An International MDS/MPN Working Group's perspective and recommendations on molecular pathogenesis, diagnosis and clinical characterization of myelodysplastic/myeloproliferative neoplasms

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ABSTRACT

In the 2008 WHO classification, chronic myeloid malignancies that share both myelodysplastic and myeloproliferative features define the myelodysplastic/myeloproliferative group, which includes chronic myelomonocytic leukemia, juvenile myelomonocytic leukemia, atypical chronic myeloid leukemia, refractory anemia with ring sideroblasts and thrombocytosis, and myelodysplastic/myeloproliferative unclassified. With the notable exception of refractory anemia with ring sideroblasts and thrombocytosis, there is much overlap among the various subtypes at the molecular and clinical levels, and a better definition of these entities, an understanding of their biology and an identification of subtype-specific molecular or cellular markers are needed. To address some of these challenges, a panel comprised of laboratory and clinical experts in myelodysplastic/myeloproliferative was established, and four independent academic MDS/MPN workshops were held on: 9th March 2013, in Miami, Florida, USA; 6th December 2013, in New Orleans, Louisiana, USA; 13th June 2014 in Milan, Italy; and 5th December 2014 in San Francisco, USA. During these meetings, the current understanding of these malignancies and matters of biology, diagnosis and management were discussed. This perspective and the recommendations on molecular pathogenesis, diagnosis and clinical characterization for adult onset myelodysplastic/myeloproliferative is the result of a collaborative project endorsed and supported by the MDS Foundation.

Introduction

The chronic myeloproliferative neoplasms are made up of diverse disorders, some with proliferative features and others with dysplastic hematopoiesis. They arise from a pluripotent lymphoid-myeloid stem cell or in some cases a more committed myeloid progenitor.[1] In an attempt to improve their classification, the World Health Organization (WHO) divided them into three distinct categories: myeloproliferative neoplasms (MPNs), myelodysplastic syndromes (MDS) and a category with overlapping characteristics of both MDS and MPNs, referred to as myelodysplastic/myeloproliferative neoplasms (MDS/MPN) or 'overlap MDS/MPN'.[2] The MDS/MPN group is made up of chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), atypical chronic myeloid leukemia (aCML), a 'provisional entity', refractory anemia with ring sideroblasts and thrombocytosis (RARS-T), and a 'by exclusion' subcategory, MDS/MPN unclassified (MDS/MPN-U) (Figure 1).[3,4] Currently there is a paucity of published registry data on the precise incidence of the various subtypes, though there is a perception that the relative incidence of MDS/MPN is quite low. The current classification defines distinct biological entities with myelodysplastic and myeloproliferative features, considerable molecular heterogeneity, and the lack of specific genotypic markers.[5] While monocytosis or eosinophilia foster recognition of CMML/JMML or chronic eosinophilic leukemia (CEL), respectively, the differentiation between aCML, MDS/MPN-U and MPN-U is often difficult. Candidate molecular pathways include JAK-STAT, mTOR, PI3K/AKT, MEK signaling cascades and epigenetic changes, most of which are of interest for developing targeted agents.[6]

To address some of the current challenges related to MDS/MPN, a panel comprised of laboratory and clinical experts in MDS/MPN was established, and four independent academic MDS/MPN workshops. These were held in Miami, Florida, USA (9th March 2013), in New Orleans, Louisiana, USA (6th December 2013), in Milan, Italy (13th June 2014), and in San Francisco, USA (5th December 2014), under the aegis of the MDS Foundation. In addition, several conference calls involving deliberations and discussions amongst the panellists took place between June 2013 and December 2014. A concise perspective and recommendations on molecular pathogenesis, diagnosis, clinical characterization and management of adult onset MDS/MPN based on the result of this collaborative initiative is summarized here; recommendations for uniform response in MDS/MPN have been submitted in a separate report.

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MDS/MPN: cytogenetic, molecular genetics and signaling abnormalities

Chromosome analysis using conventional cytogenetics and high-resolution single nucleotide polymorphism array karyotyping (SNP-A) reveals chromosome abnormalities in 70% of MDS/MPN patients.[7] Most of these are aneuploidies (trisomy 8, monosomy 7) or deletions (del7q, del13q, del20q); a minority have reciprocal translocations involving diverse tyrosine kinase (TK) fusion genes.[8,9] Some of these fusions are listed separately within the current WHO classification: 'myeloid and lymphoid neoplasms with eosinophilia' (MLN-eo) and abnormalities of *PDGFRA, PDGFRB* and *FGFR1*. Fusions involving other kinases are also seen in patients with MDS/MPN or MPNs.[10] Fusions involving *PDGFRA*, *PDGFRB* and *ABL1* are important to recognize as they confer sensitivity to TK inhibitors (TKIs), such as imatinib.[11] Other fusions involving *FGFR1* or *JAK2* are insensitive to imatinib but may respond to ponatinib or ruxolitinib, respectively.[12-16]

Most mutant genes fall into four functional classes: signaling, epigenetic, splicing and transcription (Figure 2).[17-20] Signaling mutations result in aberrant activation of proliferative and anti-apoptotic pathways normally induced by growth factors (GFs). In addition to the TK gene fusions mentioned above, mutations have been described in GF receptors (*CSF3R*), downstream cytokine receptor signaling intermediates (*JAK2, NRAS, KRAS*) and negative regulators of signaling pathways (*PTPN11, CBL, NF1*).[21-27] Mutations involving RAS are demonstrable in 90% of JMML cases and may emerge as a defining feature of this condition.[28] Signaling mutations occur in approximately 50% of CMML patients and correlate with a myeloproliferative phenotype and enhancement of *in vitro* sensitivity to GM-CSF.[29] Up to 80% of patients with RARS-T have activated JAK-STAT signaling as a consequence of the presence of *JAK2*^{V617F} or mutations in *MPL* [encoding for the thrombopoietin receptor (Tpo-R)].[30] In mice, abrogation of Notch signaling leads to a MDS/MPN phenotype, but its relevance in humans is unknown.[31]

MDS/MPN: nuclear events - epigenetics, spliceosomes and transcription factors

Mutations in genes encoding epigenetic regulators are common in MDS/MPN.[32-35] The most frequently mutated genes are TET2 and ASXL1, followed by *SRSF2, IDH1/2, EZH2, SUZ12, EED* and *UTX*.[36] The interaction between epigenetic mutations is complex, and apart from the general mutual exclusivity of *TET2* and *IDH1/2* mutations, no clear patterns have emerged.[37,38]

Mutations in elements involved in the recognition and processing of 3'-mRNA splice sites are also common in MDS/MPN.[39] Around 50% of CMML patients have mutations involving SRSF2, with a further 20% exhibiting mutations in other splicing complex genes (*SF3B1, U2AF35, U2AF65* and *SF3A1*).[34,35,40,41] In addition, *SF3B1* mutations are present in 72% of patients with RARS-T.[42,43] These *SF3B1* mutations are not always mutually exclusive and may be accompanied by *DNMT3, JAK2, ASXL1* and *TET2* mutations. Functionally, disruption of SF3B1 function leads to the formation of ring sideroblasts; however, its exact role in malignant transformation remains unclear.[20,44-47] Studies of mutant *U2AF35* in model systems indicate global impairment of splicing induction of mRNA surveillance pathways and impairment of growth. Nevertheless, it is not known if the critical effect of such mutations is indeed global or whether they impact only a small subset of genes.

The *RUNX1* gene is mutated in 15%-30% of CMML patients. *RUNX1* encodes core-binding factor alpha (CBFα), which plays a fundamental role for definitive commitment of hematopoiesis. *NPM1* and *TP53* are mutated in only a small percentage of cases. SET binding protein 1 (*SETBP1*) was recently identified as a novel oncogene mutated in 25% of aCML cases, and less frequently in other MDS/MPN.[48] The precise downstream consequences of SETBP1 mutations are unknown, but they may attenuate the activity of the tumor suppressor phosphatase, PP2A though abrogation of a ubiquitination site (functionally equivalent to overexpression). A small minority of MDS/MPN have calreticulin (CALR) mutations, which are more commonly associated with *JAK2* and *MPL* unmutated MPN.[49] Although somatically acquired in myeloid malignancies, mutations in *SETBP1, ASXL1, EZH2* and other genes are also found in rare congenital developmental disorders of variable phenotypic severity. A likely unifying factor is that these mutations alter the expression of *HOX* genes that are important for both embryonic development and adult hematopoiesis.[48,50]

Table 1. A potential diagnostic approach for patients suspected to have myelodysplastic/myeloproliferative neoplasms.

	CMML	aCML	MDS/MPN-U
Mean age	72	72	72
Sex ratio	2/1	2/1	2/1
Mean OS	~3 years	~1 year	~2 years
Incidence	1/100000	1/100 CML	Unknown
Criteria	Monocytosis > 1 G/L at least 3 months +/- bone marrow cell dysplasia	Persistent leukocytosis > 13 G/L + immature circulating myeloid precursors > 10% of leukocytes + Marked dysgranulopoiesis, and - Absent/minimal monocytosis (<1 G/L and <10% of leukocytes) - Absent/minimal basophilia (<2%)	Heterogeneous group of rare myeloid neoplasms with myeloproliferative features & myelodysplastic features that cannot be classified as JMML, CMML, RARS-T, and aCML

A definitive diagnosis of MDS/MPN requires the exclusion of: AML: BM blast cells < 20%; CML: lack of BCR-ABL; MLN-Eo: lack of PDGFR/FGFR fusion & eosinophilia. CMML: chronic myelomonocytic leukemia; aCML: acute chronic myeloid leukemia; MDS: myelodysplastic syndromes; MPN-U: myeloproliferative neoplasms-Unknown; AML: acute myeloid leukemia; myeloproliferative neoplasms; BM: bone marrow.

MDS/MPN: subtypes

Chronic myelomonocytic leukemia

The annual incidence of chronic myelomonocytic leukemia (CMML) is 1/100,000 adults, with a median age of 70 years and a male predominance.[51] The BCR-ABL1

Table 2. Principal historical CMML risk models.

Study	n (total)	n (CMML)	Treatment	CMML validation	Cytogenetics considered	Genetics considered
IPSS	816	126**	N	[1]	Y	–
IPSS(R)	7012	631**	N	–	Y	–
MDASC	1915	306	Y****	[1]	Y	–
MDAPS	213	213	Y***	[2]	–	–
DS	235	25	N	[1,2]	–	–
SS	70	70	Y*	[1,2]	–	–
mBS	53	53	N	[1,2]	–	–

*IPSS: International Prognostic Scoring System; IPSS-R: International Prognostic Scoring System Revised; MDASC: Global MD Anderson Scoring System; MDAPS: MD Anderson Scoring System for CMML; DS: Düsseldorf Score; SS: Spanish Score; mBS: Modified Bournemouth Score. *35% of patients received low-dose cytarabine, hydroxyurea or mercaptopurine. **Patients with a WBC >12x10^9/L excluded. ***Patients received either supportive care (n=71), α- or γ-interferon (n=9), low-dose or single-agent chemotherapy (hydroxyurea with or without busulfan or mithramycin, low-dose cytarabine, topotecan, fludarabine, 6-mercaptopurine, thioguanine, oral idarubicin, oral etoposide, 9-nitrocamptothecin, azacitidine) (n=68), or intensive chemotherapy (n=65). ****1097 received growth factors, chemotherapy, or transfusions (318 had received transfusions only).*

gene and rearrangements of either *PDGFRA, PDGFRB* or *FGFR1* are absent. The *JAK*^{V617F} mutation occurs in less than 10% of patients with CMML, in particular those with proliferative, rather than dysplastic features.[52] Rarely, CMML can be therapy-related or a secondary neoplasm, arising in the background of MDS or as a progression of myelofibrosis (MF), in particular in the presence of an *SRSF2* mutation.[53,54]

Although the diagnosis of CMML is based on laboratory, morphological and clinical parameters, the incorporation of molecular data is now recognized, with the notable presence of somatic mutations in *TET2* (50%-60%), *SRSF2* (40%-50%), *ASXL1* (35%-40%) and *RUNX1* (15%). Indeed, several investigators have noted that over 90% of CMML patients studied exhibited one or more mutations and that concurrent mutations in *TET2* and *SRSF2* appear to be highly specific for this entity.[34,55,56] Other mutations include those affecting cytosine methylation (*DNMT3A, IDH2, IDH1*), RNA splicing (*SF3B1, U2AF35, ZRSR2*), chromatin remodeling (*UTX, EZH2*), and signaling pathways (*NRAS, KRAS, CBL, JAK2, FLT3, CSF3R*), whereas *TP53* mutations are rare.[33,55-58] A cardinal feature is persistent peripheral blood monocytosis more than 1x10^9/L, with a WBC percentage of monocytes of more than 10%. Morphologically, these monocytes demonstrate an abnormal appearance with bizarre nuclei and cytoplasmic granules.[59] In some patients, blood cells identified as monocytes are later recognized to be dysplastic and immature granulocytes endowed with immunosuppressive properties.[60] Clinical features include splenomegaly, skin and lymph node infiltration, and serous membrane effusions. The diagnostic criteria for CMML *versus* aCML *versus* MDS/MPN-U are shown in Table 1; RARS-T is a provisional entity that remains apart.

The current WHO classification divides CMML into two risk groups, CMML-1 and CMML-2, based on the number of blasts and promonocytes in the peripheral blood and bone marrow (BM) (Figure 3A-D).[3] The BM is hypercellular with dysplasia and an increase in the 'paramyeloid cells'; some patients may also have reticulin fibrosis.[61] Recent data from the Dusseldorf registry also suggest the notion of a poorer outcome in 'proliferative' compared to 'dysplastic' CMML.[62] Cytogenetic abnormalities include trisomy 8, monosomy 7, del(7q), and rearrangements with a 12p breakpoint.

Clonal architecture analysis in CMML has demonstrated linear acquisition of candidate mutations with limited branching through loss of heterozygosity.[56] The principal CMML characteristics seem to be early clonal dominance arising within the CD34(+)/CD38(-) cells, and the subsequent granulo-monocytic differentiation skewing of progenitors. Based on this, a unique causal linkage between early clonal dominance and skewed granulo-monocytic differentiation has been proposed (Figure 4).[63]

Another important biological feature is the unique hypersensitivity to GM-CSF, as measured by hematopoietic colony formation and GM-CSF-dependent phosphorylation of STAT5.[29,64] This STAT5 pathway convergence is supported by transgenic models of mutated genes in CMML. Mouse models recapitulating mutations in *TET2, JAK2, CBL,* and *NRAS* have also been reported to up-regulate the STAT5 pathway and/or increase hematopoietic colony formation in a cytokine-dependent fashion. These novel observations support the candidacy of Janus kinase (JAK) inhibitors and other novel treatment strategies in future CMML clinical trials.

Most, if not all, of the prognostic tools in CMML have been derived from studies focused on MDS and preceded the use of hypomethylating agents (HMAs) (Table 2).[65-71] Recent efforts include genetic information and clinical features.[55,72] Solary and colleagues sequenced *ASXL1* and other genes, including epigenetic (*TET2, EZH2, IDH1, IDH2, DNMT3A*), splicing (*SF3B1, SRSF2, ZRSR2, U2AF1*), transcription (*RUNX1, NPM1, TP53*), and signaling (*NRAS, KRAS, CBL, JAK2, FLT3*) regulators in 312 patients with CMML. They noted that *ASXL1* mutations, age, hemoglobin, WBC, and platelet counts defined three prognostically distinct patient subsets with varied overall survival (Figure 5). Such and colleagues proposed a 'CMML-specific prognostic scoring system' (CPSS), based on cytogenetics and red blood cell (RBC) transfusion dependence, which divides patients into four risk groups for survival and risk of AML transformation.[73] A third group of authors identified absolute monocyte count, presence of circulating immature myeloid cells, anemia and thrombocytopenia, but not the spliceosome complex nor *ASXL1* mutations, as independent variables for survival.[74] These investigators also confirmed the independent prognostic value of the *SETBP1* mutation in CMML, initially reported by the Solary group.[60,75]

The clinical management of patients with CMML can often be a challenge since some patients have a relatively indolent disorder with median survival in excess of ten years, whilst others progress rapidly to secondary AML, which is often difficult to treat. Allogeneic stem cell transplantation (allo-SCT) remains the only treatment modality associated with long-term remissions and potential cure; for transplant ineligible patients, there is no firm consensus with regards to the optimal treatment. The French registry data suggest a 3-year overall survival (OS) of 32% in a cohort of CMML patients allografted in chronic phase.[76] Survival was negatively influenced by the presence of splenomegaly. Similar results have been reported by other groups, though few focused exclusively on patients with CMML.[77] The Seattle group reported a 10-year OS of approximately 40%.[78] Factors associated with favorable outcomes appear to be CMML risk group (CMML1 *vs.* CMML2), pre-transplant hematocrit, cytogenetic risk category, comorbidity index, and age. Intriguingly, in this series there appeared to be a gender influence on risk of relapse, with female-female transplants faring the worst. It was worthy of note that neither the type of pre-conditioning regimen [reduced intensity conditioning (RIC) *vs.* myeloablative] nor the type of pre-transplant therapy appeared to influence allo-SCT outcomes significantly. There was, however, a tendency for a lower relapse and a better survival with fludarbine and targeted busulfan conditioning. Most published results suggest disease relapse as a principal cause for transplant failure.

The historical results of conventional allo-SCT have been confounded by the substantial non-relapse transplant-related mortality (NRM). This is probably due, at least in part, to the older age of patients with CMML and the increasing presence of significant co-morbid conditions. Efforts to improve these results have led to general improvements in allo-SCT technology. These include strategies to enhance the graft-*versus*-leukemia (GvL) effects, which account for probable cure in those who achieve long-term remission, and an increased use of RIC preparative regimens. At present, the very considerable advances in the understanding of the genomic landscape in CMML, with the notable exception of *ASXL1*, appear not to have been validated sufficiently for adaptation in treatment algorithms to assess candidacy for allo-SCT compared to conventional therapy. Results of treatments for CMML patients who are either in frank AML transformation, or at high risk of transformation, remain subopti-

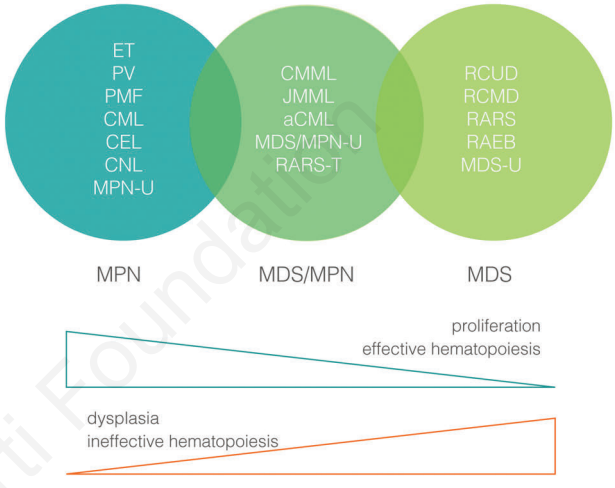


Figure 1. **Myeloproliferative neoplasms and myelodysplastic syndromes.**

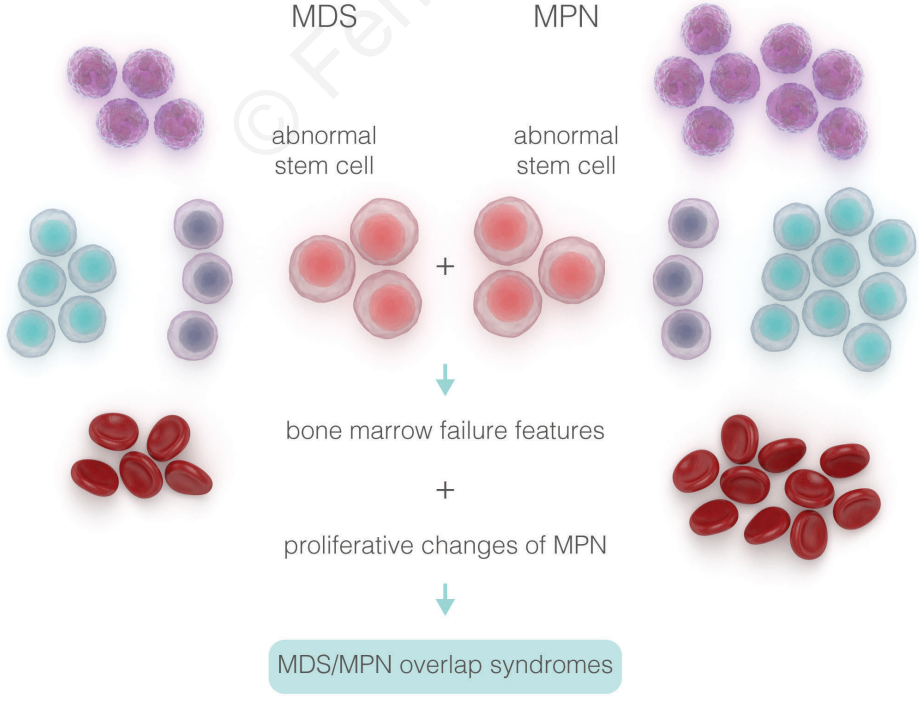


Figure 2. **A schematic description of genotypic diversity in patients with myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN).**

mal, with a median survival of 2.4 months for those who fail to achieve a complete remission following induction chemotherapy; for patients who achieve a complete remission following induction and then receive an allo-SCT, survival is about 28 months.[79]

Hypomethylating agents (HMAs) are currently the preferred non-transplant treatment option, though the response rates are relatively low, with no important impact on overall survival.[80-83] Furthermore, even when responses are achieved, most tend to be short-lived. It is of interest that, in a recent study, *ASXL1, RUNX1* and *TET2* mutations portended a better response to decitabine, whereas *MYB* and *JUN* expression negatively affected outcome.[84] Current efforts are investigating diverse agents, including JAK and MEK inhibitors, BCL-X_L and BCL-2 inhibitors, clofarabine, next generation HMAs, and other novel agents.

The notion of using HMAs in order to improve the performance status and allo-SCT eligibility appears attractive. What is not known is the impact of HMAs on short and long-term outcomes following allo-SCT. There are no CMML transplant-specific risk scores other than the time-tested Gratwohl methodology for transplant recipients in general.[85] An interesting compromise would be to offer a transplant only to patients with high-risk disease or patients with low-risk disease in whom parameters start to deteriorate. This may allow patients responding to HMAs to continue the therapy until resistance/intolerance, or indeed, progression of disease, are noted. The disadvantages with such an approach are the potential risks for leukemic transformation and the prolonged use of a therapy that has yet to demonstrate a durable survival benefit.

Atypical chronic myeloid leukemia

Atypical chronic myeloid leukemia (aCML) is an extremely rare subtype of MDS/MPN with an estimated incidence of 1% that of typical *BCR-ABL1*-positive CML.[86] It was initially described as a subtype of myeloid neoplasm resembling CML, but with the notable absence of the *BCR-ABL1* fusion gene. Diagnosis of aCML requires the exclusion of not only *BCR-ABL1*, but also rearrangement of *PDGFRA, PDGFRB* or *FGFR1*.[3,87] Patients tend to have severe anemia, thrombocytopenia, neutrophilic leukocytosis with granulocytic dysplasia, and splenomegaly; monocytosis and basophilia are not prominent in the peripheral blood.[88] In the clinic, aCML patients can be difficult to distinguish from those with another very rare MDS/MPN subtype, known as MDS/MPN-U.

Orazi and colleagues recently analyzed a series comprising 69 patients with aCML and 65 with MDS/MPN-U, in an effort to define clinical, histological and genetic characteristics which would help distinguish these two rare entities.[89] They identified aCML patients to have an aggressive disease course, with a poor prognosis and an overall survival of 12.4 months, compared with 21.8 months for patients with MDS/MPN-U (P=0.004). They attempted to subclassify the study cohort by the presence of leukocytosis more than 13x10^9/L, peripheral blood myeloid precursors more than 10%, and dysgranulopoiesis more than 10% in patients with aCML (Figure 6). Median leukocytes for aCML was 40.8x10^9/L, compared to 19.4x10^9/L for those with MDS/MPN-U (P<0.001). Bone marrow (BM) samples revealed hypercellularity and dysgranulopoiesis in all patients with aCML, compared to about half of MDS/MPN-U patients; there was variable fibrosis and osteosclerosis, and non-specific recurrent complex cytogenetic abnormalities and i(17q) appeared to be slightly more frequent in aCML. aCML patients were also found to have increased LDH, splenomegaly, severe anemia, thrombocytopenia less than 100x10^9/L, higher peripheral blood myeloid precursors, and less than 2% basophils.

Although no specific molecular abnormality has been described in aCML, recurrent mutations in *SETBP1*, located on chromosome 18q21.1, have been observed in 25% of aCML, 6%-15% of CMML and less than 3% of JMML cases.[58,90-93] The functional significance of these mutations are not yet fully understood. Recurrent somatic mutations

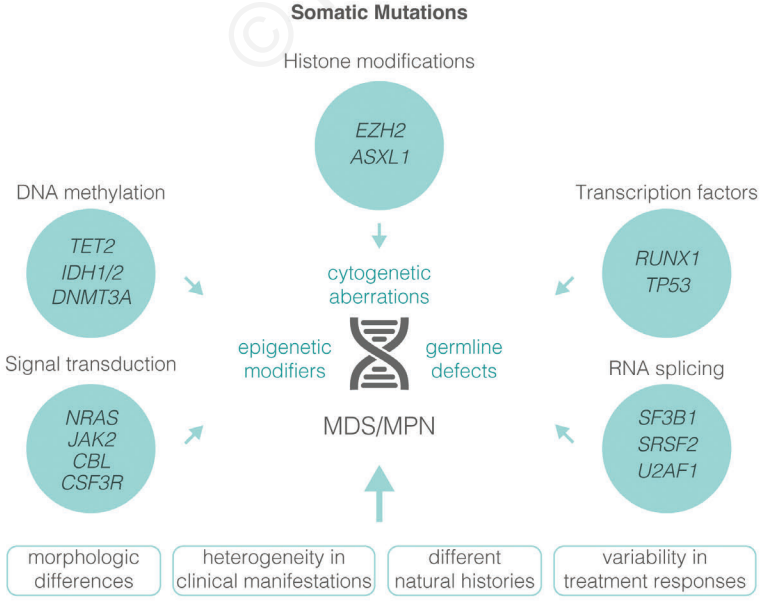


Figure 3. A photomicrograph from a patient with chronic myelomonocytic leukemia (CMML)-1. (A) Peripheral blood smear showing three abnormal monocytes and one neutrophil. (B and C) Bone marrow aspirate and the corresponding napthyl butyrate esterase image of the aspirate. (D) Bone marrow trephine biopsy.

in *JAK2, NRAS, IDH2, CBL, CSF3R* and *ETNK1* can also be detected in aCML, although at a much lower frequency; anecdotal cases with fusion genes such as *BCR-JAK2* or *NUP98-HOXA9* have also been detected.[94-97] Future studies should provide insights into the potential impact of such analyses on precision-medicine therapeutic approaches. In this regard, the recent proposal of considering the reactivation of PP2A as a therapeutic strategy in SETBP1-mutated cells is of interest.[98]

There are also clinical and morphological similarities between aCML and chronic neutrophilic leukemia (CNL), a rare subtype of MPN. The genomic landscape, however, appears to be quite distinct. A seminal observation by Maxson and colleagues demonstrated the presence of mutated *CSF3R* in about 90% of patients with CNL and 40% of those with aCML; subsequent studies confirmed the high frequency in CNL but were unable to confirm the mutations in aCML.[99-101] This gene encodes the receptor for colony-stimulating factor 3 (G-CSF).[27] Somatic *CSF3R* mutations, together with *ELANE, HAX1*, and *G6PC3* mutations have previously been described in severe congenital neutropenia (SCN).[102] A germ-line T640N *CSF3R* mutation has also been identified in hereditary neutrophilia. Interestingly, a homologous *CSF3R* somatic mutation affecting the extracellular domain and conferring autonomous signaling properties has been found in sporadic transformed SCN and *de novo* AML.[103] In sporadic cases, the most common *CSF3R* mutation is *CSF3R*^{T618I}, which strongly activates the JAK/STAT pathway; however, *CSF3R* truncating mutations were also observed and these predominantly signal through SRC family kinases.[104] Recently, a CALR mutation was reported in a case of *CSF3R*-positive CNL.[105]

Allo-SCT appears to be the only treatment that can accord aCML patients a long-term remission, though there is no firm consensus due to the extremely low incidence of this rare disease. Most of the published series, including registry data, include aCML as part of a more general series of myeloid malignancies. A recent report of 2 aCML patients with a heterozygous *CSF3R*^{T618I} mutation is of some interest as it highlights the candidacy of this mutation to be used as a disease-specific biomarker of residual disease.[106]

Patients not suitable for allo-SCT often receive HMAs with some demonstrating transient improvements in some of the clinical and pathological features. Other treatments used include hydroxyurea and lenalidomide. It is best, therefore, to offer these patients suitable clinical trials. The notion of the CSF3R mutation activating the JAK/STAT pathway and, in some instances, the SRC kinases, provides some support for clinical trials to assess JAK inhibitors, such as ruxolitinib, and SRC inhibitors, such as dasatinib, respectively. A recent case report of a *CSF3R*^{T618I} -positive-aCML patient treated with ruxolitinib showed a significant improvement in his constitutional symptoms and splenomegaly, providing additional support for such trials.[107]

Juvenile myelomonocytic leukemia

Juvenile myelomonocytic leukemia (JMML) is an uncommon WHO-defined MDS/MPN with an incidence of 0.12 per 100,000 children, a median age of two years, and a disproportionate male preponderance. It carries a poor prognosis[108,109] and shares some clinical and molecular features with CMML. Congenital JMML predisposition syndromes exist, particularly neurofibromatosis and Noonan syndrome, which converge on RAS signaling abnormalities and markedly increase the risk of developing JMML[110,111] JMML is a heterogeneous clinical entity in that some patients, particularly those with Noonan syn-

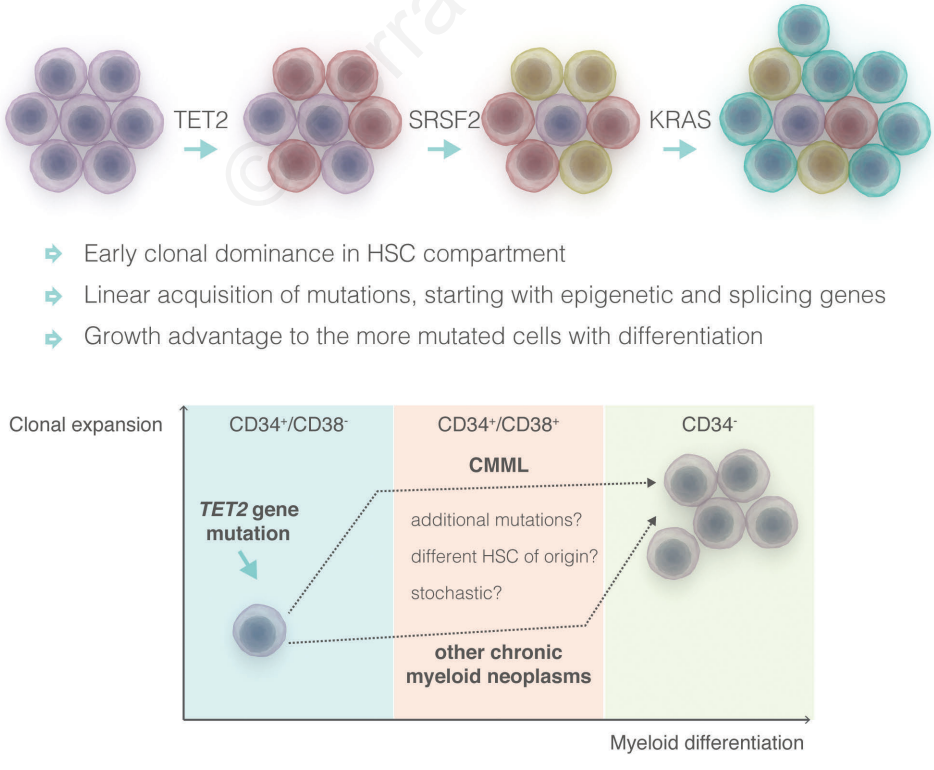


Figure 4. Early clonal dominance (CD34$^+$/CD38$^-$cells) in chronic myelomonocytic leukemia (CMML) compared to myeloproliferative neoplasms (MPN). Adapted from Itzykson *et al.*[56]

drome, have spontaneous resolution of their disease despite identification of clonal hematopoiesis, while others can have a fulminant course refractory to allo-SCT.[112,113] Although leukemic transformation is seen in JMML, it is uncommon in comparison with adult myeloid malignancies.[114]

Clinically JMML is characterized by an overproduction of monocytes that infiltrate liver, spleen lung, intestine and other organs, which may also lead to considerable morbidity and mortality. The cardinal clinical features also include fever, thrombocytopenia, monocytosis, splenomegaly, hepatomegaly, hemoglobin F elevations, and failure to thrive. Despite a readily apparent diagnostic marker of disease (peripheral monocytosis), the diagnosis of JMML is not straightforward due to the extreme rarity of disease and confounding clinical characteristics in common with more common entities (such as viral infections).

The above notwithstanding, JMML is arguably considered the most well understood hematologic malignancy after CML, at least in children. Most, if not all, children with JMML harbor either a somatic or germ-line unique mutation in the Ras pathway (*PTPN11*> *NF1*>*NRAS*/*KRAS*>*CBL*).[115,116] In rare cases, additional mutations in SETBP1 or JAK3 have been identified; these appear to confer a poorer prognosis.[117] However, the mutational landscape of JMML distinguishes it from various adult myeloid malignancies in that the genetic abnormalities appear to be restricted to a limited set of genes, particularly excluding epigenetic and alternative splicing modifiers that are enriched in adults.[118,119] It is also of some interest that most patients with JMML exhibit an increased *in vitro* sensitivity to GM-CSF.[120] Despite the virtually universal dysregulation of RAS, this hypersensitivity appears to augment signaling of other downstream effectors, particular JAK/STAT.[121,122] This has been demonstrated in human samples and murine models of *NRAS*-derived JMML.[29]

Allo-SCT remains the principal treatment for JMML, with an event-free 5-year survival of 52%.[123] The principal cause for failure is relapse, which approaches 50%, though 50% of these patients can be rescued with a second allograft.[124,125] It has been speculated that the high relapse rate might be related to an underlying fundamental immune defect or incomplete eradication of resistant disease prior to myeloablation. Strategies to rescue children post relapse remain suboptimal, with limited success of donor lymphocyte infusions (DLI).[126]

Current non-transplant alternatives are limited, and many efforts to target underlying driver mutations are in progress. Efforts to target the RAS proteins, which is involved in the vast majority of JMML patients, have met little success so far.[127] The first generation of farnesyltransferase inhibitors (FTIs) have now been tested, but in view of unacceptable toxicities and poor efficacy they are no longer under development. Clinical trials are now in progress with MEK inhibitors, such as trametinib, JAK inhibitors, such as ruxolitinib, as well as SRC inhibitors and HMAs.[128-130]

RARS-T

Considerable debate remains as to how RARS-T is best characterized among chronic myeloid malignancies. It was provisionally defined by the WHO to be part of the MDS/MPN in 2001, since patients had MDS features of refractory anemia with ring sideroblasts (RARS) in addition to thrombocytosis and megakaryocyte cytological features resembling essential thrombocythemia (ET).[131,132] By the time of the 2008 revision of the WHO classification, several reports indicated the presence of clonal *JAK2*^{V617F} and *MPL*^{W515} gene mutations in RARS-T, favoring the notion that this sub-category should be considered an

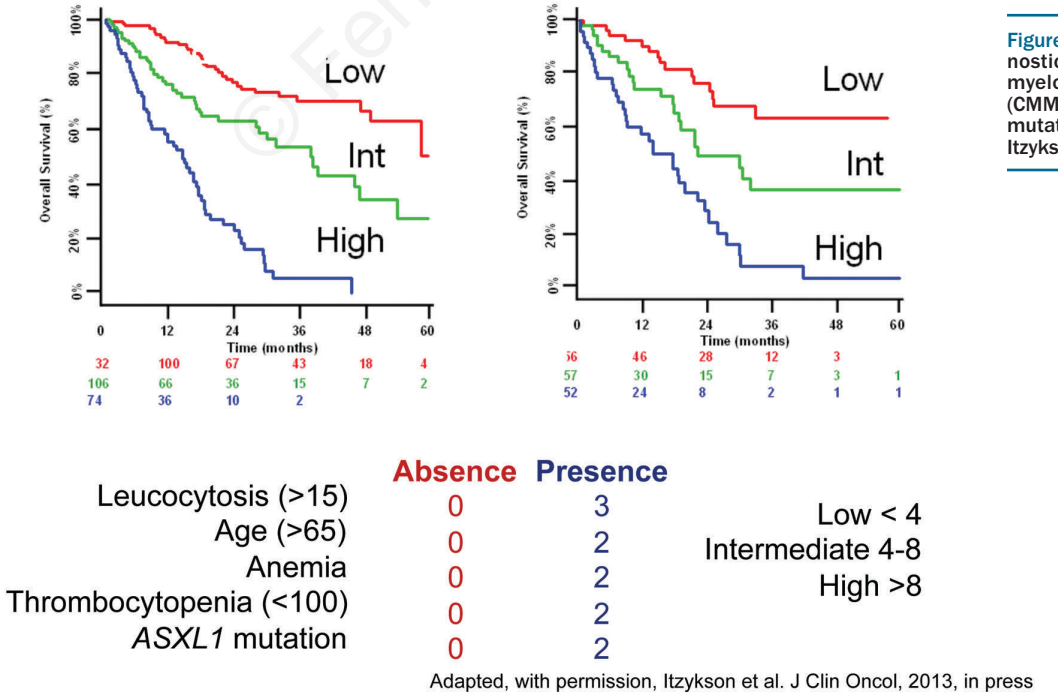


Figure 5. A simplified prognostic score for chronic myelomonocytic leukemia (CMML) that includes *ASXL1* mutations. Adapted from Itzykson *et al.*[55]

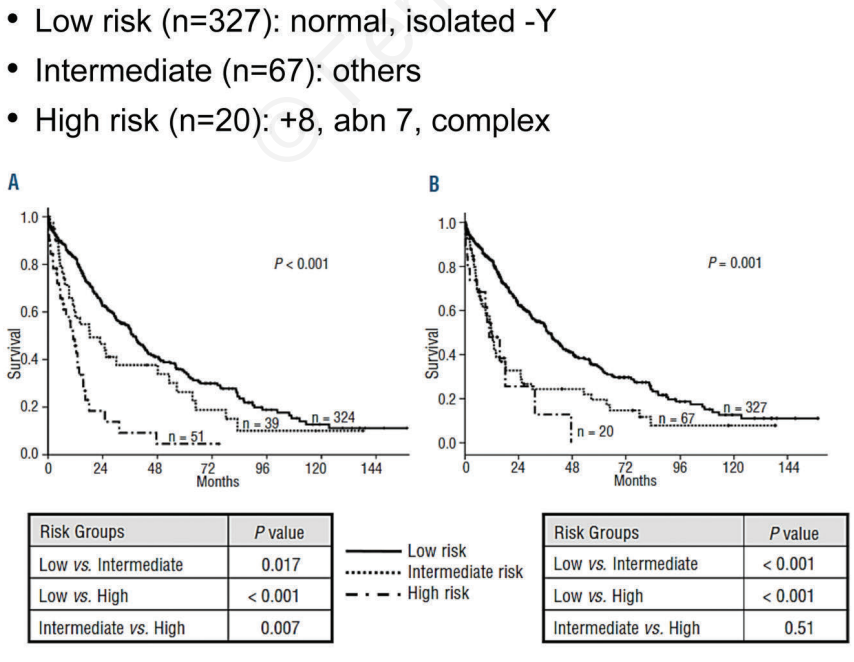
MPN akin to ET. Nonetheless, RARS-T displays poor *in vitro* colony forming capacity, a recognized feature of MDS.[3] Distinction between RARS-T and RARS with moderate thrombocytosis has become more difficult following the WHO 2008 revisions that lowered the platelet threshold for RARS-T and ET from more than 600x10^9/L to more than 450x10^9/L. Therefore, RARS-T currently remains a 'provisional' member of the MDS/MPN family, in which entity mutations in *SF3B1* (60%-80% of cases) may be responsible for mitochondrial iron overload in sideroblasts, ineffective erythropoiesis, and anemia (myelodysplastic features), while mutations in *JAK2* or *MPL* are thought to be responsible for thrombocytosis (myeloproliferative features) (Figure 7).[133,134] Furthermore, the notion of secondary RARS-T developing in RARS has also been suggested.[135]

As in the case of the other MDS/MPN, there is no firm consensus regarding optimal clinical management and supportive care remains the cornerstone of treatment. Since the thrombotic risk in RARS-T appears to be low, there is no recommendation for platelet-suppressive therapy or aspirin prophylaxis. However, a recent report noted an increased rate of thrombotic events in RARS-T patients carrying *SF3B1* mutations.[136] There are anecdotal reports of 'partial remission' following the use of imatinib or lenalidomide.[137-139] In the 60%-80% of RARS-T patients who harbor a *JAK2* or *MPL* mutation, it is reasonable to consider a JAK inhibitor.

MDS/MPN-Unclassified

MDS/MPN-Unclassified (MDS/MPN-U) is quite possibly the most heterogeneous subgroup of MDS/MPN and includes patients who lack defining characteristics of the other MDS/MPN subtypes. Some patients with MDS/MPN-U may be phenotypically similar to those with aCML, but lack isolated granulocytic dysplasia and may have basophilia and megakaryocytic hyperplasia accompanied by intense BM fibrosis.[89,140] MDS/MPN-U probably accounts for less than 5% of all myeloid malignancies. The recent MD Anderson Cancer Center (MDACC) series of 85 WHO-defined MDS/MPN-U patients is arguably the largest published series so far.[141] These investigators elected to apply both MDS and MPN prognostic scoring systems to allow for the defining dysplastic and proliferative features. This, together with the recent work of Orazi and colleagues, which included patients from multiple institutions including the MDACC, allows us to have a better understanding of the pertinent clinical and biological features of MDS/MPN-U.[89]

Both series of WHO-defined MDS/MPN-U patients showed a median age of 71 years, a male predominance (around 2:1), presence of splenomegaly, low monocyte counts, 20%-30% *JAK2*^{V617F}-positivity, and non-specific cytogenetic findings, with the exception of trisomy 8, which was the sole cytogenetic abnormality in 15% of the MDACC cohort. The principal differences in the series were the proportion of patients with thrombocytosis more than 450x10^9/L: 18% *vs.* 32%, and the median overall survival, which was considerably worse for the MDACC series: 12.4 months *vs.* 21.8 months. It is possible, but not certain, that the greater number of patients with thrombocytopenia (<100x10^9/L) in the MDACC series might reflect a biologically more aggressive disease resulting in the poorer survival. It would have been of interest to assess the AML-free survival of both series, which was 18.9 months in the Orazi series and not reported in the MDACC series. It was interesting that the MDS-IPSS model allocated 68% of the MDACC cohort as 'low-risk' despite the poor survival; conversely the MDA global model appeared to be a useful prognostic tool.[142]



Risk Groups	*P* value
Low *vs.* Intermediate	0.017
Low *vs.* High	< 0.001
Intermediate *vs.* High	0.007

Risk Groups	*P* value
Low *vs.* Intermediate	< 0.001
Low *vs.* High	< 0.001
Intermediate *vs.* High	0.51

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Figure 6. Hematologic parameters in a cohort of 121 patients with atypical chronic myeloid leukemia (aCML).

Currently, there is no optimal treatment consensus for MDS/MPN-U patients who are ineligible for an allo-SCT. In the MDACC series, the majority of patients received HMA and the overall survival was better compared to 'other' approaches (16.4 months *vs.* 11.5 months). The other non-transplant treatments included interferon alpha, cyclosporine, thalidomide, lenalidomide and anti-thymocyte globulin.[141] There is much interest in combining HMA with JAK inhibitors in the context of a clinical trial, given the moderate frequency of *JAK2* mutations.

Transformation to AML in MDS/MPN

Transformation to acute myeloid leukemia (AML), which is often refractory to conventional treatment, is a challenging complication in MDS/MPN, as it is in MDS and MPN. The rate and incidence of AML transformation in MDS/MPN is unknown, with the exception of CMML and RARS-T. Most estimates are based on MPN patients who transform into AML. A French trial in PV estimated the risk of transformation of 24% at 15 years in patients treated with hydroxyurea or pipobroman; smaller series suggest a risk of 3%-40%.[143-146]

The incidence of CMML transformation (AML-M5) is 15%-52%, with higher white blood counts, marrow cellularity, karyotype risk score, and revised IPSS score associated with greater risk.[147,148] The presence of *ASXL1* or *RUNX1* may also increase the transformation risk.[149-152] Transformation in patients with RARS-T appears comparable to RARS patients (1.8 and 2.4 per 100 patient-years, respectively) and higher than that in ET.[153] Collectively, MDS/MPN appears to have a higher risk of transformation compared to MPN, akin to that in MDS. It is, therefore, imperative to better characterize the incidence and potential for transformation risk in MDS/MPN.

Other candidate genetic events that have been linked to AML risk in MPN include *TET2, IDH1/2, DNMT3A* and *EZH2* mutations.[154,155] Cytogenetic progression, often involving abnormalities in chromosomes 7 (target genes *EZH2, IKZF1*), 8 (*MYC*), 17p (p53), 21 (*ERG, RUNX1*), and 12 (*ETV6*), is commonly observed at transformation. MDS/MPN with an isolated isochromosome (i)17p (leading to TP53 haploinsufficiency) may be a distinct disease entity with further increased risk of AML progression.[156,157] It is possible that some patients may harbor sub-clones with mutations in *TP53*, which are only detected by next generation sequencing (NGS). Clearly this is important in view of the associated high risk of transformation, and perhaps an early consideration for allo-SCT.

Results of treatments for AML transformation in MDS/MPN, including allo-SCT, remain suboptimal, with a median survival of less than five months.[158] Management is empiric and often is comprised of conventional cytotoxic combinations (used in *de novo* AML) or novel induction regimens, of both higher and lower intensity.[79,159,160] Current efforts are investigating diverse agents, including hypomethylating agents, JAK and MEK inhibitors, BCL-X_L and BCL-2 inhibitors, and clofarabine.[161-164]

Impact of symptom burden in patients with MDS/MPN

There has been considerable interest on the pathobiology of MDS and MPN-related symptoms and the potential impact of associated abnormalities, such as cytokine abnormalities and inflammation, on the overall prognosis. Furthermore, prospective assessment of disease-specific symptom burden and its impact on quality of life (QOL) has been found to be useful in clinical trials to assess benefit.[165,166] Symptoms for MPN and MDS are similar, but disparate. For MPN patients, thromboembolic (macrovascular) and metabolic/catabolic symptoms are more prominent.[167] MPN patients, particularly those with myelofibrosis (MF), frequently suffer from fever, night sweats, pruritus, bone pain, profound fatigue, weight loss, cachexia, as well as abdominal pain and distension.[168,169] MDS patients sustain debilitating fatigue, infections, and cardiovascular complications in addition to significant age-associated comorbidities.[170]

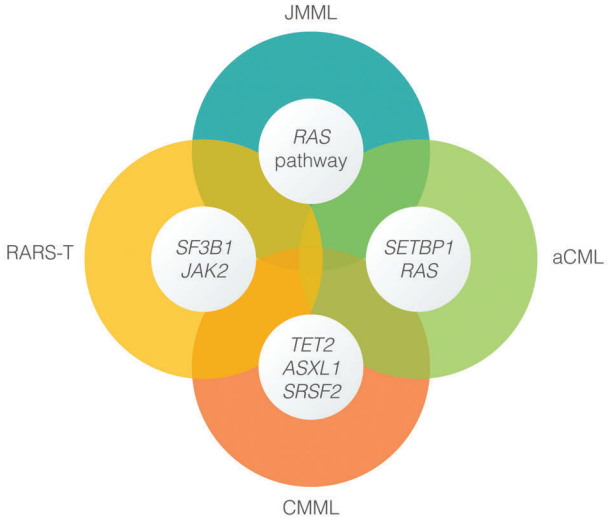

Figure 7. Emerging molecular fingerprints of myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN).

Symptom burden assessment has not been studied in MDS/MPN, which is likely to depict symptoms of both MDS and MPNs. We recommend a prospective symptom assessment study using the MPN-SAF TSS, EORTC-QOL-C30 and EQ-5D as initial symptom scales/questionnaires, along with an open-ended cognitive feedback tool capturing patients' answers to specific symptom questions. Such efforts should lead to the collation of candidate questions from which a refined MDS/MPN specific symptom assessment tool could be developed and then prospectively validated.[171]

Novel strategies and future clinical trial designs for the treatment of MDS/MPN

Since there is no treatment consensus for patients with MDS/MPN, strategies to improve outcomes must focus on rationally developed predictive and prognostic biomarkers based on molecular and clinical perspectives.[172] Questions remain as to whether eligibility for future clinical studies should be restricted to WHO subtypes of MDS/MPN, such as a study for CMML patients alone *versus* broad inclusion of MDS/MPN-U patients, or whether the focus should be based on clinical disease phenotype or proliferative *versus* non-proliferative features. As we move forward with clinical studies based on targeted molecular pathways, these should ideally determine patient selection based upon 'founder' tyrosine kinase signaling pathway mutations or those with transcription factor mutations. The underlying molecular complexity of these diseases will be a significant challenge. It is critical to identify

MDS/MPN specific therapeutic response criteria and suitable end points correlated with survival and AML risk for uniform assessment of treatment benefit. Moreover, criteria for disease progression or stability while on therapy ought to be introduced and vetted among experts.

The high frequency of *SF3B1* mutations in RARS-T subtypes suggests that spliceosome inhibition may offer the prospect of selective synthetic lethality. Trials are currently evaluating the benefit of the JAK1/JAK2 inhibitor ruxolitinib in CMML patients, either as monotherapy or in combination with 5-azacytidine. Based on the pre-clinical data suggestive of GM-CSF dependent STAT-5 hypersensitivity in CMML, it would be reasonable to design trials assessing GM-CSF neutralizing antibodies (KB003) or JAK inhibitors. Other putative targets include small-molecule inhibitors directed against STAT3/5, MAPK, AKT, MEK and PI3K-mTORC pathways.[173-175]

Conclusion

The MPN/MDS group is a very heterogeneous group defined by WHO mainly on morphological grounds, especially the concomitance of cytopenia(s) and at least one "cytosis". Current studies suggest considerable genetic complexity and heterogeneity in MDS/MPN.[176] Most patients with JMML, and up to 50% of cases with other subtypes, have mutations that directly activate proliferative signaling pathways. Over 30 recurrent gene mutations have now been identified, whereas in the case of CMML, there may be 5-20 such gene mutations *per case*, suggesting a multi-step and highly variable molecular pathogenesis. Collectively, *TET2, ASXL1* and SRSF2 represent the most commonly mutated genes.

Importantly, at present there are no specific mutations in MDS/MPN that stringently define particular subtypes. Nevertheless, some clear associations have emerged, including *SF3B1* and *JAK2* mutations in RARS-T, and *SETBP1* aCML. Understanding clonal hierarchies should serve as a cornerstone for development of a robust molecular classification of MDS/MPN, as well as molecular predictors of prognosis and therapeutic response. An immediate initiative to consider is the set-up of large registries for these rare hematologic malignancies, along with collaborative efforts to define risk models and suitable end points for clinical trials. Outside of clinical trials, allo-SCT remains the most viable treatment options for the eligible patients with about one-third of patients achieving long-term remission and probable cure. For those who are not transplant candidates and who have no recourse to a clinical trial, it appears reasonable to consider HMAs in the first instance, except for those who have *JAK2*-mutant disease, for whom a trial of a JAK inhibitor might be indicated.

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