: one with 2% and 37%, respectively, and the other with 34% and 15%, respectively, suggesting that these mutations exist in distinct clones. This observation could be of importance in designing future targeted therapies for patients with myeloid neoplasms with mutated splicing factor genes; however, further characterization is limited in our study due lack of single cell genomics data.

The results of this study highlight that concurrent MPN-driver mutations and *SRSF2* mutations can occur in a variety of myeloid neoplasms with a predominance of a MPN-like phenotype and clinical features. *SRSF2* mutation in myeloid neoplasms may precede the acquisition of MPN driver mutations and might affect the histologic features and clinical course of the disease; however, *SRSF2* mutation is not a disease-defining alteration or restricted to any specific entity. Rather, *SRSF2* mutation cooperates with other genomic alterations to exert its phenotypic effects.

DATA AVAILABILITY

All data are available upon request.

REFERENCES

1. Kvasnicka, H. M., Thiele, J., Orazi, A., Horny, H. P. & Bain, B. J. Myeloproliferative neoplasm, unclassifiable. In: S.H. Swerdlow *et al.* (eds). *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues* 57–59 (IARC Lyon, 2017).
2. Orazi, A., Bennett, J. M., Bain, B. J., Baumann, I., Thiele, J., Bueso-Ramos, C. *et al.* Myelodysplastic I myeloproliferative neoplasm, unclassifiable. In: S.H. Swerdlow *et al.* (eds). *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues* 95–96 (IARC: Lyon, 2017).

3. Grinfeld, J., Nangalia, J., Baxter, E. J., Wedge, D. C., Angelopoulos, N., Cantrill, R. *et al*. Classification and Personalized Prognosis in Myeloproliferative Neoplasms. *New England Journal of Medicine* **379**, 1416–1430 (2018).
4. Papaemmanuil, E., Gerstung, M., Malcovati, L., Tauro, S., Gundem, G., Van Loo, P. *et al*. Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood* **122**, 3616–3627; quiz 3699 (2013).
5. Yoshida, K., Sanada, M., Shiraishi, Y., Nowak, D., Nagata, Y., Yamamoto, R. *et al*. Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature* **478**, 64–69 (2011).
6. Kim, E., Ilagan, J. O., Liang, Y., Daubner, G. M., Lee, S. C., Ramakrishnan, A. *et al*. SRSF2 Mutations Contribute to Myelodysplasia by Mutant-Specific Effects on Exon Recognition. *Cancer Cell* **27**, 617–630 (2015).
7. Patnaik, M. M., Lasho, T. L., Finke, C. M., Hanson, C. A., Hodnefield, J. M., Knudson, R. A. *et al*. Spliceosome mutations involving SRSF2, SF3B1, and U2AF35 in chronic myelomonocytic leukemia: prevalence, clinical correlates, and prognostic relevance. *Am J Hematol* **88**, 201–206 (2013).
8. Makishima, H., Visconte, V., Sakaguchi, H., Jankowska, A. M., Abu Kar, S., Jerez, A. *et al*. Mutations in the spliceosome machinery, a novel and ubiquitous pathway in leukemogenesis. *Blood* **119**, 3203–3210 (2012).
9. Wu, S. J., Kuo, Y. Y., Hou, H. A., Li, L. Y., Tseng, M. H., Huang, C. F. *et al*. The clinical implication of SRSF2 mutation in patients with myelodysplastic syndrome and its stability during disease evolution. *Blood* **120**, 3106–3111 (2012).
10. Federmann, B., Abele, M., Rosero Cuesta, D. S., Vogel, W., Boiocchi, L., Kanz, L. *et al*. The detection of SRSF2 mutations in routinely processed bone marrow biopsies is useful in the diagnosis of chronic myelomonocytic leukemia. *Hum Pathol* **45**, 2471–2479 (2014).
11. Vannucchi, A. M., Lasho, T. L., Guglielmelli, P., Biamonte, F., Pardanani, A., Pereira, A. *et al*. Mutations and prognosis in primary myelofibrosis. *Leukemia* **27**, 1861–1869 (2013).
12. Zhang, S. J., Rampal, R., Manshouri, T., Patel, J., Mensah, N., Kayserian, A. *et al*. Genetic analysis of patients with leukemic transformation of myeloproliferative neoplasms shows recurrent SRSF2 mutations that are associated with adverse outcome. *Blood* **119**, 4480–4485 (2012).
13. Chapman, J., Geyer, J. T., Khanlari, M., Moul, A., Casas, C., Connor, S. T. *et al*. Myeloid neoplasms with features intermediate between primary myelofibrosis and chronic myelomonocytic leukemia. *Mod Pathol* **31**, 429–441 (2018).
14. Gur, H. D., Loghavi, S., Garcia-Manero, G., Routbort, M., Kanagal-Shamanna, R., Quesada, A. *et al*. Chronic Myelomonocytic Leukemia With Fibrosis Is a Distinct Disease Subset With Myeloproliferative Features and Frequent JAK2 p.V617F Mutations. *Am J Surg Pathol* **42**, 799–806 (2018).
15. Hu, Z., Ramos, C. E. B., Medeiros, L. J., Zhao, C., Yin, C. C., Li, S. *et al*. Utility of JAK2 V617F allelic burden in distinguishing chronic myelomonocytic Leukemia from Primary myelofibrosis with monocytosis. *Hum Pathol* **85**, 290–298 (2019).
16. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. (IARC Lyon, 2017).
17. Della Porta, M. G., Travaglino, E., Boveri, E., Ponzoni, M., Malcovati, L., Papaemmanuil, E. *et al*. Minimal morphological criteria for defining bone marrow dysplasia: a basis for clinical implementation of WHO classification of myelodysplastic syndromes. *Leukemia* **29**, 66–75 (2015).
18. Thiele, J., Kvasnicka, H. M., Facchetti, F., Franco, V., van Der Walt, J. & Orazi, A. European consensus on grading bone marrow fibrosis and assessment of cellularity. *Haematologica* **90**, 1128–1132 (2005).
19. Khoury, J. D., Sen, F., Abruzzo, L. V., Hayes, K., Glassman, A. & Medeiros, L. J. Cytogenetic findings in blastoid mantle cell lymphoma. *Hum Pathol* **34**, 1022–1029 (2003).
20. McGowan-Jordan J, S. A., Schmid M. *ISCN 2016: An International System for Human Cytogenomic Nomenclature (2016)*. (Basel: S. Karger Publishing, 2016).
21. Ok, C. Y., Loghavi, S., Sui, D., Wei, P., Kanagal-Shamanna, R., Yin, C. C. *et al*. Persistent IDH1/2 mutations in remission can predict relapse in patients with acute myeloid leukemia. *Haematologica* **104**, 305–311 (2019).
22. Lee, S. C., North, K., Kim, E., Jang, E., Obeng, E., Lu, S. X. *et al*. Synthetic Lethal and Convergent Biological Effects of Cancer-Associated Spliceosomal Gene Mutations. *Cancer Cell* **34**, 225-241 e228 (2018).
23. Taylor, J., Mi, X., North, K. D., Binder, M., Penson, A., Lasho, T. L. *et al*. Single-cell genomics reveals the genetic and molecular bases for escape from mutational epistasis in myeloid neoplasms. *Blood* https://doi.org/10.1182/blood.2020006868 (2020).

AUTHOR CONTRIBUTIONS

M.T. and S.L. designed the study, reviewed the pathology, collected and analyzed data. J.D. K., S.A.W., S.H., P.L. C.B.R., L.J.M. collected pathology data, M.J.R., R.L. K.P.P. C.Y.O., R.K.S. collected molecular data, SHE assisted in manuscript preparation, N.P., P.B. and S.V. manages patients and collected clinical data. All authors were involved in manuscript preparation and approved the final draft.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Institutional Review Board (IRB) at MD Anderson Cancer Center (MDACC) and conducted in accord with the Declaration of Helsinki. Consent is not applicable for this retrospective study.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41379-022-01118-3.

Correspondence and requests for materials should be addressed to Sanam Loghavi.