Genomics of myelodysplastic syndrome/myeloproliferative neoplasm overlap syndromes

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Myelodysplastic syndrome (MDS)/myeloproliferative neoplasm (MPN) overlap syndromes are uniquely classified neoplasms occurring in both children and adults. This category consists of 5 neoplastic subtypes: chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), *BCR-ABL1*–negative atypical chronic myeloid leukemia (aCML), MDS/MPN-ring sideroblasts and thrombocytosis (MDS/MPN-RS-T), and MDS/MPN-unclassifiable (U). Cytogenetic abnormalities and somatic copy number variations are uncommon; however, >90% patients harbor gene mutations. Although no single gene mutation is specific to a disease subtype, certain mutational signatures in the context of appropriate clinical and morphological features can be used to establish a diagnosis. In CMML, mutated coexpression of *TET2* and *SRSF2* results in clonal hematopoiesis skewed toward monocytosis, and the ensuing acquisition of driver mutations including *ASXL1*, *NRAS*, and *CBL* results in overt disease. MDS/MPN-RS-T demonstrates features of *SF3B1*-mutant MDS with ring sideroblasts (MDS-RS), with the development of thrombocytosis secondary to the acquisition of signaling mutations, most commonly *JAK2*V617F. JMML, the only pediatric entity, is a bona fide RASopathy, with germline and somatic mutations occurring in the oncogenic RAS pathway giving rise to disease. *BCR-ABL1*–negative aCML is characterized by dysplastic neutrophilia and is enriched in *SETBP1* and *ETNK1* mutations, whereas MDS/MPN-U is the least defined and lacks a characteristic mutational signature. Molecular profiling also provides prognostic information, with truncating *ASXL1* mutations being universally detrimental and germline *CBL* mutations in JMML showing spontaneous regression. Sequencing information in certain cases can help identify potential targeted therapies (*IDH1*, *IDH2*, and splicing mutations) and should be a mainstay in the diagnosis and management of these neoplasms.

LEARNING OBJECTIVES

- Define the landscape of cytogenetic and molecular abnormalities in patients with MDS/MPN overlap neoplasms including chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), *BCR-ABL1*–negative atypical chronic myeloid leukemia (aCML), MDS/MPN-ring sideroblasts and thrombocytosis (MDS/MPN-RS-T), and MDS/MPN-unclassifiable (U)
- Characterize molecular signatures that can be used in the context of appropriate clinical and morphological features to help diagnose CMML, JMML, MDS/MPN-RS-T and *BCR-ABL1*–negative aCML
- Underscore the importance of molecular profiling in MDS/MPN overlap syndromes with regard to diagnosis, prognosis, and clinical therapeutics

Case

A 71-year-old man presents with a 6-month history of effort intolerance, weakness, intermittent drenching night sweats, and low-grade fevers. His last complete blood count 2 years ago had demonstrated mild thrombocytopenia. On examination his vital signs are stable. He his spleen is palpable 10 cm below the left costal margin. He has no hepatomegaly or lymphadenopathy. His past medical history is significant for hypertension controlled with lisinopril. His blood counts reveal hemoglobin of 9.6 g/dL, white blood cell count 15 × 10^9/L, absolute monocyte count 2.3 × 10^9/L, and platelet count 110 × 10^9/L. His blood smear did not have elevated blasts or promonocytes, but there were circulating metamyelocytes and myelocytes. A bone marrow biopsy was 90% cellular with megakaryocytic atypia and hyperplasia. Bone marrow blasts were estimated at 7%. The karyotype was normal, and next-generation sequencing identified

mutations involving *ASXL1*: c.1934dup; p.Gly646Trpfs*12 (20%), *TET2* c.1648C>T; p.Arg550* (41%), *SRSF2* c.284C>T; p.Pro95Leu (43%); and *NRAS*c.38G>A; p.Gly13Asp (46%) (variant allele frequency for each mutation added in parentheses).

What is the diagnosis, and how would you risk stratify this patient?

Introduction

Myelodysplastic syndrome (MDS)/myeloproliferative neoplasm (MPN) overlap syndromes are well-defined myeloid neoplasms characterized by overlapping features of MDS and MPN.[1] This uniquely classified entity consists of 4 adult-onset subtypes: chronic myelomonocytic leukemia (CMML), *BCR-ABL1*–negative atypical chronic myeloid leukemia (aCML), MDS/MPN-ring sideroblasts and thrombocytosis (MDS/MPN-RS-T), and MDS/MPN-unclassifiable (MDS/MPN-U). There is also one pediatric subtype: juvenile myelomonocytic leukemia (JMML) (Table 1).[1] Although the classification of these neoplasms relies largely on clinical features and peripheral blood and bone marrow (BM) morphology, the incorporation of next-generation sequencing (NGS) techniques has helped in defining the molecular landscape and ability to diagnose, risk stratify, and plan appropriate treatment strategies. Among the subtypes, CMML is the most common, demonstrating marked clinical heterogeneity and an inherent tendency to transform to acute myeloid leukemia (AML).[2]

Whereas CMML and JMML are defined by the presence of clonal monocytosis, aCML presents with dysplastic neutrophilia, MDS/MPN-RS-T with anemia and thrombocytosis, and MDS/MPN-U with poorly defined overlapping features. Table 1 outlines the 2016 World Health Organization (WHO) criteria for the diagnosis of MDS/MPN overlap syndromes, including key associated genes and epidemiologic features (incidence, median age, and median overall survival [OS]).[1] Although cytogenetic abnormalities are seen in a small fraction of patients with overlap neoplasms, molecular aberrations occur in most (>90%). In this review, we highlight salient features with regard to the genetic landscape of MDS/MPN overlap neoplasms.

Molecular aberrations in MDS/MPN overlap neoplasms

To establish a diagnosis of an MDS/MPN overlap syndrome, molecularly defined neoplasms that present with similar or overlapping features must be ruled out.[1] These include *BCR-ABL1*–driven CML

Table 1. WHO diagnostic criteria, epidemiology, and gene mutations in MDS/MPN overlap neoplasms

MDS/MPN Overlap Neoplasms	WHO Diagnostic Criteria	Epidemiology	Somatic Gene Mutations (Frequency %)
Diagnosis of MDS/MPN overlap syndromes must exclude Philadelphia chromosome *(BCR/ABL rearrangement), PDGFRA, PDGFRB, FGFR1, PCM1- JAK2* rearrangements and have PB and BM blast counts <20%			

JMML	**Clinical Features (mandatory):**
	• PB monocyte count ≥ 1 x 10^9/L
	• Splenomegaly
	Presence of 1+ Molecular Feature:
	• Somatic mutations in *PTPN11** or *KRAS** or *NRAS**
	• Clinical diagnosis of neurofibromatosis-1 or *NF1* mutation
	• Germ-line *CBL* mutation and loss of heterozygosity of CBL**
	If Molecular Feature is not present, the following criteria must be met:
	• Monosomy 7 or other chromosomal abnormality or at least 2 of the following:
	  • Myeloid or erythroid precursors on PB smear
	  • Hemoglobin F increased for age
	  • GM-CSF hypersensitivity in CFA;
	  • hyperphosphorylation of STAT5
		• Incidence: Rare; 0.67/million/yr (Cases with neurofibromatosis have 200-fold increased risk of JMML)
		• Median Age: 1.1-1.8 yrs
		• Median OS: 10-12 months; In those without HSCT = <12 months; cases that received HSCT = 5-yr OS rate is 64%, event free survival of 52%.The 5-yr cumulative incidence of relapse is 35%, while the 5-yr cumulative incidence of transplantation-related mortality is 13%
		• Rate of LT: Infrequent
			• *PTPN11** (38%)
			• *NRAS** (18%)
			• *KRAS** (14%)
			• *CBL* (12-18%)
			• *NF1* (5-10%)

CMML	• Persistent PB monocyte count ≥ 1x10^9/L (≥3 months) • Dysplastic changes in one or more lineages, if no dysplasia then must include: • An acquired clonal cytogenetic or molecular genetic abnormality (*TET2*, *ASXL1*, *SRSF2*, and/or *SETBP1*) **Sub-types:** • CMML-0 (<2% PB blasts and <5% BM blasts) • CMML-1 (2-4% PB blasts and 5-9% BM blasts) • CMML-2 (5-19% PB blasts and 10-19% BM blasts and/or when any Auer rods are present).	• Incidence: Rare; 1 case/100000/yr • Median Age: 71-74 yrs • Median OS: CMML-1 (38 months), CMML-2 (24 months) • Rate of LT: 15-30%	• *TET2* (60%) • *SRSF2* (50%) • *ASXL1* (40%) • *NRAS* (4-16%) • *CBL* (10-22%) • *RUNX1* (15%) • *SETBP1* (15%) • *KRAS* (7-18%) • ≤10%: *JAK2, SF3B1, U2AF1, EZH2, DNMT3A, PTPN11, ZRSR2, FLT3; NF1; IDH1/2*
MDS/MPN RS-T	• Platelet count ≥ 450 x 10^9/L • ≥ 15% ring sideroblasts in the bone marrow or > 5% with *SF3B1* mutation • Presence of megakaryocytic atypia resembling ET or MF	• Incidence: Rare; <1% of all MDS • Median Age: 71-75 yrs • Median OS: 5-7 yrs • Rate of LT: <5%	• *SF3B1* (90%) • *JAK2* (50%) • *TET2* (10%) • *DNMT3A* (13%) • *SETBP1* (13%) • *ASXL1* (29%) • <10% of: *SRSF2, CBL*
aCML	• WBC ≥ 13x10^9/L with increased and dysplastic neutrophils • No or minimal absolute basophils and monocytosis • Hypercellular BM with granulocyte dysplasia (neutrophil precursors >10%)	• Incidence: Rare; not established • Median Age: >60 yrs • Median OS: 22 months • Rate of LT: 30-40%	• *ASXL1* (60%) • *SETBP1* (48%) • *N/KRAS* (35%) • *TET2* (30%) • *EZH2* (13%) • <10% of: *ETNK1, CBL, FLT3, RUNX1, CEBPA*
MDS/MPN-U	• Features of MDS • Prominent myeloproliferative features, no preceding history of MPN or MDS and no recent cytotoxic or growth factor therapy • No isolated del(5q),	• Incidence: Rare; not established • Median Age: 70 yrs • Median OS: 12-28 months • Rate of LT: unknown	• *ASXL1* (50%) • *SRSF2* (37%) • *SETBP1* (21%) • *JAK2* (19%) • *N/KRAS* (15%) • *TET2* (15%) • <10% of the following: *CBL*

WHO : World Health Organization (according to 2016 revision, *Blood 2016; 127:2391*); yr = year; JMML - Juvenile myelomonocytic leukemia; CMML = Chronic myelomonocytic leukemia; MDS/MPN-RS-T = Myelodysplastic syndrome myeloproliferative neoplasm with ring sideroblasts and thrombocytosis; aCML = Atypical chronic myeloid leukemia; MDS/MPN-U = Myelodysplastic syndrome myeloproliferative neoplasm unclassified; PB = peripheral blood; BM = bone marrow; OS = Overall survival; LT = Leukemic transformation; WBC = White blood cell count; HSCT = Hematopoietic Stem Cell Transplant

* Germ line mutations (indicative of Noonan syndrome) need to be excluded.
** Occasional cases may harbor heterozygous splice site mutations.

(especially the p190 variant), *PDGFRA*, *PDGFRB*, *FGFR1*, and *PCM1-JAK2* rearranged myeloid neoplasms.[1,3] In patients with overlap who present with monocytosis and eosinophilia, aberrations in *PDGFRA*, *PDGFRB*, *FGFR1*, and the *PCM1-JAK2* fusion should be assessed by fluorescence in situ hybridization or quantitative polymerase chain reaction studies. Of note, whereas the most common *PDGFRA* abnormality, the *FIP1L1-PDGFRA* fusion secondary to *CHIC2* deletion, is karyotypically occult, the *ETV6-PDGFRB* fusion and *FGFR1* rearrangements are regularly detected by conventional karyotyping.[4]

Chronic myelomonocytic leukemia

Gene mutations are seen in >90% of patients with CMML and most commonly involve *TET2* (60%), *SRSF2* (50%), *ASXL1* (40%), and the oncogenic RAS pathway (30%).[5-7] Additional genes mutated at lower frequencies include *SETBP1* (15%), *RUNX1* (15%), and *JAK2*V617F (10%), with *DNMT3A*, *IDH1*, *IDH2*, *STAG2*, *PHF6*, *CEBPA*, *ETNK1*, and *EZH2* occurring at < 5%.[2,8,9] Unlike in MPN and chronic neutrophilic leukemia (CNL), driver mutations in *MPL*, *CALR*, and *CSF3R* (CNL) are very infrequent, and if found they suggest an alternative diagnosis.[10] Similarly, leukemia-associated driver mutations including *NPM1* and *FLT3* are very uncommon and if present suggest AML in evolution.[11]

CMML is a disease of aging, resulting from the acquisition of clonal hematopoiesis–related mutations (*TET2*, *ASXL1*, and *SRSF2*), resulting in monocyte-biased hematopoiesis and disease progression secondary to acquisition of additional driver mutations along with cell-intrinsic and -extrinsic factors.[12] Among mutations seen in CMML, truncating (frameshift and nonsense) *ASXL1* mutations are universally deleterious, adversely affecting both OS and leukemia-free survival (LFS),[2,13,14] whereas *TET2* mutations are associated with favorable outcomes, especially in the absence of *ASXL1* mutations, with the *ASXL1*wt/*TET2*mt genotype predicting best survival rates.[10,15] In fact, this genotype is also most predictive of responses to hypomethylating agent therapy (HMA) in CMML.[10,15] Heterozygous splicing mutations (*SRSF2*, *SF3B1*, *U2AF1*, and *ZRSR2*) are common, with *SRSF2* (P95 hot spot) being most frequent, with no clear impact on survival.[16] Acquisition of oncogenic RAS pathway mutations (*NRAS*, *CBL*, *KRAS*, *PTPN11*, and *NF1*) drives a proliferative phenotype (MPN-CMML), with marked leukocytosis/monocytosis, pronounced constitutional symptoms, splenomegaly, and lower survival.[13] *RUNX1* and *SETBP1* mutations are seen in 15% of patients, with *RUNX1* mutations associated with severe thrombocytopenia, and both mutations negatively affect outcomes.[2,17] Of note, *TP53* mutations are uncommon in CMML (<1%) and if seen are usually present in the context of therapy-related CMML.[18]

Juvenile myelomonocytic leukemia

JMML is the only pediatric-onset neoplasm in this category and is considered a bona fide RASopathy, with germline and somatic mutation in the RAS/RAF/MEK/ERK pathway giving rise to disease.[19] Germline mutations associated with JMML include *NF1*, *RAS* mutations in the context of Noonan syndrome (*PTPN11, KRAS, NRAS, RIT1*), and *CBL*, with *CBL* mutant JMML often demonstrating spontaneous regression.[20,21] Somatic mutations that give rise to JMML include *PTPN11* (38%), *NRAS* (18%), *KRAS* (14%), *RRAS* and *RRAS2* (<10%). Unlike in CMML, mutations in epigenetic regulators including *ASXL1* and *SETBP1* and in signaling genes such as *JAK3* are late events and are often responsible for disease progression to AML.[19,22]

MDS/MPN-ring sideroblasts and thrombocytosis

This is a unique overlap neoplasm, most recently formally assigned to this category in the 2016 WHO classification, characterized largely by features of MDS with ring sideroblasts (MDS-RS) and concomitant thrombocytosis.[23] Unlike for other neoplasms in this category, the median OS is favorable at 5 to 7 years, with AML transformation rates of <5%.[23,24] The disease is defined by the specific presence of *SF3B1* (90%) and *JAK2*V617F (50%) mutations, and apart from BM RS it also demonstrates atypical megakaryocytes in the BM with peripheral blood thrombocytosis.[23,24] It is believed that *SF3B1*-mutant MDS-RS clonally evolves into MDS/MPN-RS-T, because of the acquisition of signaling mutations such as *JAK2*V617F. Of note, although *CALR* mutations have been documented in a small fraction of patients with JAK2/MPL wildtype MDS/MPN-RS-T, they tend to be infrequent (<5%).[24,25] Additional mutations seen in MDS/MPN-RS-T include *ASXL1* (29%), *DNMT3A* (13%), *SETBP1* (13%), and *TET2* (10%),[23,24] with the *ASXL1*wt/*SETBP1*wt genotype associated with better outcomes.[23,24]

BCR-ABL1–negative atypical CML

This is a rare overlap neoplasm characterized by dysplastic neutrophilia in the absence of monocytosis and basophilia.[1] Gene mutations encountered include *ASXL1* (28%), *TET2* (16%), *EZH2* (15%), *NRAS* (15%), *SETBP1* (12%), and *RUNX1* (12%), with *ETNK1* mutations seen in 10%.[26,27] Initial data ascribed *CSF3R* mutations to 30% of patients with aCML, but in our experience these mutations are uncommon in WHO-defined aCML and are more reflective of CNL.[28] aCML and CMML share overlapping mutational profiles largely differentiated by the frequencies of *NRAS*, *CBL*, *TET2*, *SRSF2*, and *ETNK1* mutations.[15,29]

MDS/MPN-U

This subtype consists of a conglomerate of poorly defined MDS/MPN overlap syndromes, not meeting criteria for other well-defined entities in this group[1]. Frequencies of gene mutations encountered include *ASXL1* (30% to 50%), *SRSF2* (23% to 37%), *SETBP1* (11% to 21%), *JAK2* (19% to 25%), *NRAS* (10% to 15%), and *TET2* (15% to 27%).[30,31] Less frequent occurrences of *TP53* and *CBL* mutations have also been documented, with a negative impact on survival.[30] Although MDS/MPN-U does not have a specific prognostic scoring system, 2 studies have shown that MDS-centered prognostic models such as the international prognostic scoring system can be used to risk stratify affected patients.[30,31]

Functional categories of mutated genes encountered in MDS/MPN overlap neoplasms

These categories are listed in Table 2 and illustrated in Figure 1.

Epigenetic regulator genes

Key altered epigenetic regulator genes include *TET2*, *ASXL1*, *EZH2*, *DNMT3A*, *IDH1*, and *IDH2*. *TET2* is a critical dioxygenase that helps convert 5-methylcytosine to 5-hydroxymethylcytosine and other oxidative metabolites, which regulate the state of DNA accessibility (methylation).[10] *TET2* is mutated in 60% of patients with CMML, and in the absence of *ASXL1* mutations it has a favorable prognostic impact. *ASXL1* regulates chromatin dynamics through its interaction with the polycomb group repressive

Table 2. Gene mutations and cytogenetic abnormalities seen in MDS/MPN overlap neoplasms

		Gene	Chr Position	JMML	CMML	aCML	MDS/MPN RS-T	MDS/MPN U	Mutation Info
GENE SPECIFIC ALTERATIONS	SIGNALING	*PTPN11*	12q24.13	38%	5%[a]	4%			• Somatic mutations usually involve exons 3, 4, and 13, but can be distributed throughout • In JMML, most common somatic mutations include codons: E76, A72, Q79, and D61
		NRAS	1p13.2	18%	4-16%[a]	16%		15%	• Hot spots include point mutations at codons: G12, G13, and Q61
		KRAS	12p12.1	14%	7-18%[a]				• Hot Spots include point mutations at codons: G12, G13, and Q61
		CBL	11q23.3	12-18%	10-22%[a]	0-10%	<4%[a]	>10%	• Mutations typically occur in the linker and RING finger domains (intron 7, exons 8 and 9) • In JMML, germline HET mutations lead to a Noonan syndrome-like phenotype, somatic mutations include LOH due to UPD with common change: Y732H • In CMML, can be associated with high allelic burden
		NF1	17q11.2	5-10%	1-7%[a]				• Mutations found throughout gene and consist of missense, frameshift, and nonsense • Hot spot: T676 (seen in AML) • In JMML, germline NF1 mutation with loss of second allele due to UPD; in some cases compound heterozygosity / somatic interstitial deletions have been seen
		JAK2	9p24.1		10%	4-8%	50%	19%	• *JAK2*V617F is most common and presence associated with higher hemoglobin/plt counts
		FLT3	13q12.2		<5%	<5%			• Internal tandem duplications in ITD (juxtamembrane region) /TKD mutations (activation loop)
	SPLICING	*SF3B1*	2q33.1		5-10%	8%	90%		• Hot spots include codon K700, and less frequent codons K666, H662, E622, and R625
		SRSF2	17q25.2		50%	12-40%		37%	• Hot spot is codon P95; small in/del are also reported involving this region
		U2AF1	21q22.3		5-15%	0-20%			• Hot spots include codons S34 and Q157
		ZRSR2	Xp22.2		8-10%	4%			• Somatic mutations occur throughout gene
	EPIGENETIC	*ASXL1*	20q11.21	rare	40%[a,b]	28%[b]	29%[a,b]	50%[a,b]	• Heterozygous nonsense/frameshift mutations most often along or upstream of exon 12, resulting in C-terminal truncation of the protein upstream of the PHD finger • Most frequent mutation is G646Wfs12
		TET2	4q24		60%[b]	16%[a,b]	10%[b]	15%[b]	• Somatic mutations occur throughout gene • Favorable outcome especially in ASXL1wt cases • In CMML, ASXL1wt/TET2mut genotype most responsive to HMA
		IDH1/2	IDH1: 2q34 IDH2: 15q26.1		<5%	<4%		5-10%	• Mutation hot spots include: (*IDH1*) R132, and (*IDH2*) R140 and R172 • Targeted therapy available
		EZH2	7q36.1		<5%	8-20%		10%	• Somatic mutations occur throughout gene
		DNMT3A	2p23.3		<5%[a,b]	rare[b]	13%[b]	4%[b]	• Mutation hot spot includes R882 • Other mutations can occur throughout gene
	TRAN.	*RUNX1*	21q22.12		15%[a]	12%		14%	• Mutations located throughout gene • Familial conditions present with germline mutations
	OTHER	*SETBP1*	18q12.3	rare	15%[a]	12%	13%[a]	21%	• Mutations frequently are located in SKI homologous region, codons: E858-I871 • Other mutations can occur throughout gene • In CMML, strongly overlap *ASXL1* mutations • Presence usually mutually exclusive with *JAK2* and *TET2* • In MDS/MPN-RS-T, *ASXL1wt/SETBP1wt* genotype associated with better survival
		ETNK1	12p12.1		<5%	10%		rare	• Hotspots are in kinase domain codons: H243-G245
		STAG2	Xq25		<5%	<4%			• Mutations can be located throughout gene • Splice site mutations are frequent

	JMML	CMML	aCML	MDS/MPN RS-T	MDS/MPN U
Abnormal karyotype	30%	~30%	30-40%	20%	50%
CHROMOSOMAL ALTERATIONS:	• Monosomy 7 (clusters with *KRAS*-mutant cases) • Deletion 7q	• -Y • Monosomy 7 and del7q • Trisomy 8 (+8) or +21 • Interstitial deletions of chr 20q, 11q, and 12p • 50% of cases may have large areas of UPD • Complex karyotypes	• Trisomy 8 • Monosomy7 • Deletion 12p • Less common are: idic(Xq), del(5q), t(6;8)(p23;q22), -9, del(11q), del(12q), del(15q), del(17p), t(17;20), and add(21q)	• Trisomy 8 • Less common are: complex/monosomal, del(5q31), del(7q), del(20q), inv(3)(q21q26.2)	• Trisomy 8 • Complex karyotype (15%) • Monosomy 7 (10%)

Key: [a] = Gene alterations which have been shown to have independent adverse prognostic impact on survival outcomes; [b] = Genes common for clonal hematopoiesis of indeterminate potential (CHIP)/age-related clonal hematopoiesis (ARCH); GOF = Gain of function mutation; CMML = ; JMML = ; aCML = ; MDS/MPN-RS-T = ; MDS/MPN-U = ; Chr = Chromsome; RING = Really interesting new gene domain; HET = heterozygous; LOH = loss of heterozygosity; UPD = Uniparental disomy; ITD = Internal tandem duplication domain; TKD = Tyrosine kinase domain; PHD = Pleckstrin homology domain; del = deletion; Diseases with nothing n the frequency column indicate that mutations are not typically seen, and they may be rare or acquired as disease progresses. TRAN. = Transcription Factor

complex proteins (PRC1 and PRC2).[32] It is believed that *ASXL1* mutations result in loss of PRC2-mediated H3K27 (histone 3 lysine 27) tri-methylation.[33] In addition, recent data suggest that *ASXL1* truncations confer enhanced activity on the ASXL1-BAP1 (BRCA associated protein 1) complex. Both of these pathways result in a global erasure of H2AK119Ub and depletion of H327Kme3, promoting dysregulated transcription.[34] EZH2 is a key catalytic component of PRC2, and loss-of-function mutations result in

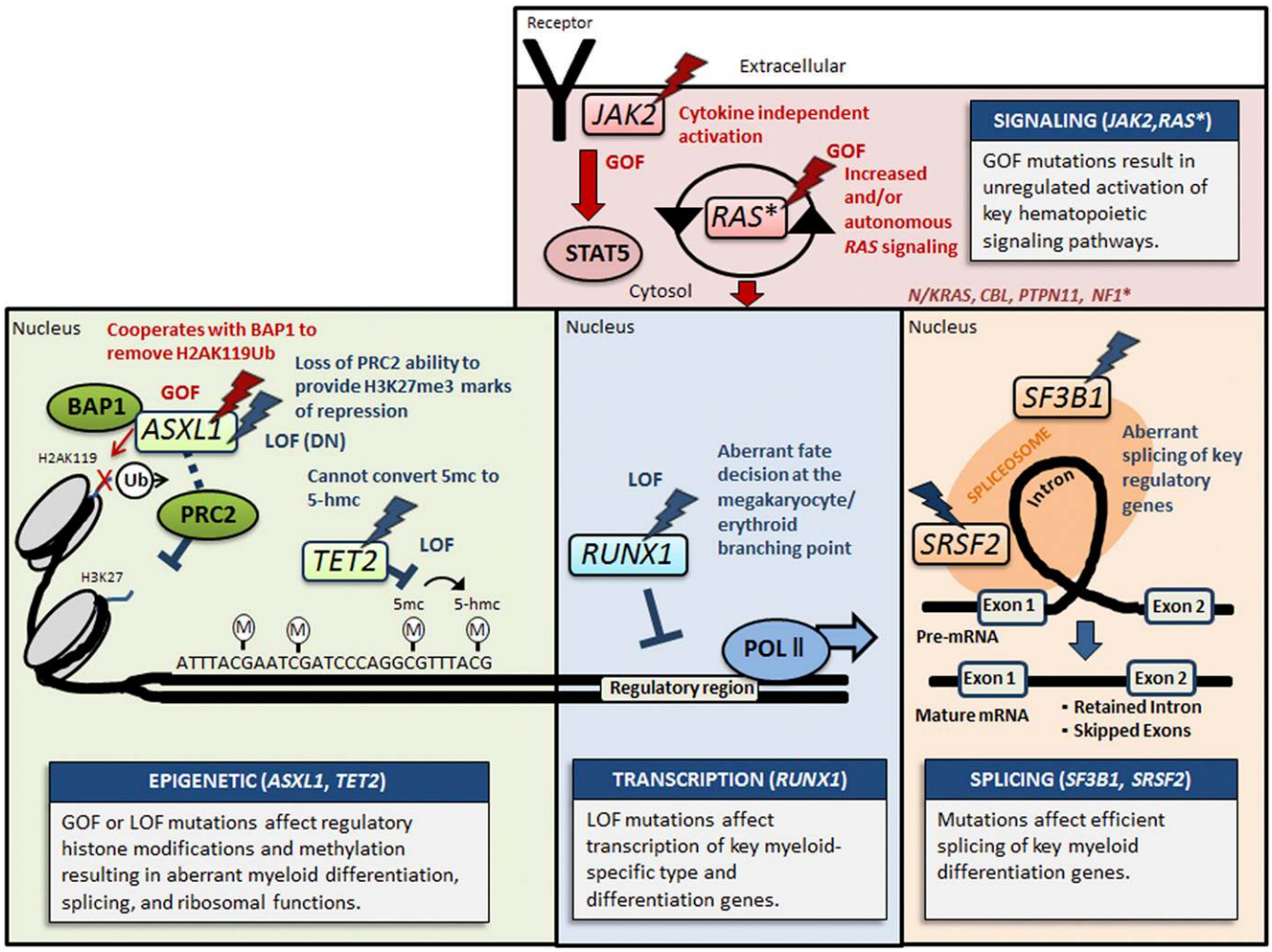

Figure 1. Mechanisms of key mutations in gene categories represented in MDS/MPN overlap syndromes. This figure illustrates the top represented mutated genes in each of four categories: signaling (pink), epigenetic (green), transcription (blue), and splicing (orange). Key mutated genes in each panel are highlighted by lightning, and the color red corresponds to gain-of-function (GOF) mutations, whereas the color blue denotes a loss-of-function (LOF) or dominant negative (DN) mutant effect. Other RAS pathway genes* include *NRAS*, *KRAS*, *CBL*, *PTPN11*, and *NF1*.

dysregulated chromatin dynamics. *EZH2* mutations are seen in aCML (15%) but are uncommon in CMML (<5%), where they often co-occur with *ASXL1* mutations and are associated with poor outcomes.[9,35] *DNMT3A* encodes for the DNA methyltransferase responsible for the conversion of cytosine to 5-methylcytosine. *DNMT3A* mutations are seen in <5% of patients with CMML and are associated with poor outcomes.[8] *IDH1* and *IDH2* are key components of oxidative phosphorylation, with mutant *IDH1/2* generating the oncometabolite 2-hydroxyglutarate (2-HG). 2-HG in turn suppresses *TET2* activity, mimicking a *TET2* mutant effect on methylation, with *IDH1/2* mutations being infrequent (<5%).[5] It is believed that because of the convergence of pathways (2-HG–mediated suppression of TET activity), *TET2* and *IDH1/2* mutations are largely mutually exclusive.

Splicing mutations

Spliceosome components are critical regulators of pre-mRNA splicing, with gene mutations involving *SRSF2*, *SF3B1*, *U2AF1*, and *ZRSR2* implicated in myeloid oncogenesis.[16] In CMML, *SRSF2* mutations are seen in 50% of patients, with no clear impact on survival, whereas *SF3B1* mutations are less common and phenotypically associated with BM RS.[16,36] *SF3B1* mutations are seen in 90% of patients with MDS/MPN-RS-T, often co-occurring with *JAK2*V617F.[24]

Signaling mutations

Aberrant signaling in overlap neoplasms involves mainly the oncogenic RAS pathway and is secondary to mutations involving *NRAS*, *KRAS*, *CBL*, *PTPN11*, and *NF1*.[37] In MPN-CMML, these mutations can be early clonal/dominant events and are associated with poor outcomes,[5] whereas they occur later and impose transformation risk in MDS-CMML. *JAK2* is the other common signaling mutation seen, with *JAK2*V617F identified in 50% of patients with MDS/MPN-RS-T and in 10% of patients with CMML.[38] *CSF3R*, *MPL*, *CALR*, and *FLT3* mutation are uncommon (<5%). Signaling mutations in MDS/MPN overlap neoplasms are associated with cytokine deregulation and inflammation. Mutations involving the JAK/STAT pathway (*JAK2/CALR/MPL*) and the oncogenic RAS pathway (*NRAS*, *KRAS*, *CBL*, *PTPN11*, and *NF1*) result in complex ligand-independent deregulation in cytokine

production and secretion. Cytokines significantly elevated in patients with CMML and signaling mutations include IL-10, CCL2/MCP-1, CD44, IL-1RA, and CXCL7, whereas lower IL-6 levels have been seen in *TET2*-mutant CMML.[39] Although granulocyte–macrophage colony-stimulating factor (GM-CSF) levels were not statistically different between patients with CMML and controls, GM-CSF hypersensitivity has been well documented in both JMML and RAS pathway mutant CMML patient samples.[40]

Transcription factors

RUNX1, a critical transcription factor gene, can be mutated in CMML (15%), MDS/MPN-U (14%), and aCML (12%).[17,30] *RUNX1* mutations are associated with lower platelet counts and a shortened LFS.[17] These mutations should be curated manually to ensure that they are not germline, given that RUNXI-FPD (familial platelet disorder) is associated with an inherent risk for myeloid neoplasms.[17] This is particularly relevant when *RUNX1* mutation variant allele frequencies are in the heterozygous range (40% to 60%). Based on a family history of thrombocytopenia and myeloid neoplasms, personal history of antecedent thrombocytopenia, and the clinical scenario (eg, choosing matched related donors), germline tissue (skin biopsy–derived fibroblast or hair follicle–derived DNA) assessments should be considered. In addition, RUNX1-FPD can result from gene deletions, which are often missed by amplicon based-NGS assays.[17] In these circumstances, copy number analysis can be carried out with array comparative genome hybridization assays.

Others

SETBP1 mutations are found in 15% of patients with CMML and aCML and are associated with inferior outcomes.[2,41] Various oncogenic mechanisms have been proposed, including binding to the SET region and interfering with methylation of lysine residues on histone tails. *TP53* is a critical tumor suppressor gene, and mutations are infrequent in MDS/MPN overlap syndromes. *STAG2* and *RAD21* are components of the cohesion complex, with mutations seen in <10% of patients, with no clear impact on outcomes.

Cytogenetic abnormalities in MDS/MPN overlap neoplasms

Clonal cytogenetic abnormalities are seen in ~30% of patients with CMML, with common alterations including trisomy 8 (+8), −Y, abnormalities of chromosome 7 (monosomy 7 and del7q), trisomy 21, and complex karyotypes.[42-44] The CMML-specific cytogenetic risk stratification (CPSS) system categorizes patients in three groups: high risk (+ 8, chromosome 7 abnormalities, or complex karyotype), intermediate risk (all except for those in the high- and low-risk categories), and low risk (normal karyotype or −Y), with 5-year OS of 4%, 26%, or 35%, respectively.[43] The Mayo–French cytogenetic risk stratification system was developed to refine this prognostication and has three distinct risk categories: high (complex and monosomal karyotypes), intermediate (all abnormalities not in the high- or low-risk groups), and low (normal, sole −Y, and sole der(3q)), with median survivals of 3 (hazard ratio, 8.1; 95% confidence interval, 4.6-14.2), 21 (hazard ratio, 1.7; 95% confidence interval, 1.2-2.3) and 41 months, respectively.[45]

Cytogenetic abnormalities in JMML are uncommon; monosomy 7 is the most common, with this abnormality clustering with *KRAS*-mutant JMML.[21] Although cytogenetic abnormalities are uncommon in MDS/MPN-RS-T (80% with normal karyotype), approximately 50% of patients with MDS/MPN-U have cytogenetic aberrations (+8 and complex karyotypes, 15% each, and monosomy 7, 10%).[23,30] Cytogenetic changes are seen in approximately 30% to 40% of patients with aCML, with +8 being most common.[26]

Integration of molecular and cytogenetic abnormalities for diagnosis, prognostication, and therapeutics of MDS/MPN overlap syndromes

Diagnosis

Although none of the aforementioned gene mutations or cytogenetic abnormalities are specific to a single MDS/MPN subtype, molecular signatures can be used in combination with clinical and morphological features to help establish a diagnosis (Figure 2). Data from clonal hematopoiesis and clonal architectural studies in CMML have shown that coexpression of *TET2* and *SRSF2* mutations result in clonal monocytosis, with the acquisition of subsequent driver mutations defining dysplastic (*RUNX1*, *SETBP1*, *DNTM3A*, *ASXL1*) or proliferative (*NRAS*, *KRAS*, *CBL*, *PTPN11*, *JAK2*) CMML subtypes.[46,47] Based on their frequency and co-occurrence, the presence of *ASXL1*, *TET2*, and *SRSF2* mutations in the presence of adult-onset sustained monocytosis (>3 months) can be used to establish a diagnosis of CMML.[1] As mentioned before, *MPL* and *CALR* mutations are uncommon in CMML, and their presence points toward a differential diagnosis of MPN with monocytosis.[38] In MDS/MPN-RS-T, there is acquisition of driver signaling mutations, most commonly *JAK2*V617F in the context of antecedent *SF3B1* mutant MDS-RS, giving rise to anemia and thrombocytosis.[48] *SF3B1* mutations correlate strongly with the presence of BM ring sideroblasts, and the presence of *JAK2/SF3B1* mutations with BM RS and thrombocytosis can be used to establish a diagnosis of MDS/MPN-RS-T.[36] The presence of germline or somatic RAS pathway mutations, in the context of early-onset monocytosis (infants and children), can be used to establish a diagnosis of JMML, whereas subsequent clonal hematopoiesis (*SETBP1*, *ASXL1*, and *JAK3*) is usually a marker of disease progression. Although aCML and MDS/MPN-U do not have classic molecular features, the relative enrichment of *SETBP1* and *ETNK1* mutations in aCML can be helpful in the presence of dysplastic neutrophilia.

Prognosis

Gene mutations have prognostic value in MDS/MPN overlap neoplasms. *ASXL1* mutations are universally detrimental across myeloid neoplasms and have a particularly poor outlook in CMML.[2,13,14] In CMML, these mutations have been incorporated into three molecularly integrated prognostic models: Mayo Molecular Model, CPSS-molecular, and the Groupe Francophone des Myelodysplasies model.[2,13,14] All three models effectively integrate clinical and molecular features and help risk stratify patients with regard to OS and LFS (Table 3). In addition to *ASXL1* mutations, the CPSS-molecular model includes *NRAS*, *RUNX1*, and *SETBP1* mutations and also incorporates clonal cytogenetic abnormalities (genetic score).[13] In JMML, the presence of germline mutations in *CBL* and *PTPN11* can be associated with spontaneous regressions, and the secondary acquisition of *SETBP1* and *JAK3* mutations is associated with disease progression and inferior OS.[19,22] In fact, in JMML, knowledge on specific nucleotide changes is informative, with somatic *NRAS* and *KRAS*G12S mutations being associated with better outcomes

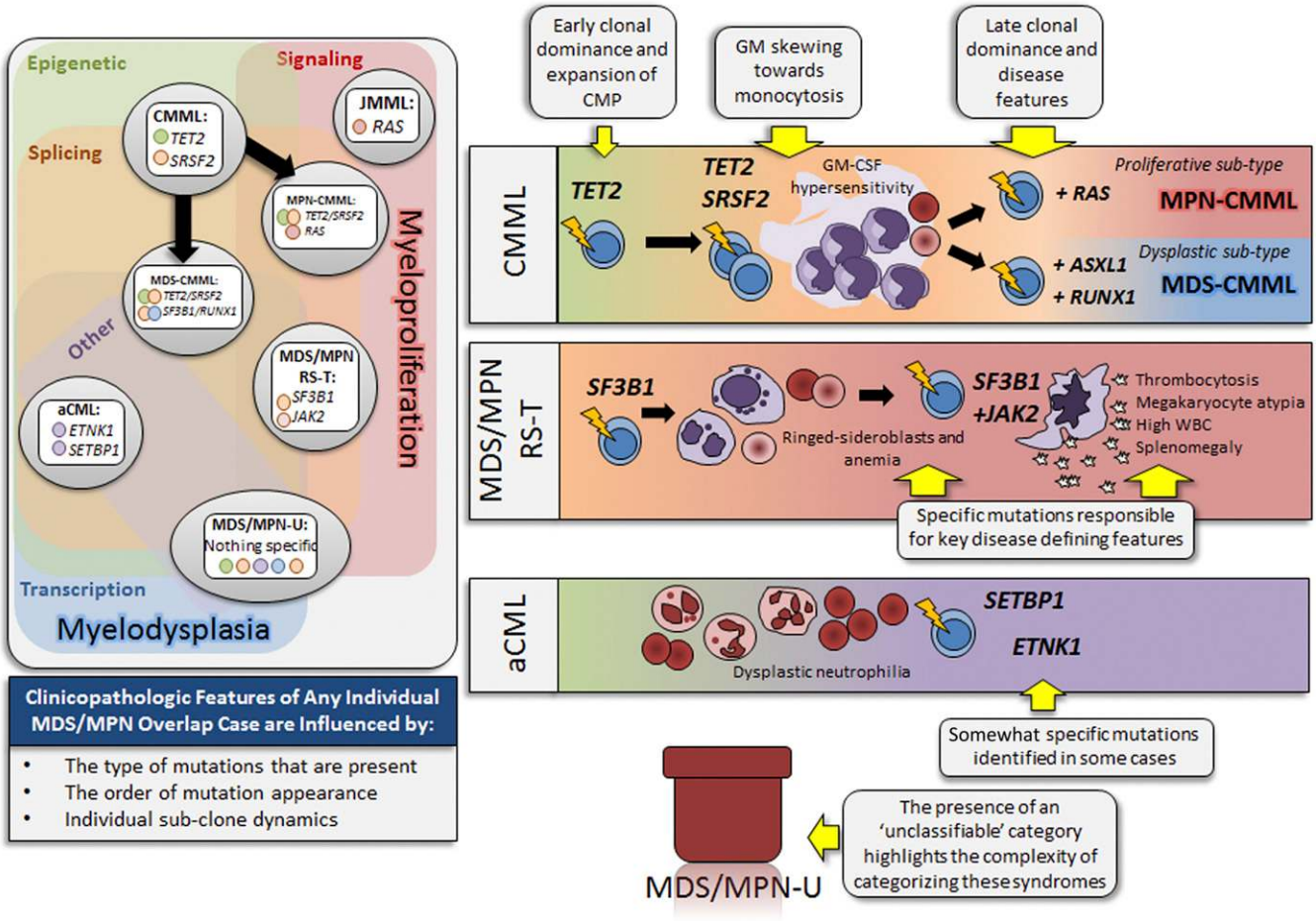

Figure 2. Clonal architecture and molecular signatures of MDS/MPN overlap syndromes. The panel on the left illustrates all 5 MDS/MPN overlap syndrome entities with corresponding specific mutational signatures. CMML has additional subcategories based on the relative enrichment of mutation types in proliferative (MPN-CMML) or dysplastic (MDS-CMML) CMML. Each entity is spatially placed according to mutation type in relation to myeloproliferative (on the right) and myelodysplastic (on bottom) features. The five mutated gene categories are represented in the left panel: epigenetic (green), signaling (pink), splicing (orange), other (purple), and transcription (blue). The panels on the right depict the influence of mutations on each MDS/MPN overlap subtype. aCML, atypical chronic myeloid leukemia; CMML, chronic myelomonocytic leukemia; CMP, common myeloid progenitor; GM, granulocytic-monocytic; JMML, juvenile myelomonocytic leukemia; MDS/MPN-RS-T, MDS/MPN-ring sideroblasts and thrombocytosis; MDS/MPN-U, MDS/MPN-unclassifiable.

than the typical G12D mutations.[49] In MDS/MPN-U we recently demonstrated the negative prognostic impact of *TP53* and *CBL* mutations, and the *ASXL1*mt/*SETBP1*mt genotype is associated with adverse outcomes in aCML.[26,30] Gene mutations are also predictive of allogenic hematopoietic cell transplantation (HCT) outcomes. In a molecularly annotated cohort of 52 CMML patients who underwent HCT, *NRAS* mutations were associated with higher relapse rates, whereas *ATRX* and *WT1* mutations were associated with relapse and an inferior OS.[50] This study also showed that higher mutational burdens (≥10) and mutations involving ≥4 epigenetic regulator genes were associated with poor outcomes.[50]

Clinical therapeutics

Currently, allogenic HCT remains the only curative option for higher-risk MDS/MPN overlap neoplasms, with HMA being used for HCT-ineligible patients. Although HMA epigenetically restores hematopoiesis in a subset of patients with CMML (30% to 40%), serial monitoring of somatic mutations has shown that they do not affect mutational allele burdens, with disease progression occurring in most.[51] Gene mutations that serve as therapeutic targets in myeloid neoplasms are uncommon in MDS/MPN overlap neoplasms. Effective targets such as mutations involving *IDH1*, *IDH2*, and *FLT3* are seen in <10% of patients,[8] and emerging targets such as *TP53* are even more uncommon (<5%). Given the ubiquitous nature of splicing mutations in these diseases, spliceosome component inhibitors in clinical trials are being eagerly watched. MEK inhibition in RAS mutant subtypes has not proven to be an effective strategy.[52] In CMML, the presence of the *ASXL1*wt/*TET2*mt genotype is best associated with responses to HMA,[10,53] whereas clonal RAS pathway mutations (MPN-CMML) are associated with resistance. Gene mutations affecting prognosis (*ASXL1*, *NRAS*, *RUNX1*, and *SETBP1*) in CMML also help with important decisions with regard to timing and the need for allogenic HCT.

Table 3. Genetically integrated prognostic models in MDS/MPN overlap neoplasms

CMML

Model		Risk Categories	Survival (m)	Risk Factors
CMML-Specific Cytogenetic Risk Stratification (CPSS)		Low risk	35% 5-yr OS	• Normal or isolated loss of Y
		Intermediate risk	26% 5-yr OS	• All others
		High risk	4% 5-yr OS	• Trisomy 8, chr 7 abnormalities, or complex karyotype
Mayo-French Cytogenetic Risk Stratification System		Low risk	41	• Normal, sole -Y, and sole der(3q)
		Intermediate risk	21	• All abnormalities not in low or high categories
		High risk	3	• Complex and monosomal karyotypes
Mayo Molecular Model (MMM)	MMM	Low risk (0 pts)	97	• AMC > $10x10^9$/L (2 pts)
		Intermediate-1 risk (≥ 2 pts)	59	• Presence of circulating IMCs (2 pts)
		Intermediate-2 risk (2.5-4.5 pts)	31	• Hemoglobin level < 10g/dL (2 pts) • *ASXL1* mutation (1.5 points)
		High risk (≥ 5 pts)	16	• Platelet count < $100x10^9$/L (1.5 pts)
Groupe Francophone de Myelodysplasies (GFM)	GFM	Low risk (0-4 pts)	65	• WBC > $15x10^9$/L (3 pts)
		Intermediate risk (5-7 pts)	28	• *ASXL1* mutations (2 pts) • Age > 65 years (2 pts)
		High risk (8-12 pts)	17	• Platelet count < $100x10^9$/L (2 pts) • Hemoglobin <10g/dL in females and <11g/dL in males (2 pts)

CMML-Specific Prognostic Scoring System (CPSS-Mol)

Genetic Risk* Score for CPSS

Points for Mutation Status	Unmut	Mut
ASXL1	0	1
NRAS	0	1
RUNX1	0	2
SETBP1	0	1

+

Points for Karyotype Status based on CPSS	Pts
Normal or -Y	0
Anything between	1
Trisomy 8, Monosomal, Complex	2

=

Genetic Risk* for CPSS mol Model	Pts
Low	0
Int-1	1
Int-2	2
High	≥3

CPSS-Mol

Risk Categories	Rate of AML	Survival (m)	CPSS-Mol Score	0 pts	1 pt	2 pts	3 pts
Low (0 pts)	0%	Not reached	• WHO Subtype:	CMML-1	CMML-2		
Intermediate-1 (1 pt)	8%	64	• FAB Sub-type:	MDS-CMML	MPN-CMML		
Intermediate-2 (2-3 pts)	24%	37	•Genetic Risk*:	Low	Inter-1	Inter-2	High
High (≥ 4pts)	52%	18	• RBC transfusion dependence:	No	Yes		

aCML

Model	Risk Categories	Survival (m)	Risk Factors
Mayo Prognostic Model for aCML	Low risk, (0-1 risk factors)	~18	• Age > 67 years • Hemoglobin < 10 g/dL • Presence of *TET2* mutations
	High risk, (≥ 2 risk factors)	~7	

MDS/MPN-RS-T

Model	Risk Categories	Survival (m)	Risk Factors
Mayo Prognostic Model for MDS/MPN-RS-T	Low risk, (0 pts)	80	• Abnormal karyotype (2 pts)
	Intermediate risk (1 pt)	42	• *ASXL1* or *SETBP1* (1 pt each)
	High risk, (≥ 2 pts)	11	• Hemoglobin < 10 g/dL (1 pt)

Key: Pts = Points; CMML = Chronic myelomonocytic leukemia; m = Month; Y = y-chromosome; der = Derivative chromosome; OS = Overall survival; AMC = Absolute monocyte count; IMCs = Immature myeloid cells; WHO = World Health Organization; WBC = White blood cell count; RBC = Red blood cells; CMML-MDS = Dysplastic CMML; CMML-MPN = Proliferative CMML; FAB = French-American-British; *Corresponds to CMML-Specific Cytogenetic Risk Stratification; Inter = Intermediate; depend. = Dependent; Rate of AML = Rate of Transformation (cumulative at 48 months)

Our patient is a 71-year-old man who presented with constitutional symptoms, splenomegaly, anemia, leukocytosis, monocytosis, and thrombocytopenia (Figure 3). His BM has features of dysplasia, and NGS testing has identified mutations involving *ASXL1*, *TET2*, *SRSF2*, and *NRAS*. These features suggest a diagnosis of CMML-1. According to the CPSS-molecular model, he fits into the intermediate-2 risk category, with an estimated median OS of 18 months and a 48% cumulative incidence of AML at 48 months.[13] According to the Mayo Molecular Model, he fits into the high-risk category, with a median OS of 16 months.[2] This patient will benefit from an allogenic transplant consult and will probably need pretransplant cytoreductive therapy with HMA.

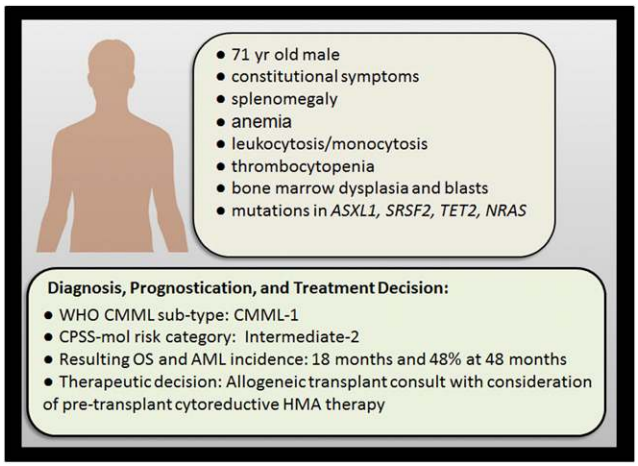

Figure 3. MDS/MPN overlap case study. Shown is the current clinical vignette with symptoms, laboratory results, diagnosis, and resulting prognostication. AML, acute myeloid leukemia; HMA, hypomethylating agent; OS, overall survival.

Conclusions

MDS/MPN overlap neoplasms are a well-defined group of myeloid neoplasms with unique molecular signatures. Mutations in *ASXL1*, *TET2*, and *SRSF2* are common in CMML, whereas the *SF3B1*/*JAK2V617F* genotype often defines the pathobiology of MDS/MPN-RS-T. JMML is a RAS-driven disease, with germline and somatic mutations in the RAS pathway accounting for most cases. aCML is enriched in *SETBP1* and *ETNK1* mutations, and MDS/MPN-U is the least defined in this group. Understanding the molecular landscape in overlap neoplasms is important, because it helps with establishing a diagnosis, helps with disease prognostication, and in certain cases allows selection of appropriate treatment strategies.

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Conflict-of-interest disclosure

M.M.P. has served on the advisory boards for Kura Oncology and Stemline Therapeutics. T.L.L. has no competing interests to declare.

Off-label drug use

None disclosed.

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