REVIEW ARTICLE

Chronic myelomonocytic leukemia: molecular pathogenesis and therapeutic innovations

Ludovica Marando, Clifford M. Csizmar and Mrinal M. Patnaik

Division of Hematology, Department of Internal Medicine, Mayo Clinic, MN, USA.

Correspondence: L. Marando
Marando.Ludovica@mayo.edu

M.M. Patnaik
Patnaik.Mrinal@mayo.edu

Received: June 12, 2024.
Accepted: October 4, 2024.
Early view: October 17, 2024.

https://doi.org/10.3324/haematol.2024.286061

Abstract

Chronic myelomonocytic leukemia (CMML) is an aggressive clonal stem cell disorder categorized among myelodysplastic/myeloproliferative overlap neoplasms. While sharing features with both myelodysplastic syndromes and myeloproliferative neoplasms, CMML has distinct molecular and clinical profiles. The presence of CMML-specific prognostic models, response criteria, and dedicated clinical trials underscores a unique and complex biology. Age-related changes affecting the bone marrow microenvironment, immune responses, and the intricate balance between epigenetic deregulation and proinflammatory signaling are characteristic of this disease, collectively posing significant scientific and clinical challenges in its management. CMML is an aging-related, clinically heterogeneous neoplasm with limited approved therapeutic options, representing an area of unmet medical need. This review offers a comprehensive analysis of the current understanding of the molecular mechanisms driving CMML evolution and its clinical manifestations within the ever-evolving landscape of precision medicine. In light of the most recent molecular discoveries, we highlight the shortcomings of existing therapies and underscore promising investigational agents. Many of the biological findings discussed are shared across a spectrum of acute and chronic myeloid neoplasms, as well as clonal hematopoiesis, broadening the scope of this review.

Aging and myeloid malignancies

In addition to sustained peripheral blood monocytosis and bone marrow dysplasia[1] (Figure 1), CMML is associated with an inherent risk of transformation to secondary acute myeloid leukemia (AML) of about 15-20% over 3 to 5 years.[2] Like patients with AML, patients with CMML have about 10-15 mutations per kilobase of coding DNA. Although this is several-fold lower than in other malignancies (melanoma ~1,000, lung cancer ~150),[3,4] CMML has a very heterogeneous clinical course.[4] While only *ASXL1* mutations reproducibly predict inferior outcomes in CMML,[5] higher levels of interleukin (IL)-10 (human cytokine synthesis inhibitory factor) have also been shown to improve prognosis,[6] emphasizing a complex pathophysiology that extends beyond somatic alterations, including cytokine diversity and epigenetic deregulation.[6]

Among all hematologic neoplasms, CMML displays the most striking skewing towards older age with a median age at presentation of >70 years.[1,2] Aging hematopoietic stem cells (HSC) steadily accumulate mutations (mean of 17 per year after birth).[7] While most are inconsequential, between one in 34 and one in 12 non-synonymous mutations provide a selective advantage and drive clonal expansion by cell-intrinsic and extrinsic selection pressures.[7,8] Driver mutations commonly occur in leukemia genes, and lead to clonal hematopoiesis of indeterminate potential (CHIP) when such mutations can be detected at a variant allele fraction of ≥2%, in the absence of blood count abnormalities. There is significant overlap between clonal hematopoiesis mutations and CMML driver mutations, with the majority of CMML patients showing mutations in ≥2 clonal hematopoiesis-associated genes (e.g., *ASXL1*, *TET2*, *SRSF2*). This suggests that in most cases, CMML develops on the background of age-related clonal hematopoiesis, with subsequent mutations shaping the CMML phenotype and AML transformation rates.[9]

Oxidative stress, telomere shortening, and activation of tumor suppressor genes all contribute to an abrupt re-

duction in the diversity of the HSC pool, such that by the age of 70 years, most peripheral blood cells are derived from only 10-20 HSC clones.[7] Interestingly, only 20% of the HSC clones remaining in people aged >70 years have identifiable mutations in known driver genes.[7] While CHIP is associated with an increased risk of developing hematologic malignancies,[10-12] the more prevalent, presumed "driverless" clonal expansions noted in the elderly, might underlie other blood- and immune-related signs of aging.[13-15] Notably, while CHIP is present in >90% of individuals ≥85 years,[8] hematologic malignancies continue to be a rare entity, supporting the hypothesis that extrinsic non-cell-autonomous mechanisms are pivotal in shaping the natural history of CHIP.

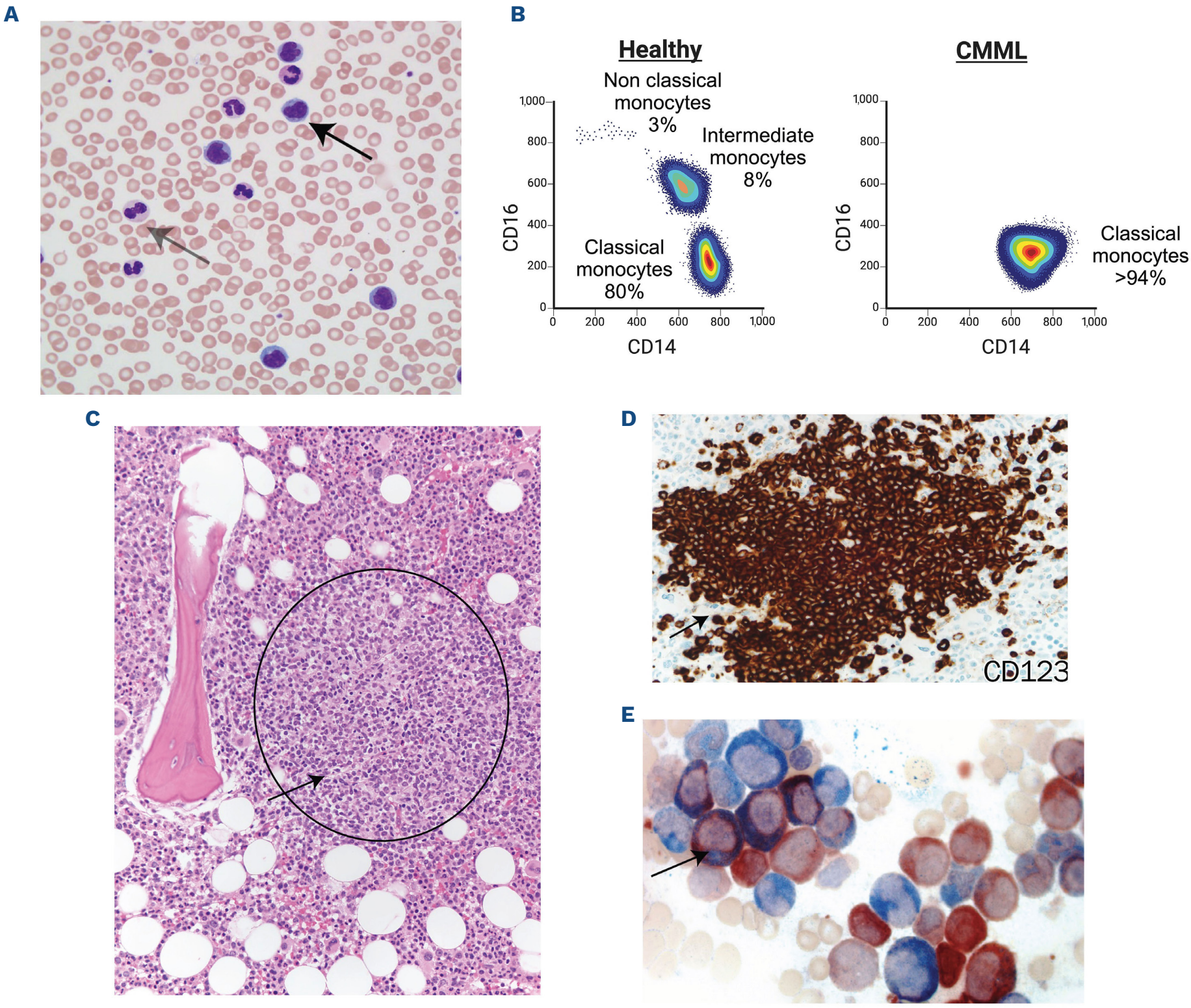


Figure 1. Morphological and immunophenotypic characteristics of chronic myelomonocytic leukemia. Peripheral blood and bone marrow morphology, immunohistochemistry, and cytochemical analysis in chronic myelomonocytic leukemia (CMML). (A) Wright-Giemsa ×200 magnification. Peripheral blood smear of a patient with CMML demonstrating promonocytes (bold arrow) along with dysplastic neutrophils (gray arrow). (B) Monocyte repartitioning flow cytometry demonstrating a normal M01 fraction (classical monocytes, $CD14^+$, $CD16^-$) in a healthy control (~80%), while the right scatter plot shows an expanded M01 fraction (>94%) considered characteristic for a diagnosis of CMML. (C) Bone marrow core biopsy of a patient with CMML demonstrating a plasmacytoid dendritic cell nodule/aggregate (black circle). (D) Image at a higher magnification showing the same nodule brightly positive for CD123 by immunohistochemistry (×1,000 magnification). (E) Cytochemistry on a bone marrow aspirate in a patient with CMML using a dual esterase stain (α-naphthyl butyrate esterase and choloroacetate esterase) demonstrating dysplastic monocytes taking up both colors (blue and brick red). Normal granulocytes stain bright blue, while normal monocytes stain brick red (×400 magnification).

Epigenetic/splicing dysregulation in chronic myelomonocytic leukemia

Somatic mosaicisms form the structural background in >90% of CMML patients.[16,17] Recurrently mutated genes include epigenetic regulators (*TET2* ~50% and *ASXL1* ~40%), spliceosome components (*SRSF2* ~50%) and cell signaling pathways (*RAS* ~30% and *CBL* ~15%)[18-21] (Figure 2A). While these mutations are not exclusive to CMML and can be found across the spectrum of acute and chronic myeloid neoplasms as well as CHIP, specific mutations can define a dysplastic or a more aggressive proliferative phenotype (Figure 2B-D). In addition, studies in larger cohorts have highlighted that a pattern of *TET2-SRSF2* (~46%) co-mutations,[22] as well as biallelic *TET2* mutations/alterations (*biTET2*, ~45%), are commonly identified in CMML, secondary to granulocyte-monocyte progenitor (GMP)-biased hematopoesis.[23] These events occur either when a secondary *TET2* variant is subclonal to an ancestral *TET2* variant or following loss of heterozygosity at 4q24.[23] Notably, truncating *TET2* variants are more likely to occur in the context of *biTET2*,[23] highlighting that clonal selection for a complete loss of *TET2* plays an important role in the evolution of CMML. At the protein level, TET2 plays a pleiotropic role in hematopoiesis, with mutations found in both myeloid and lymphoid malignancies. TET2 initiates DNA demethylation and regulates chromatin modifications at regions critical for lineage commitment and differentiation of HSC and progenitor cells.[24] In murine models, loss of *Tet2* (in particular, the catalytic domain[25]) induces a strong myeloid bias[26] (Figure 3), with increased accessibility at enhancers of pro-myeloid differentiation genes thought to contribute to this phenotype.[27] The strong myeloid bias induced by *TET2* variants is also recapitulated in patients, as suggested by the observation that, at single-cell resolution, *TET2* mutations mostly expand in the myeloid lineage.[28]

Variants in *SRSF2*, a component of the spliceosome machinery, are the second most common ancestral variants in CMML, and also promote a myeloid bias (Figure 3). Mutations at the hotspot proline residue 95 (P95) alter messenger RNA (*mRNA*) splicing by changing the RNA binding affinity of SRSF2, leading to mis-regulation of exon inclusion.[29] These variants, however, lead to only modest changes in global mRNA splicing. Even if a subset of mis-spliced transcripts has been proposed to be relevant for myelodysplastic syndromes,[29-31] the molecular mechanisms whereby *SRSF2* variants are implicated in MDS and CMML remain elusive. Using a conditional murine model of *Srsf2*^{P95H}, it was shown that *Srsf2*$^{P95H/+}$ native chimeras showed clonal expansion at the expense of wild-type HSC only when transplanted into lethally irradiated recipients without an external competitor, suggesting that the specific characteristics of a microenvironment that is aged matched to the HSC plays a crucial role in allowing this variant to establish clonal dominance.[32] In addition, *Srsf2*$^{P95H/+}$ mice were observed to develop monocytosis and dysplastic neutrophils on aging (~12 months after induction of the recombinant allele), and eventually succumbed to a myeloproliferative disorder characterized by the presence of additional somatic mutations observed in CMML, including Ras pathway mutations.[32]

Among all the epigenetic drivers, truncating *ASXL1* variants are associated with adverse outcomes in CMML, proliferative disease features (Figure 2B), and resistance to epigenetic therapies.[5] Most *ASXL1* variants are frameshift or nonsense mutations in exon 12 (last exon) resulting in truncation of the protein at the C-terminus and loss of the plant homeodomain (PHD).[33] *ASXL1* mutations have been shown to cause both loss of polycomb repressive complex 2 (PRC2)-mediated histone methylation (H3K27me3) at HOXA cluster genes,[34] and loss of polycomb repressive complex 1 (PRC1)-mediated histone deubiquitination (H2AK119Ub).[35,36]

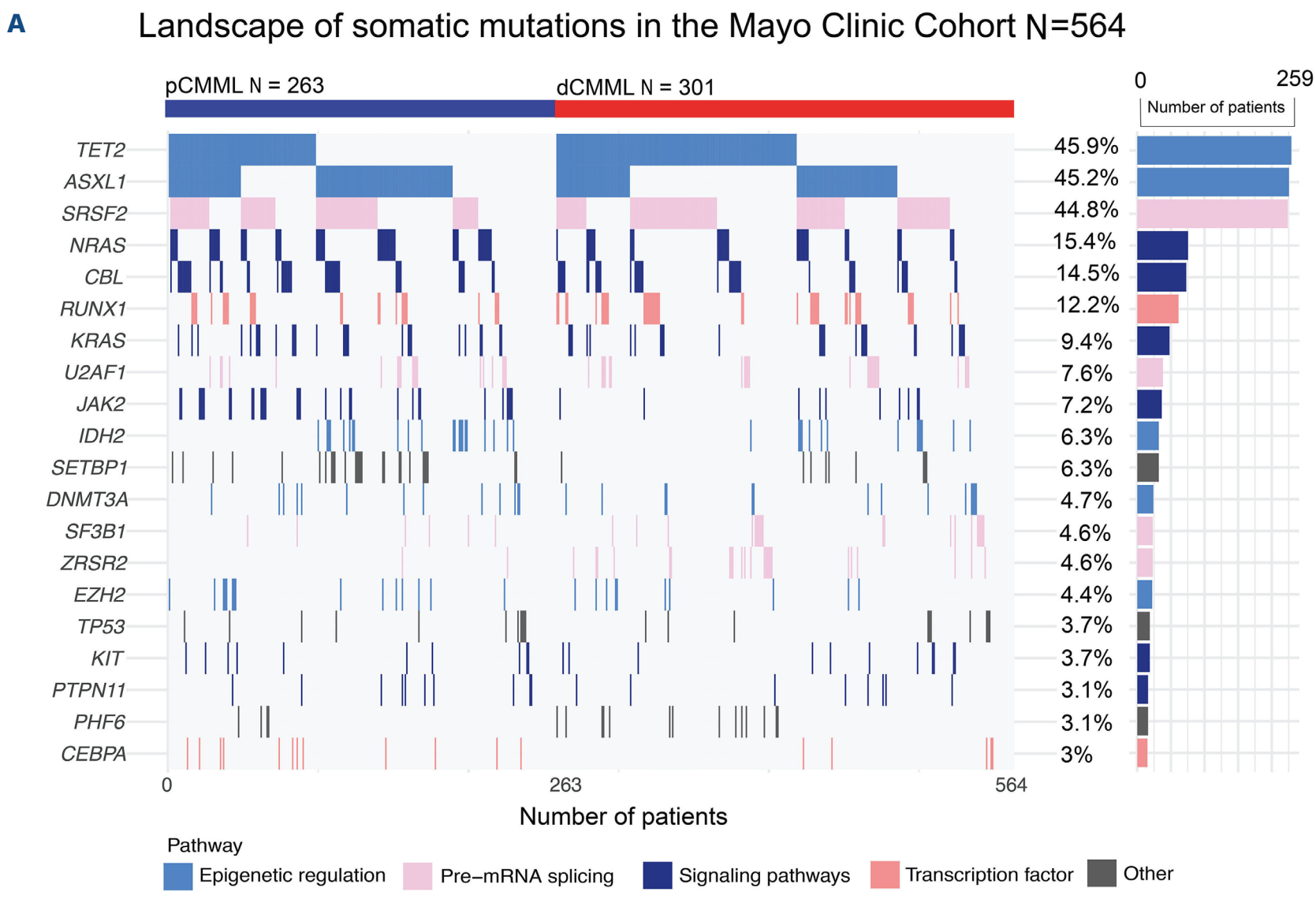
Given the complex ASXL1 interactions, our group attempted to further define the role of ASXL1 in CMML by interrogating the genome, transcriptome, and epigenome of wild-type *ASXL1 versus* truncating *ASXL1*-mutated primary CMML samples. We found that *ASXL1*-mutated patients gained accessibility at several enhancers enriched in ETS and BRD4 complex motifs, with upregulation in genes involved in cell cycle progression, DNA replication, and leukemogenesis.[37]

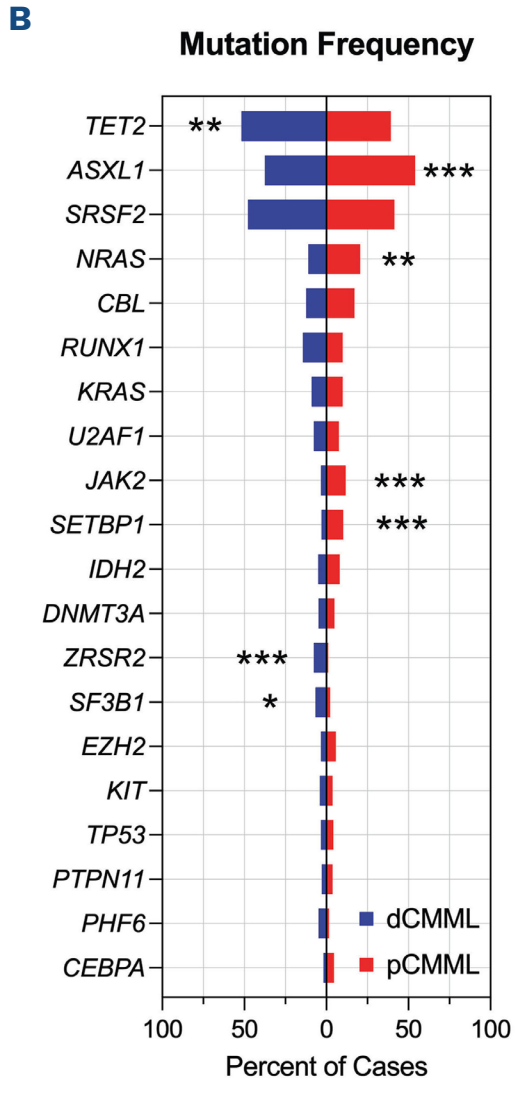
Signaling pathway mutations in chronic myelomonocytic leukemia

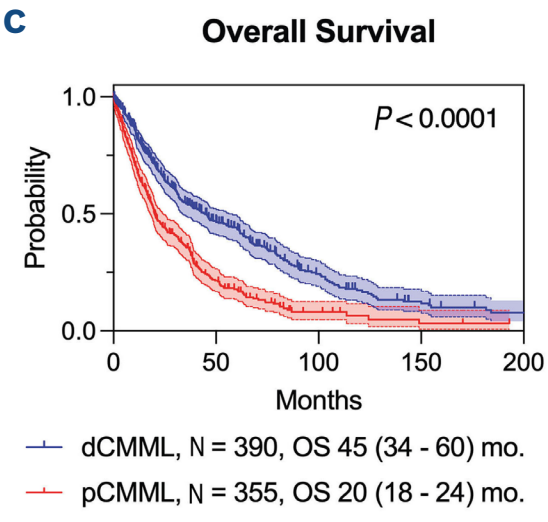
Signaling mutations are frequent in CMML and are usually associated with myeloproliferative features (so-called proliferative CMML).[38] These are largely dominated by oncogenic RAS pathway mutations (>70%; *NRAS*, *CBL*, *PTPN11*, *KRAS*, *NF1*, *BRAF*) but also include *JAK2*^{V617F}, *FLT3* and *CSF3R*. The latter two are very infrequent, with the presence of an *FLT3*-internal tandem duplication usually heralding transformation to AML.[39]

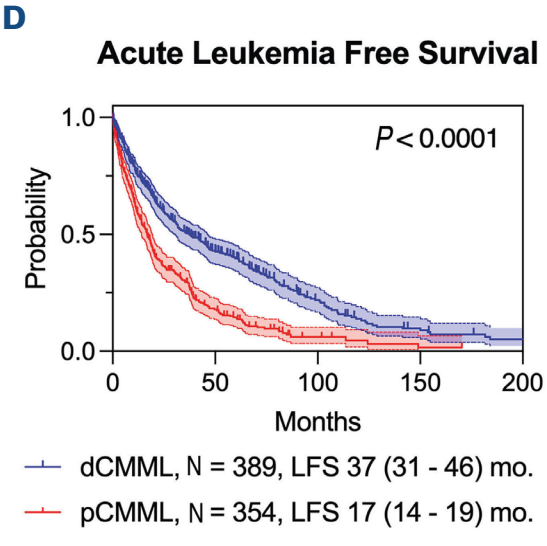
RAS pathway mutations (*NRAS*, *CBL*, *PTPN11*, *KRAS*, *NF1*)

Mutations in the epigenetic machinery are not specific to CMML, but are generally considered ancestral to signaling pathway mutations.[38,40,41] Germline and somatic RAS pathway mutations are associated with juvenile myelomonocytic leukemia, an aggressive pediatric myeloproliferative neoplasm that resembles proliferative CMML.[42] Features of proliferative CMML include constitutional symptoms, extramedullary hematopoiesis and myeloproliferation. This contrasts with dysplastic CMML, which is instead characterized by cytopenia(s) and a more indolent course (Figure 2B-D). Leveraging a large cohort of >1,000 CMML patients, our group demonstrated that oncogenic *RAS* mutations are more prevalent in, and occur at higher variant allele fractions in proliferative CMML than in dysplastic CMML.[38] Interestingly, in murine models, *Nras*^{G12D} has been shown to have a bimodal effect on HSC, both increasing and decreasing the rate at which some HSC divide; besides, *Nras*^{G12D} can also increase reconstitution and self-renewal potential of HSC on serial transplantation.[43]









Continued on following page.

Figure 2. Characteristics of the Mayo Clinic cohort. (A) Landscape of pathogenic somatic mutations in 564 individuals with chronic myelomonocytic leukemia (CMML) followed at Mayo Clinic. Note only the top 20 most recurrently mutated genes are plotted. (B) Frequency of gene mutations in dysplastic and proliferative subtypes of CMML. Only the 20 most recurrently mutated genes are shown. Asterisks depict significance by Fisher exact test as **P*<0.05, ***P*<0.01, or ****P*<0.001. (C, D) Overall survival (C) and acute leukemia-free survival (D) of the dysplastic and proliferative subtypes of CMML in the Mayo Clinic cohort with a median follow-up duration of 79 months. Data from the Kaplan-Meier analysis are presented as median overall survival and leukemia-free survival (with 95% confidence intervals) and compared via the log-rank (Mantel-Cox) method. pCMML: proliferative CMML; dCMML: dysplastic CMML; OS: overall survival; LFS: leukemia-free survival.

In mice, during emergency and leukemic myelopoiesis, GMP aggregate in self-renewing GMP clusters. These are transcriptionally defined by the activation of an inducible *Irf8* and β-catenin self-renewal network.[44] Novel insights into CMML-related phenotypes come from the identification, in a subset of CMML patients carrying RAS mutations and high-risk disease features, of a GMP-like inflammatory population, transcriptionally similar to the cluster of self-renewing GMP described above[45] (Figure 3). Besides its canonical RAS/MEK/ERK oncogenic signaling (Figure 4), mutant RAS induces the

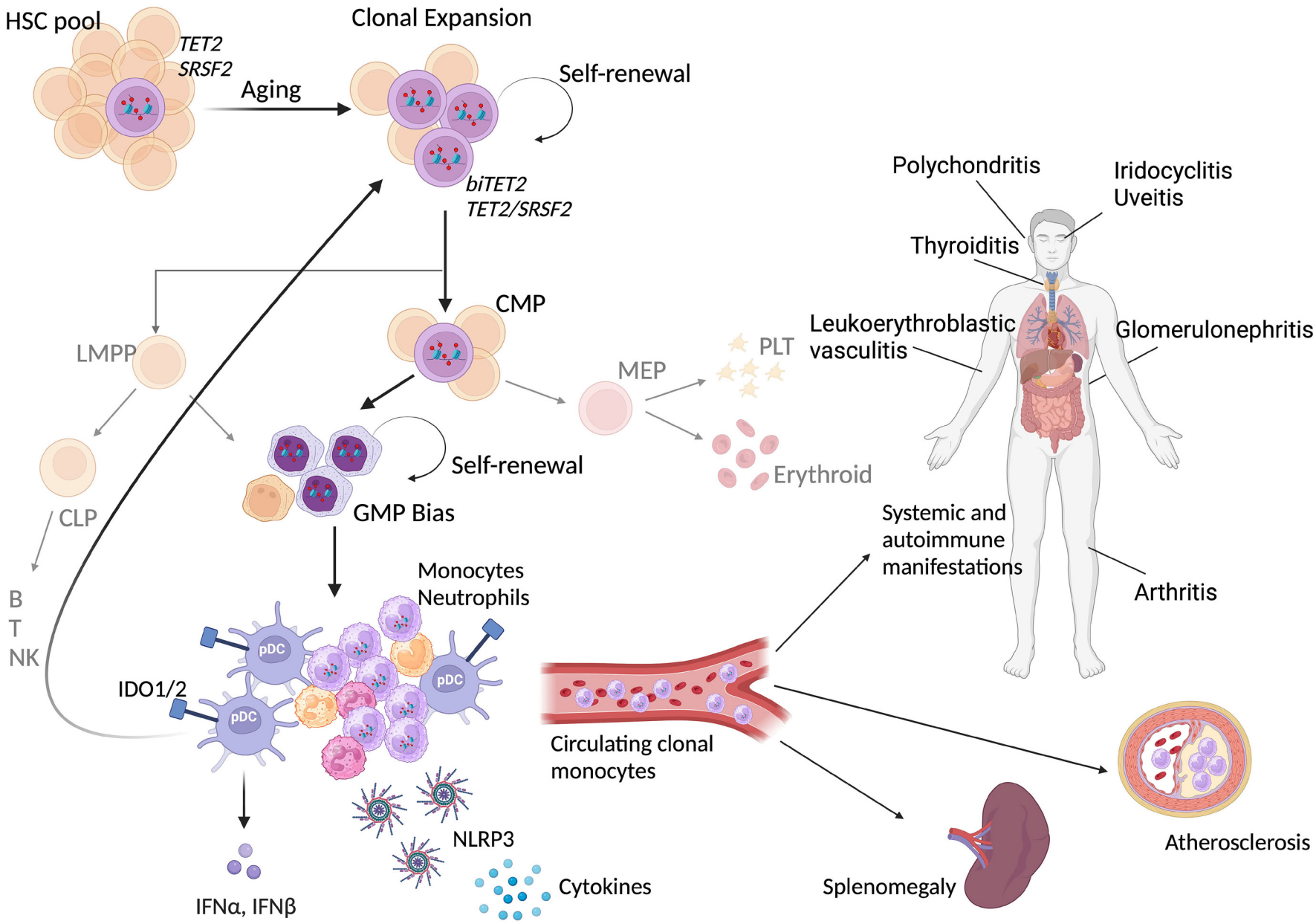


Figure 3. Mechanisms driving the evolution of chronic myelomonocytic leukemia and its clinical manifestations. Clonal expansion of biallelic*TET2* or *TET2/SRSF2*-mutated hematopoietic stem cells causes a granulocyte-monocyte progenitor bias and preferential production of clonal inflammatory myeloid cells (monocytes and neutrophils). Clusters of self-renewing granulocyte-monocyte progenitors might be implicated in further clonal propagation. Among other mechanisms, activation of the NLRP3 inflammasome, especially in association with mutant RAS, mediates release of cytokines and contributes to an inflammatory microenvironment. Clusters of clonal indoleamine 2,3-dioxygenase-positive plasmacytoid dendritic cells induce immunotolerance and clonal propagation, and can contribute to leukemia transformation. Circulating inflammatory monocytes are responsible for the systemic manifestations of chronic myelomonocytic leukemia. HSC: hematopoietic stem cell; CMP: common myeloid progenitor; LMPP: lymphoid-primed multipotent progenitor; CLP: common lymphoid progenitor; *biTET2*: biallelic *TET2* mutations; B: B cell; T: T cell; NK: natural killer cell; GMP: granulocyte-monocyte progenitor; IDO: indoleamine 2,3-dioxygenase; IFN: interferon; NLRP3: NOD-, LRR- and pyrin domain-containing protein 3; MEP: megakaryocyte-erythroid progenitor; PLT: platelet.

generation of reactive oxygen species, which in turn, promote the activation of the NLRP3 inflammasome.[46] The latter has a key role in the development of several clinical manifestations associated with *KRAS*-mutant myeloproliferative neoplasms.[46] In response to various signals from pathogens and internal damage, activation of NLRP3 results in release of proinflammatory cytokines (e.g., IL-1β and IL-18)[47] through gasdermin D-mediated permeabilization of the plasma membrane. If gasdermin D pores cannot be repaired, cells undergo pyroptosis[48] with the potential to amplify inflammatory responses (Figure 3).[49,50] In addition to the classical mitogen-activated protein kinase (MAPK) pathway, GTP-bound RAS also binds to p110 to activate the PI3K-AKT-mTOR signaling cascade[51] (Figure 4). Thus, inhibition of the phosphoinositol-3 kinase (PI3K) pathway has been explored in multiple RAS-mutated tumor types, both as an initial strategy and in subsequent efforts to overcome resistance to RAS inhibition.[52] RAS mutations might also have roles in modulating adaptive immune responses to tumor cells. Using an *Asxl1*$^{-/-}$ *Nras*$^{G12D/+}$ Vav-Cre mouse model, we have shown that *Nras* and *Asxl1* cooperate to accelerate progression of CMML to AML, with AML cells overexpressing all the inhibitory immune checkpoint ligands (PD-L1/PD-L2, CD155, and CD80/CD86), highlighting that a suppressive microenvironment could play an important role in transformation to secondary AML.[53] We have also shown accumulation of clonal RAS mutant CD123/CD303^{+} plasmacytoid dendritic cells in CMML patients (Figures 1C, D and 3), and have documented their association with transformation to AML.[54] Further work by our group has shown that these plasmacytoid dendritic cell clusters are positive for indoleamine 2,3-dioxygenase 1/2 (IDO1/2),[55] with IDO being an immune-checkpoint enzyme that induces systemic immune tolerance through multiple mechanisms, including regulatory T-cell expansion and tryptophan catabolism[56,57] (Figure 3).

JAK2^{V617F} mutations in chronic myelomonocytic leukemia

JAK2^{V617F} mutations are encountered in ~10% CMML patients. By studying a large cohort of CMML patients, we found that *JAK2*-mutated CMML is associated with higher hemoglobin/hematocrit levels and platelet counts, and frequently co-occurs with *TET2* mutations. However, we did not identify an increased risk of thrombosis, acute leukemia transformation, or impact on overall survival.[58] *JAK2*^{V617F} mutant CMML can at times be difficult to distinguish from *JAK2*-mutant myeloproliferative neoplasms with monocytosis, although the use of monocyte partitioning flow cytometry (M01/classical monocytes >94% in CMML) and focus on megakaryocytic morphology can help to resolve this dilemma in several cases.[59] Irrespective of signaling pathway mutations of signaling pathway mutations, CMML patients demonstrate granulocyte-macrophage colony-stimulating factor (GM-CSF)-dependent hypersensitivity in colony formation assays and by phospho-STAT5 (pSTAT5) flow cytometry, as compared to healthy controls, an effect more pronounced in patients with RAS mutations.[60] Lenzilumab (KB003) is a novel engineered human immunoglobulin G1κ monoclonal antibody with high affinity for human GM-CSF (Figure 5) and has activity in preclinical models of CMML.[60] In a phase I study of lenzilumab in CMML, we documented that the drug was well tolerated and had a durable clinical benefit in 33% of the patients.[61] An interim analysis of the PREACH-M trial (ACTRN12621000223831) evaluating the combination of lenzilumab and 5-azacitidine in CMML patients with RAS pathway mutations has shown encouraging results, with complete remissions achieved within three cycles of treatment in 55% of subjects.[62] JAK2 is a primary kinase regulating all the known activities of GM-CSF.[63] This, along with its constitutive activation in CMML, provides the rationale for the use of JAK inhibitors, with ruxolitinib having completed early phase testing in CMML (Table 1, Figure 5).[64]

The role of inflammatory monocytes in chronic myelomonocytic leukemia

In CMML, classical monocytes (CD14^{+}/CD16^{-}) represent the predominant monocyte subset (>94% of total) (Figure 1B).[65] These have highly inflammatory transcriptional signatures[66] and, as a result, CMML patients have substantially different cytokine expression levels compared to healthy controls.[6] Accordingly, CMML patients have a >2-fold increased risk of cardiovascular events,[67] and ~20% have an associated systemic inflammatory and autoimmune disease (Figure 3).[68] The effects of clonal monocytes go beyond the role of these cells in inflammation and organ infiltration. CMML commonly co-occurs with histiocytic malignancies that share ancestral mutations with CMML.[69-71] While this suggests a common cell of origin, it also raises the possibility that neoplastic monocyte-derived macrophages contribute to the phenotypic origins of histiocytic neoplasms.

Beyond clinical manifestations, inflammation is also implicated in disease progression (Figure 3). Chronic inflammation can fuel clonal expansion of mutated HSC while inhibiting the function of wild-type HSC.[72] In a murine model of *Tet2*$^{-/-}$, increased IL-6 levels, due to blood dissemination of gut bacteria from a dysfunctional small-intestinal barrier, were associated with a CMML-like disease.[73] In addition, when inflammation is active, TET2-mediated regulation of active chromatin facilitates histone deacetylation and suppresses IL6 and IL-1β expression, leading to resolution of inflammation in innate myeloid cells[74] and macrophages.[75] As a result, in the presence of *TET2* loss-of-function variants, myeloid cells show a reduced capacity to resolve inflammation. In addition, *Tet2*-deficient murine and *TET2*-mutant human HSC, when exposed to high levels of pro-inflammatory tumor necrosis factor-α *in vitro,* have a strong proliferative advantage compared with wild-type cells.[76] Equally, *in vivo*, under inflammatory "stress", murine *Tet2*-deficient HSC expand rapidly, which results in enhanced production of inflammatory cytokines, including IL-6, and resistance to apoptosis.[77]

Table 1. Results of investigational therapies in chronic myelomonocytic leukemia.

Trial number (Eponym)	Phase	Target(s)	Agent(s)	N	ORR %	CR %	mOS months	Reference
Standard of care								
NCT02214407 (DACOTA)	III	DNMT1	Decitabine	84	63	8	18.4	Itzykson, 2023[97]
Epigenetic modifiers								
NCT01613976	I	HDAC	Azacitidine + panobinostat	4	0	0	-	Kobayashi, 2018[98]
NCT02877277	I	TET2	Azacitidine + ascorbic acid	3	-	-	-	Gillberg, 2019[99]
NCT03418038	II	TET2	Ascorbic acid	2	-	-	-	Xie, 2023[100]
Chromatin modifiers								
-	I	Aurora kinase A	ENMD-2076	1	0	0	-	Yee, 2016[101]
NCT01926587	I	PLK1	Azacitidine + rigosertib	1	0	0	-	Navada, 2020[102]
RNA processing inhibitors								
NCT02159872	II	Ribosome A-site	Omacetaxine mepesuccinate (homoherringtonine)	8	33	0	7.5	Short, 2019[103]
NCT02841540	I	SF3B1	H3B-8800	4	0	0	-	Steensma, 2021[104]
JAK inhibitors								
NCT01787487	II	JAK2	Ruxolitinib + azacitidine	17	65	-	15.1	Assi, 2018[105]
NCT01776723	I/II	JAK2	Ruxolitinib	50	38	2	24.7	Hunter, 2021[106]
NCT01776723	II	JAK2	Ruxolitinib	29	17	0	24	Padron, 2022[107]
RAS superfamily agents								
NCT00920140	I/II	MEK1/2	Trametinib	11	27	9	14.5	Borthakur, 2016[108]
NCT00034684	II	Farnesyl-transferase	Lonafarnib	35	29	6	-	Feldman, 2008[109]
EudraCT 2004-004685-32	II	Farnesyl-transferase	Lonafarnib	2	0	0	-	Ravoet, 2008[110]
Protein degradation agents								
NCT00744536	II	E3 ubiquitin ligase	Lenalidomide + melphalan	12	25	0	8.5	Buckstein, 2014[111]
ACTRN-12607000283471	II	E3 ubiquitin ligase	Azacitidine + thalidomide	16	63	19	-	Kenealy, 2017[112]
NCT01368757 (AGMT CMML-1)	I	E3 ubiquitin ligase	Lenalidomide	20	8	0	28.9	Burgstaller, 2018[113]
NCT01522976 (SWOG S1117)	II	E3 ubiquitin ligase HDAC	Azacitidine + lenalidomide Azacitidine + vorinostat	19 16	68 12	-	NR	Sekeres, 2017[114]
NCT01342692 (AZA PLUS)	II	E3 ubiquitin ligase HDAC dsDNA	Azacitidine + lenalidomide Azacitidine + valproic acid Azacitidine + idarubicin	4 5 6	35	18	19.7	Ades, 2022[115]
NCT00580242	I	E3 ubiquitin ligase, proteasome	Lenalidomide + bortezomib	-	0	0	-	Attar, 2013[116]
NCT02610777	II	NEDD8	Azacitidine + pevonedistat	9	78	33	-	Sekeres, 2021[117]
NCT03862157	I/II	NEDD8	Azacitidine + venetoclax + pevonedistat	2	100	0	-	Short, 2023[118]
Cytokine & growth factor derivatives								
NCT02268253	I/II	CD123 (IL-3R)	Tagraxofusp	36	11	0	-	Patnaik, 2021[119]
NCT02546284	I	GM-CSF	Lenzilumab	15	33	0	-	Patnaik, 2020[120]
ACTRN-12621000223831 (PREACH-M)	II/III	GM-CSF	Lenzilumab + azacitidine ± ascorbic acid	11	73	55	-	Hiwase, 2023[121]
Immune checkpoint inhibitors								
NCT03066648	I	TIM-3	Sabatolimab + azacitidine/ decitabine	15	67	27	7.6*	Brunner, 2024[122]

Many studies have enrolled diverse populations including patients with both myelodysplastic syndromes and chronic myelomonocytic leukemia (CMML). The data tabulated here represent only prospective studies that reported CMML-specific outcomes. Three retrospective studies (with 6, 27, and 51 patients) have described the combination of venetoclax with either azacitidine or decitabine, with overall response rates ranging from 50-90%, complete response rates of 0-22%, and median overall survivals of 10.3-25.1 months.[123-125] *The study by Brunner *et al.* reported only median progression-free survival. The hyphen symbol (-) indicates that these data were not reported for the CMML-specific population. ORR: overall response rate; CR: complete response; mOS: median overall survival; HDAC: histone deacetylase. NR: not reached; GM-CSF: granulocyte-macrophage colony-stimulating factor.

Likewise, mutations affecting pre-mRNA splicing, including *SRSF2* mutations, have also been shown to result in the hyperactivation of nuclear factor-KB signaling pathways.[78,79] The presence of clonal myeloid cells is therefore likely to sustain a "vicious circle" of inflammation and clonal expansion in which an inflammatory milieu confers a selective advantage to the mutant clone, while at the same time, the mutant clone is able to perpetuate inflammation. Further underscoring this concept, autocrine or paracrine activation of MAPK though C-C chemokine receptor type 2 (CCR2) has been implicated in defective apoptosis of circulating classical monocytes in CMML.[80]

The progression of chronic myelomonocytic leukemia to acute myeloid leukemia

Rates of progression to secondary AML remain high,[2] with current survival outcomes after blast transformation remaining dismal (median overall survival, <9 months).[2] Acquisition of additional somatic driver mutations in the coding DNA, or increments in variant allele fraction of existing driver mutations, explain transformation in only 40-60% of patients.[38,81] Mutations in the non-coding genome, somatic copy number alterations, and epigenetic mechanisms, likely in the context of reduced immune surveillance, emerge as plausible mechanisms that need to be explored. Understanding steps that lead to AML transformation is a much-needed area of research, and a critical one to improve outcomes in patients.

Contemporary therapeutic approaches for chronic myelomonocytic leukemia

Allogeneic hematopoietic stem cell transplant is the only potential cure for CMML; however, due to older age and comorbidities, this approach is unfeasible for the majority of patients. DNA methyltransferase (DNMT) inhibitors, also referred to as hypomethylating agents, remain the only Food and Drug Administration-approved treatment options for CMML, but overall response rates are <50%, with true remissions being achieved in <20% patients.[82,83] In a large multicenter study, we showed that the best predictor of response to DNMT inhibitors is the presence of *TET2* mutations in the absence of *ASXL1* mutations.[84] Although use of DNMT inhibitors can lead to demethylation at promoters and CpG islands,[83] response to these inhibitors occurs in the absence of significant variation in the clonal structure.[85] Several studies have shown a lack of correlation between differences in promoter methylation and transcriptional changes in CMML,[37,38] a phenomenon that might explain why, despite an epigenetic effect, DNMT inhibitors do not affect the mutant allelic burdens, nor alter the natural history of the disease.[86] Ascorbic acid is an important cofactor for TET dioxygenase activity, prompting the use of parenteral ascorbic acid in combination with DNMT inhibitors in *TET2*-mutant CMML. In this setting, ascorbic acid has been postulated to enhance TET2 activity generated from the unmutated allele and/or exploit functional redundancies with TET3 in the hematopoietic system.[87]

The synergistic effect of venetoclax and 5-azacitidine is much less pronounced in CMML than in AML.[88,89] Some mechanistic insights come from studies in AML patients. Monocytic and/or *RAS*-mutated AML is more resistant to BCL2 inhibition, and thus venetoclax can favor the outgrowth of monocytic subpopulations that arise from low variant allele fraction *NRAS*-mutated and *KRAS*-mutated clones. These clones activate an MLL-specific leukemia stem cell signature and show dependency on the anti-apoptotic protein MCL-1.[90] CMML monocytes too show dependency on MCL-1, resulting in defective apoptosis, with a combination of MCL1 and MEK inhibitors showing early promise in xenografts models.[80] Although, traditionally, the RAS/RAF/MEK/ERK pathway has been difficult to target in hematologic malignancies due to the lack of effective drugs and the late and subclonal nature of its mutations, CMML is an exception, given that in proliferative CMML, RAS mutations are often early and dominant clonal events.[38] Novel RAS-directed therapies including the on and off KRAS G12C and G12D inhibitors and the pan-RAS GTPase inhibitors can play an important role in the management of proliferative CMML,[91,92] and can inform safety and dosing in myeloid neoplasms, in which RAS mutations play a role in disease progression and contribute to resistance to FLT3, IDH1, IDH2, and BCL2 inhibitors.

Inhibition of the RAS-activated PI3K pathway is an enticing yet underexplored approach in CMML. As dual MAPK/PI3K pathway inhibition resulted in dose-limiting toxicities in solid tumors,[52] other combinatorial strategies are needed. For example, the combination of the PI3Kδ inhibitor, umbralisib, and the JAK1/2 inhibitor, ruxolitinib, was synergistic in pre-clinical colony-forming assays using primary CMML samples.[93] This combination has since entered early phase clinical trials (NCT02493530). Moreover, in addition to their impact on methylation, DNMT inhibitors form covalent DNA-DNMT1 adducts that invoke an ATR-CHK1-mediated DNA damage response.[94-96] Thus, combinations of DNMT inhibitors with inhibitors of cell cycle checkpoints are of particular interest. Specifically, polo-like kinase 1 (PLK1) is upregulated in RAS-mutated proliferative CMML and PLK1 inhibition was efficacious in patient-derived xenograft models of proliferative CMML.[38] Accordingly, the PLK1 inhibitor, onvansertib, has entered

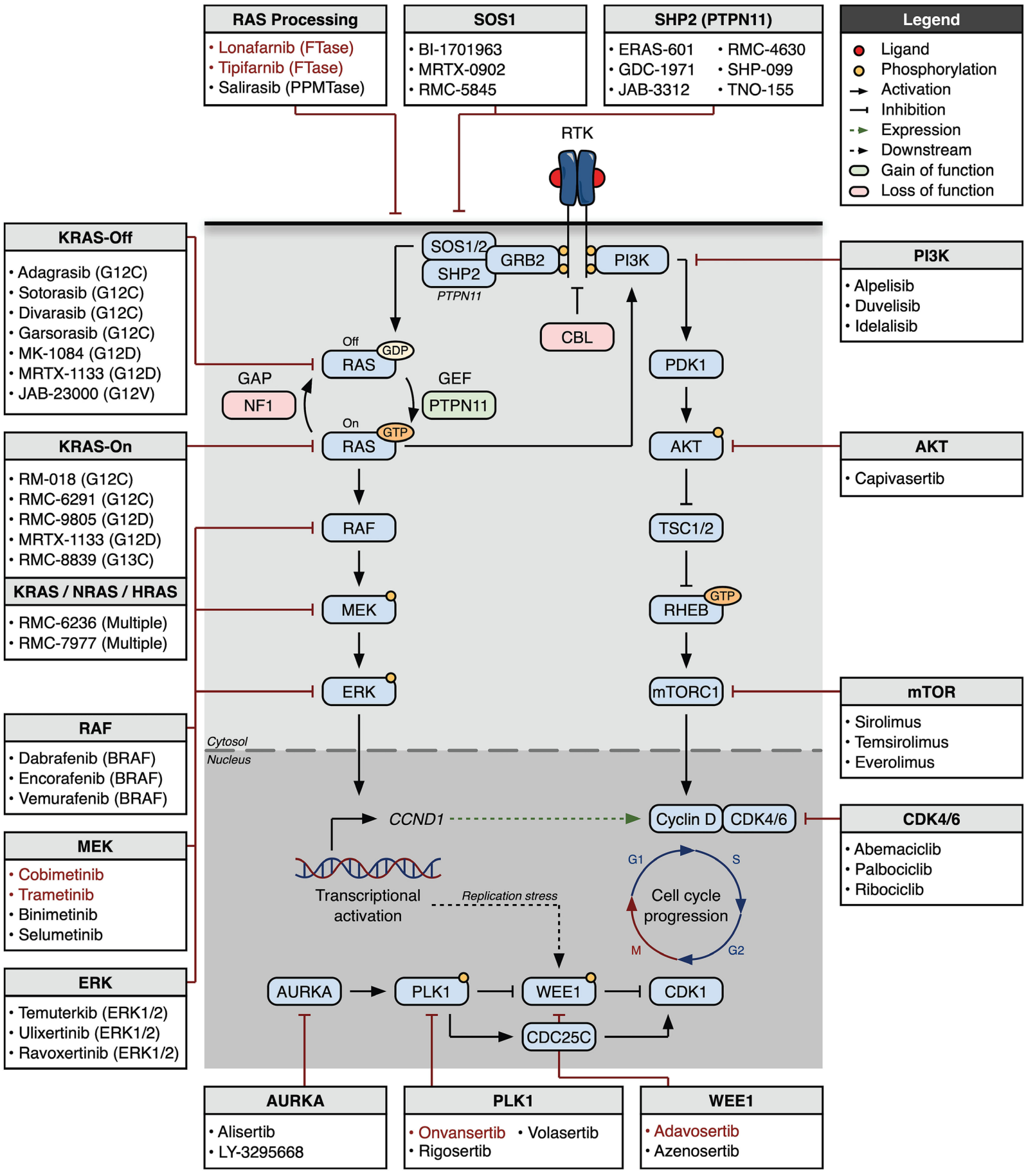


Figure 4. RAS pathway activation and downstream effects in chronic myelomonocytic leukemia. Oncogenic RAS pathway activation is associated with an aggressive tumor biology in many solid malignancies and a proliferative phenotype in chronic myelomonocytic leukemia (CMML). Accordingly, numerous inhibitors of upstream RAS activators, inactive RAS-off states, active RAS-on states, and downstream effectors of RAS signaling are in various phases of development. The central panel provides an overview of the canonical RAS pathway, wherein post-transcriptional processing of RAS via farnesyl-transferase (FTase) and prenylated protein methyltransferase (PPMTase) localize inactive RAS to the cell membrane. There, RAS GTP exchange factors (GEF) such as SOS1, SOS2, and SHP2 (also known as PTPN11) facilitate the transition from inactive GDP-bound RAS-off states to active GTP-bound RAS-on states. Conversely, as RAS has very weak intrinsic GTP hydrolase activity, GTPase-activating proteins (GAP) such as neurofibromin 1 (NF1) facilitate GTP-to-GDP hydrolysis to deactivate RAS. Active RAS signals through RAF, MEK, and ERK family proteins to promote transcription of cell-proliferation genes, including *CCND1* which encodes cyclin D1. Meanwhile, gain-of-function mutations in *PTPN11* and loss-of-function mutations in *NF1* also serve to augment RAS signaling by promoting GEF and impairing GAP function, respec-

Continued on following page.

tively. Similarly, inactivating mutations in *CBL*, which encodes the ubiquitin ligase responsible for degradation of many receptor tyrosine kinases (RTK), also sustain oncogenic RAS signaling. Additionally, RAS activates the non-canonical phosphoinositol-3 kinase (PI3K) pathway, which signals through AKT and mTORC1 to further promote cyclin D expression and, by extension, progression through the G1/S cell cycle checkpoint. Collectively, the transcriptional activation and cell cycle progression driven by the canonical and non-canonical pathways contribute to replication stress and activation of WEE1, which serves to inhibit cyclin dependent kinase 1 (CDK1) and halt progression through the G2/M checkpoint. However, dysregulation of upstream effectors such as aurora kinase A (AURKA) and polo-like kinase 1 (PLK1) mitigate this checkpoint and permit aberrant cell proliferation. The panels along the periphery depict selected agents being developed to target the various aspects of oncogenic RAS signaling. Agents interfering with RAS processing and initial activation are organized along the top. Direct inhibitors of RAS-off and RAS-on states are organized along the left upper edge, while inhibitors of downstream effectors RAF, MEK, and ERK are along the left lower edge. Inhibitors of the non-canonical PI3K pathway are organized along the right. Finally, inhibitors of the DNA damage response pathway and G2/M checkpoint are along the bottom. Agents under investigation in CMML specifically are highlighted in dark red.

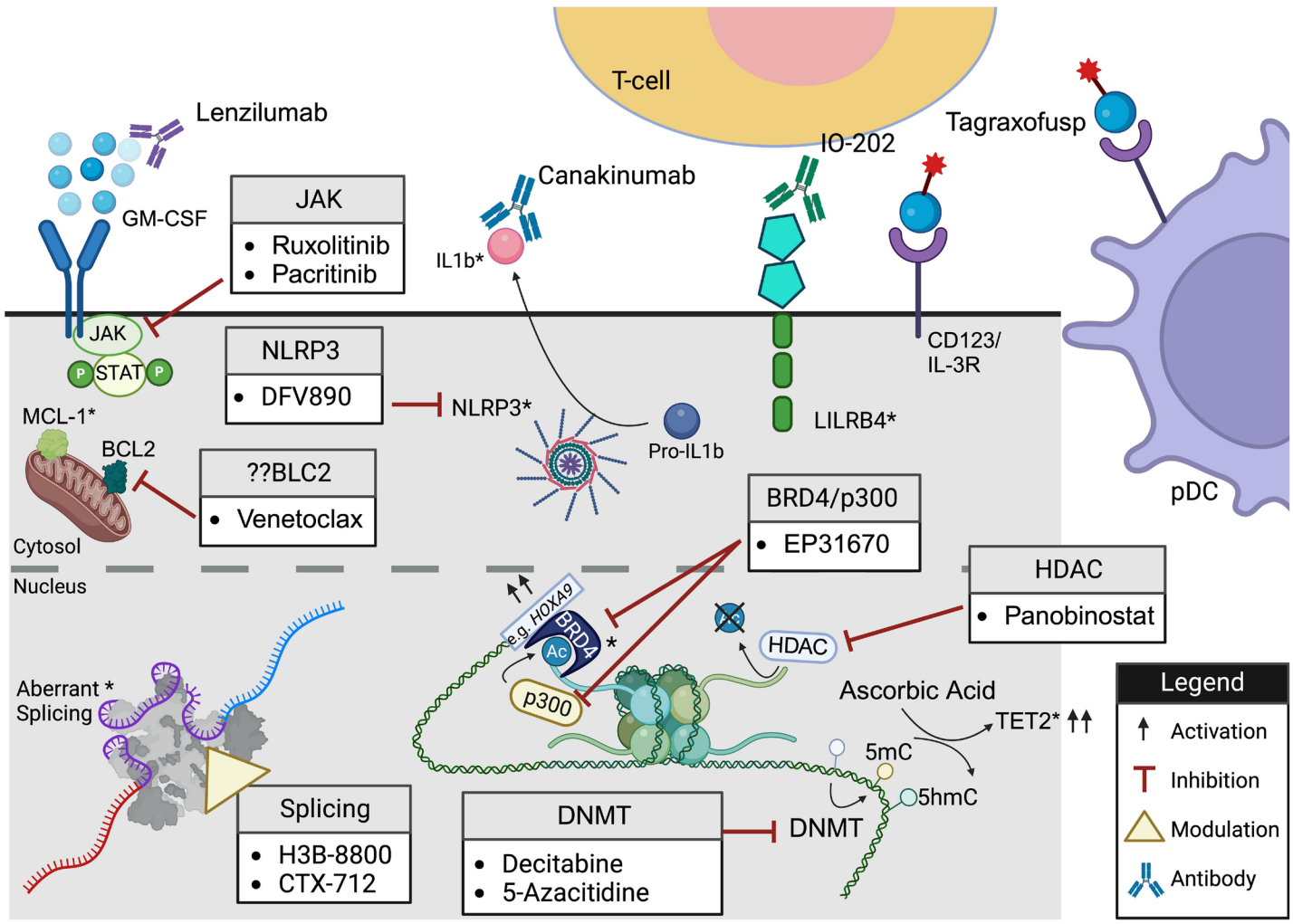


Figure 5. Additional established and investigational therapeutic targets in chronic myelomonocytic leukemia. *Indicates targets that have shown promise in preclinical models and are in early phase clinical trials. Chronic myelomonocytic leukemia (CMML) cells exhibit hypersensitivity to granulocyte-macrophage colony-stimulating factor (GM-CSF) in hematopoietic progenitor colony-formation assays. Activation of the GM-CSF receptor leads to activation of the JAK/STAT pathway, which leads to a specific STAT-5 signature in CMML, giving the rationale for JAK2 inhibitors currently in clinical trials. Lenzilumab is an engineered human immunoglobulin monoclonal antibody, with high affinity for human GM-CSF, which has shown activity in preclinical models of CMML and in phase I clinical trials. Increased activation of the NLRP3 inflammasome, especially in the presence of mutant RAS, contributes to the release of proinflammatory cytokines, interleukin (IL)-1β in particular. Canakinumab is a human anti-IL-1β monoclonal antibody and DFV890 is a new oral NLRP3 inhibitor, both currently in clinical trials. Leukocyte immunoglobulin-like receptor (LILRB4) negatively regulates immune cell activation via T-cell suppression and has been shown to have increased expression in both CMML and acute myeloid leukemia (AML) with a monocytic component. IO-202 is an antagonist antibody targeting LILRB4 in a phase I clinical trial in AML and CMML. CD123 is the α-subunit of IL-3 receptor. Its expression on islands of clonal plasmacytoid dendritic cells in the bone marrow of CMML patients correlates with an increased risk of AML transformation. CD123 is also found on myeloid progenitors and monocytes, and is involved in the proliferation and differentiation of myeloid cells. Tagraxofusp is a recombinant cytotoxin which consists of human IL-3 fused to a truncated diphtheria toxin currently approved for blastic plasmacytoid dendritic cell neoplasm and in clinical trials for CMML. Aberrant splicing is a common feature in CMML. H3B-8800 and CTX-712 are modulators of the spliceosome, believed to induce synthetic lethality in cells that already have a spliceosome dysfunction. These agents are currently in clinical trials in CMML and other myeloid malignancies. Epigenetic dysregulation plays an important role in CMML and other hematologic malignancies. EP31670 is a dual BRD4 and CBP/p300 inhibitor that is currently in early phase clinical trials. Similarly, DNA-methyltransferase inhibitors and histone deacetylase inhibitors, such as panobinostat, attempt to re-establish impaired epigenetic regulation. Ascorbic acid enhances the activity of TET2 also aiming to improve epigenetic deregulation, and is currently in clinical trials. Although targetable, IDH1/2 and FLT3 are very infrequently mutated in CMML, and inhibitors of these proteins are rarely prescribed in CMML. pDC: plasmacytoid dendritic cells; HDAC: histone deacetylase; DNMT: DNA-methyltransferase; 5mC: 5-methylcytosine; 5hmC: 5-hydroxymethylcytosine.

early phase clinical trials (NCT05549661). Combinations with other cell cycle checkpoint and DNA damage repair inhibitors remain to be explored. Additional targets of interest are outlined in Figures 4 and 5, with reported results in CMML cohorts summarized in Table 1.

Conclusion

Epigenetic dysregulation in CMML leads to myeloid bias and clonal monocytosis. Subsequent acquisition of epigenetic/transcription factor mutations typically results in dysplastic CMML, whereas signaling mutations are more commonly associated with the more aggressive proliferative CMML.[86] Increased activation of pro-inflammatory pathways in clonal monocytes as well as accumulation of leukemia-derived plasmacytoid dendritic cells, causing suppression of the adaptive immune system, drive more severe clinical manifestations with inferior outcomes. Despite active research, there remains an unmet clinical need to improve outcomes for CMML patients. Single-agent therapy fails to alter disease biology and the complex pathophysiology of CMML highlights the need to explore combination strategies, with several clinical trials currently underway.

Disclosures

MMP has received research funding from Kura Oncology, Stem Line, Epigenetix, Polaris, and Solutherapeutics.

Contributions

LM, CMC and MMP reviewed existing data, wrote the manuscript and prepared the Figures and Table.

Funding

This work was supported in part by funding from the NIH National Cancer Institute (R01CA272496) to MMP.

References

1. Arber DA, Orazi A, Hasserjian RP, et al. International Consensus Classification of myeloid neoplasms and acute leukemias: integrating morphologic, clinical, and genomic data. Blood. 2022;140(11):1200-1228.
2. Patnaik MM, Tefferi A. Atypical chronic myeloid leukemia and myelodysplastic/myeloproliferative neoplasm, not otherwise specified: 2023 update on diagnosis, risk stratification, and management. Am J Hematol. 2023;98(4):681-689.
3. Merlevede J, Droin N, Qin T, et al. Mutation allele burden remains unchanged in chronic myelomonocytic leukaemia responding to hypomethylating agents. Nat Commun. 2016;7:10767.
4. Ball M, List AF, Padron E. When clinical heterogeneity exceeds genetic heterogeneity: thinking outside the genomic box in chronic myelomonocytic leukemia. Blood. 2016;128(20):2381-2387.
5. Gelsi-Boyer V, Trouplin V, Roquain J, et al. ASXL1 mutation is associated with poor prognosis and acute transformation in chronic myelomonocytic leukaemia. Br J Haematol. 2010;151(4):365-375.
6. Niyongere S, Lucas N, Zhou JM, et al. Heterogeneous expression of cytokines accounts for clinical diversity and refines prognostication in CMML. Leukemia. 2019;33(1):205-216.
7. Mitchell E, Spencer Chapman M, Williams N, et al. Clonal dynamics of haematopoiesis across the human lifespan. Nature. 2022;606(7913):343-350.
8. Fabre MA, de Almeida JG, Fiorillo E, et al. The longitudinal dynamics and natural history of clonal haematopoiesis. Nature. 2022;606(7913):335-342.
9. Mason CC, Khorashad JS, Tantravahi SK, et al. Age-related mutations and chronic myelomonocytic leukemia. Leukemia. 2016;30(4):906-913.
10. Genovese G, Kähler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med. 2014;371(26):2477-2487.
11. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371(26):2488-2498.
12. Abelson S, Collord G, Ng SWK, et al. Prediction of acute myeloid leukaemia risk in healthy individuals. Nature. 2018;559(7714):400-404.
13. Kuranda K, Vargaftig J, de la Rochere P, et al. Age-related changes in human hematopoietic stem/progenitor cells. Aging Cell. 2011;10(3):542-546.
14. Plowden J, Renshaw-Hoelscher M, Engleman C, Katz J, Sambhara S. Innate immunity in aging: impact on macrophage function. Aging Cell. 2004;3(4):161-167.
15. Chambers SM, Shaw CA, Gatza C, Fisk CJ, Donehower LA, Goodell MA. Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation. PLoS Biol. 2007;5(8):e201.
16. Patnaik MM, Lasho TL, Vijayvargiya P, et al. Prognostic interaction between ASXL1 and TET2 mutations in chronic myelomonocytic leukemia. Blood Cancer J. 2016;6(1):e385.
17. Mei M, Pillai R, Kim S, et al. The mutational landscape in chronic myelomonocytic leukemia and its impact on allogeneic hematopoietic cell transplantation outcomes: a Center for Blood and Marrow Transplantation Research (CIBMTR) analysis. Haematologica. 2023;108(1):150-160.
18. Itzykson R, Kosmider O, Renneville A, et al. Prognostic score including gene mutations in chronic myelomonocytic leukemia. J Clin Oncol. 2013;31(19):2428-2436.
19. Patnaik MM, Itzykson R, Lasho TL, et al. ASXL1 and SETBP1 mutations and their prognostic contribution in chronic myelomonocytic leukemia: a two-center study of 466 patients. Leukemia. 2014;28(11):2206-2212.
20. Patnaik MM, Parikh SA, Hanson CA, Tefferi A. Chronic myelomonocytic leukaemia: a concise clinical and pathophysiological review. Br J Haematol. 2014;165(3):273-286.
21. Papaemmanuil E, Gerstung M, Malcovati L, et al. Chronic Myeloid Disorders Working Group of the International Cancer

Genome Consortium. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2013;122(22):3616-3627.
22. Palomo L, Meggendorfer M, Hutter S, et al. Molecular landscape and clonal architecture of adult myelodysplastic/myeloproliferative neoplasms. Blood. 2020;136(16):1851-1862.
23. Awada H, Nagata Y, Goyal A, et al. Invariant phenotype and molecular association of biallelic TET2 mutant myeloid neoplasia. Blood Adv. 2019;3(3):339-349.
24. Rasmussen KD, Jia G, Johansen JV, et al. Loss of TET2 in hematopoietic cells leads to DNA hypermethylation of active enhancers and induction of leukemogenesis. Genes Dev. 2015;29(9):910-922.
25. Ito K, Lee J, Chrysanthou S, et al. Non-catalytic roles of Tet2 are essential to regulate hematopoietic stem and progenitor cell homeostasis. Cell Rep. 2019;28(10):2480-2490.e4.
26. Li Z, Cai X, Cai CL, et al. Deletion of Tet2 in mice leads to dysregulated hematopoietic stem cells and subsequent development of myeloid malignancies. Blood. 2011;118(17):4509-4518.
27. Ostrander EL, Kramer AC, Mallaney C, et al. Divergent effects of Dnmt3a and Tet2 mutations on hematopoietic progenitor cell fitness. Stem Cell Rep. 2020;14(4):551-560.
28. Jakobsen NA, Turkalj S, Zeng AGX, et al. Selective advantage of mutant stem cells in human clonal hematopoiesis is associated with attenuated response to inflammation and aging. Cell Stem Cell. 2024;31(8):1127-1144.
29. Zhang J, Lieu YK, Ali AM, et al. Disease-associated mutation in SRSF2 misregulates splicing by altering RNA-binding affinities. Proc Natl Acad Sci U S A. 2015;112(34):E4726-4734.
30. Kim E, Ilagan JO, Liang Y, et al. SRSF2 mutations contribute to myelodysplasia by mutant-specific effects on exon recognition. Cancer Cell. 2015;27(5):617-630.
31. Kon A, Yamazaki S, Nannya Y, et al. Physiological Srsf2 P95H expression causes impaired hematopoietic stem cell functions and aberrant RNA splicing in mice. Blood. 2018;131(6):621-635.
32. Smeets MF, Tan SY, Xu JJ, et al. Srsf2P95H initiates myeloid bias and myelodysplastic/myeloproliferative syndrome from hemopoietic stem cells. Blood. 2018;132(6):608-621.
33. Gelsi-Boyer V, Trouplin V, Adélaïde J, et al. Mutations of polycomb-associated gene ASXL1 in myelodysplastic syndromes and chronic myelomonocytic leukaemia. Br J Haematol. 2009;145(6):788-800.
34. Abdel-Wahab O, Adli M, LaFave LM, et al. ASXL1 mutations promote myeloid transformation through loss of PRC2-mediated gene repression. Cancer Cell. 2012;22(2):180-193.
35. Asada S, Goyama S, Inoue D, et al. Mutant ASXL1 cooperates with BAP1 to promote myeloid leukaemogenesis. Nat Commun. 2018;9(1):2733.
36. Campagne A, Lee MK, Zielinski D, et al. BAP1 complex promotes transcription by opposing PRC1-mediated H2A ubiquitylation. Nat Commun. 2019;10(1):348.
37. Binder M, Carr RM, Lasho TL, et al. Oncogenic gene expression and epigenetic remodeling of cis-regulatory elements in ASXL1-mutant chronic myelomonocytic leukemia. Nat Commun. 2022;13(1):1434.
38. Carr RM, Vorobyev D, Lasho T, et al. RAS mutations drive proliferative chronic myelomonocytic leukemia via a KMT2A-PLK1 axis. Nat Commun. 2021;12(1):2901.
39. Bezerra ED, Lasho TL, Finke CM, et al. CSF3R T618I mutant chronic myelomonocytic leukemia (CMML) defines a proliferative CMML subtype enriched in ASXL1 mutations with adverse outcomes. Blood Cancer J. 2021;11(3):54.
40. Corces-Zimmerman MR, Hong WJ, Weissman IL, Medeiros BC, Majeti R. Preleukemic mutations in human acute myeloid leukemia affect epigenetic regulators and persist in remission. Proc Natl Acad Sci U S A. 2014;111(7):2548-2553.
41. Shlush LI, Zandi S, Mitchell A, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature. 2014;506(7488):328-333.
42. Lasho T, Patnaik MM. Juvenile myelomonocytic leukemia - a bona fide RASopathy syndrome. Best Pract Res Clin Haematol. 2020;33(2):101171.
43. Li Q, Bohin N, Wen T, et al. Oncogenic Nras has bimodal effects on stem cells that sustainably increase competitiveness. Nature. 2013;504(7478):143-147.
44. Hérault A, Binnewies M, Leong S, et al. Myeloid progenitor cluster formation drives emergency and leukaemic myelopoiesis. Nature. 2017;544(7648):53-58.
45. Ferrall-Fairbanks MC, Dhawan A, Johnson B, et al. Progenitor hierarchy of chronic myelomonocytic leukemia identifies inflammatory monocytic-biased trajectory linked to worse outcomes. Blood Cancer Discov. 2022;3(6):536-553.
46. Hamarsheh S, Osswald L, Saller BS, et al. Oncogenic KrasG12D causes myeloproliferation via NLRP3 inflammasome activation. Nat Commun. 2020;11(1):1659.
47. Hurtado-Navarro L, Cuenca-Zamora EJ, Zamora L, et al. NLRP3 inflammasome activation and symptom burden in KRAS-mutated CMML patients is reverted by IL-1 blocking therapy. Cell Rep Med. 2023;4(12):101329.
48. Broz P, Pelegrín P, Shao F. The gasdermins, a protein family executing cell death and inflammation. Nat Rev Immunol. 2020;20(3):143-157.
49. Baroja-Mazo A, Martín-Sánchez F, Gomez AI, et al. The NLRP3 inflammasome is released as a particulate danger signal that amplifies the inflammatory response. Nat Immunol. 2014;15(8):738-748.
50. Franklin BS, Bossaller L, De Nardo D, et al. The adaptor ASC has extracellular and 'prionoid' activities that propagate inflammation. Nat Immunol. 2014;15(8):727-737.
51. Schmid MC, Avraamides CJ, Dippold HC, et al. Receptor tyrosine kinases and TLR/IL1Rs unexpectedly activate myeloid cell PI3kγ, a single convergent point promoting tumor inflammation and progression. Cancer Cell. 2011;19(6):715-727.
52. Moore AR, Rosenberg SC, McCormick F, Malek S. RAS-targeted therapies: is the undruggable drugged? Nat Rev Drug Discov. 2020;19(8):533-552.
53. You X, Liu F, Binder M, et al. Asxl1 loss cooperates with oncogenic Nras in mice to reprogram the immune microenvironment and drive leukemic transformation. Blood. 2022;139(7):1066-1079.
54. Lucas N, Duchmann M, Rameau P, et al. Biology and prognostic impact of clonal plasmacytoid dendritic cells in chronic myelomonocytic leukemia. Leukemia. 2019;33(10):2466-2480.
55. Mangaonkar AA, Reichard KK, Binder M, et al. Bone marrow dendritic cell aggregates associate with systemic immune dysregulation in chronic myelomonocytic leukemia. Blood Adv. 2020;4(21):5425-5430.
56. Munn DH, Mellor AL. IDO in the tumor microenvironment: inflammation, counter-regulation, and tolerance. Trends Immunol. 2016;37(3):193-207.
57. Munn DH, Zhou M, Attwood JT, et al. Prevention of allogeneic fetal rejection by tryptophan catabolism. Science. 1998;281(5380):1191-1193.

58. Patnaik MM, Pophali PA, Lasho TL, et al. Clinical correlates, prognostic impact and survival outcomes in chronic myelomonocytic leukemia patients with the JAK2V617F mutation. Haematologica. 2019;104(6):e236-e239.
59. Patnaik MM, Timm MM, Vallapureddy R, et al. Flow cytometry based monocyte subset analysis accurately distinguishes chronic myelomonocytic leukemia from myeloproliferative neoplasms with associated monocytosis. Blood Cancer J. 2017;7(7):e584.
60. Padron E, Painter JS, Kunigal S, et al. GM-CSF-dependent pSTAT5 sensitivity is a feature with therapeutic potential in chronic myelomonocytic leukemia. Blood. 2013;121(25):5068-5077.
61. Patnaik MM, Sallman DA, Mangaonkar AA, et al. Phase 1 study of lenzilumab, a recombinant anti-human GM-CSF antibody, for chronic myelomonocytic leukemia. Blood. 2020;136(7):909-913.
62. Hiwase D, Ross DM, Steven W, et al. Lenzilumab in addition to azacitidine improves complete response rates in chronic myelomonocytic leukemia. Blood. 2023;142(Supplement 1):1847.
63. Watanabe S, Itoh T, Arai K. Roles of JAK kinases in human GM-CSF receptor signal transduction. J Allergy Clin Immunol. 1996;98(6 Pt 2):S183-191.
64. Padron E, Dezern A, Andrade-Campos M, et al. Myelodysplastic Syndrome Clinical Research Consortium. A multi-institution phase I trial of ruxolitinib in patients with chronic myelomonocytic leukemia (CMML). Clin Cancer Res. 2016;22(15):3746-3754.
65. Selimoglu-Buet D, Wagner-Ballon O, Saada V, et al. Characteristic repartition of monocyte subsets as a diagnostic signature of chronic myelomonocytic leukemia. Blood. 2015;125(23):3618-3626.
66. Franzini A, Pomicter AD, Yan D, et al. The transcriptome of CMML monocytes is highly inflammatory and reflects leukemia-specific and age-related alterations. Blood Adv. 2019;3(20):2949-2961.
67. Elbæk MV, Sørensen AL, Hasselbalch HC. Cardiovascular disease in chronic myelomonocytic leukemia: do monocytosis and chronic inflammation predispose to accelerated atherosclerosis? Ann Hematol. 2019;98(1):101-109.
68. Mekinian A, Grignano E, Braun T, et al. Systemic inflammatory and autoimmune manifestations associated with myelodysplastic syndromes and chronic myelomonocytic leukaemia: a French multicentre retrospective study. Rheumatology (Oxford). 2016;55(2):291-300.
69. Papo M, Diamond EL, Cohen-Aubart F, et al. High prevalence of myeloid neoplasms in adults with non-Langerhans cell histiocytosis. Blood. 2017;130(8):1007-1013.
70. Bonnet P, Chasset F, Moguelet P, et al. Erdheim-Chester disease associated with chronic myelomonocytic leukemia harboring the same clonal mutation. Haematologica. 2019;104(11):e530-e533.
71. Ghobadi A, Miller CA, Li T, et al. Shared cell of origin in a patient with Erdheim-Chester disease and acute myeloid leukemia. Haematologica. 2019;104(8):e373-e375.
72. Pietras EM, Mirantes-Barbeito C, Fong S, et al. Chronic interleukin-1 exposure drives haematopoietic stem cells towards precocious myeloid differentiation at the expense of self-renewal. Nat Cell Biol. 2016;18(6):607-618.
73. Meisel M, Hinterleitner R, Pacis A, et al. Microbial signals drive pre-leukaemic myeloproliferation in a Tet2-deficient host. Nature. 2018;557(7706):580-584.
74. Zhang Q, Zhao K, Shen Q, et al. Tet2 is required to resolve inflammation by recruiting Hdac2 to specifically repress IL-6. Nature. 2015;525(7569):389-393.
75. Cull AH, Snetsinger B, Buckstein R, Wells RA, Rauh MJ. Tet2 restrains inflammatory gene expression in macrophages. Exp Hematol. 2017;55:56-70.
76. Abegunde SO, Buckstein R, Wells RA, Rauh MJ. An inflammatory environment containing TNFα favors Tet2-mutant clonal hematopoiesis. Exp Hematol. 2018;59:60-65.
77. Cai Z, Kotzin JJ, Ramdas B, et al. Inhibition of inflammatory signaling in Tet2 mutant preleukemic cells mitigates stress-induced abnormalities and clonal hematopoiesis. Cell Stem Cell. 2018;23(6):833-849.
78. Lee SC, North K, Kim E, et al. Synthetic lethal and convergent biological effects of cancer-associated spliceosomal gene mutations. Cancer Cell. 2018;34(2):225-241.
79. Smith MA, Choudhary GS, Pellagatti A, et al. U2AF1 mutations induce oncogenic IRAK4 isoforms and activate innate immune pathways in myeloid malignancies. Nat Cell Biol. 2019;21(5):640-650.
80. Sevin M, Debeurme F, Laplane L, et al. Cytokine-like protein 1-induced survival of monocytes suggests a combined strategy targeting MCL1 and MAPK in CMML. Blood. 2021;137(24):3390-3402.
81. Castaño-Díez S, López-Guerra M, Bosch-Castañeda C, et al. Real-world data on chronic myelomonocytic leukemia: clinical and molecular characteristics, treatment, emerging drugs, and patient outcomes. Cancers (Basel). 2022;14(17):4107.
82. Santini V, Allione B, Zini G, et al. A phase II, multicentre trial of decitabine in higher-risk chronic myelomonocytic leukemia. Leukemia. 2018;32(2):413-418.
83. Coston T, Pophali P, Vallapureddy R, et al. Suboptimal response rates to hypomethylating agent therapy in chronic myelomonocytic leukemia; a single institutional study of 121 patients. Am J Hematol. 2019;94(7):767-779.
84. Coltro G, Mangaonkar AA, Lasho TL, et al. Clinical, molecular, and prognostic correlates of number, type, and functional localization of TET2 mutations in chronic myelomonocytic leukemia (CMML) - a study of 1084 patients. Leukemia. 2020;34(5):1407-1421.
85. Schnegg-Kaufmann AS, Thoms JAI, Bhuyan GS, et al. Contribution of mutant HSC clones to immature and mature cells in MDS and CMML, and variations with AZA therapy. Blood. 2023;141(11):1316-1321.
86. Itzykson R, Solary E. An evolutionary perspective on chronic myelomonocytic leukemia. Leukemia. 2013;27(7):1441-1450.
87. Guan Y, Greenberg EF, Hasipek M, et al. Context dependent effects of ascorbic acid treatment in TET2 mutant myeloid neoplasia. Commun Biol. 2020;3(1):493.
88. DiNardo CD, Jonas BA, Pullarkat V, et al. Azacitidine and venetoclax in previously untreated acute myeloid leukemia. N Engl J Med. 2020;383(7):617-662.
89. Tremblay D, Csizmar CM, DiNardo CD, et al. Venetoclax (VEN) improves response rates but not survival in patients with chronic myelomonocytic leukemia (CMML) treated with hypomethylating agents (HMA): a multicenter, propensity score analysis. Blood. 2023;142(Supplement 1):321.
90. Pei S, Pollyea DA, Gustafson A, et al. monocytic subclones confer resistance to venetoclax-based therapy in patients with acute myeloid leukemia. Cancer Discov. 2020;10(4):536-551.
91. Punekar SR, Velcheti V, Neel BG, Wong KK. The current state of the art and future trends in RAS-targeted cancer therapies. Nat Rev Clin Oncol. 2022;19(10):637-655.

92. Holderfield M, Lee BJ, Jiang J, et al. Concurrent inhibition of oncogenic and wild-type RAS-GTP for cancer therapy. Nature. 2024;629(8013):919-926.
93. Villaume MT, Arrate MP, Ramsey HE, et al. The delta isoform of phosphatidylinositol-3-kinase predominates in chronic myelomonocytic leukemia and can be targeted effectively with umbralisib and ruxolitinib. Exp Hematol. 2021;97:57-65.
94. Palii SS, Van Emburgh BO, Sankpal UT, Brown KD, Robertson KD. DNA methylation inhibitor 5-Aza-2'-deoxycytidine induces reversible genome-wide DNA damage that is distinctly influenced by DNA methyltransferases 1 and 3B. Mol Cell Biol. 2008;28(2):752-771.
95. Thépot S, Lainey E, Cluzeau T, et al. Hypomethylating agents reactivate FOXO3A in acute myeloid leukemia. Cell Cycle. 2011;10(14):2323-2330.
96. Yabushita T, Chinen T, Nishiyama A, Asada S, Shimura R, Isobe T. Mitotic perturbation is a key mechanism of action of decitabine in myeloid tumor treatment. Cell Rep. 2023;42(9):113098.
97. Itzykson R, Santini V, Thepot S, et al. Decitabine versus hydroxyurea for advanced proliferative chronic myelomonocytic leukemia: results of a randomized phase III trial within the EMSCO network. J Clin Oncol. 2023;41(10):1888-1897.
98. Kobayashi Y, Munakata W, Ogura M, et al. Phase I study of panobinostat and 5-azacitidine in Japanese patients with myelodysplastic syndrome or chronic myelomonocytic leukemia. Int J Hematol. 2018;107(1):83-91.
99. Gillberg L, Ørskov AD, Nasif A, et al. Oral vitamin C supplementation to patients with myeloid cancer on azacitidine treatment: normalization of plasma vitamin C induces epigenetic changes. Clin Epigenetics. 2019;11(1):143.
100. Xie Z, McCullough K, Lasho TL, et al. Phase II study assessing safety and preliminary efficacy of high dose intravenous ascorbic acid in patients with TET2 mutant clonal cytopenias. Blood. 2023;142(Supplement 1):4611.
101. Yee KW, Chen HW, Hedley DW, et al. A phase I trial of the aurora kinase inhibitor, ENMD-2076, in patients with relapsed or refractory acute myeloid leukemia or chronic myelomonocytic leukemia. Invest New Drugs. 2016;34(5):614-624.
102. Navada SC, Garcia-Manero G, OdchimarReissig R, et al. Rigosertib in combination with azacitidine in patients with myelodysplastic syndromes or acute myeloid leukemia: results of a phase 1 study. Leuk Res. 2020;94:106369.
103. Short NJ, Jabbour E, Naqvi K, et al. A phase II study of omacetaxine mepesuccinate for patients with higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia after failure of hypomethylating agents. Am J Hematol. 2019;94(1):74-79.
104. Steensma DP, Wermke M, Klimek VM, et al. Phase I first-in-human dose escalation study of the oral SF3B1 modulator H3B-8800 in myeloid neoplasms. Leukemia. 2021;35(12):3542-3550.
105. Assi R, Kantarjian HM, Garcia-Manero G, et al. A phase II trial of ruxolitinib in combination with azacytidine in myelodysplastic syndrome/myeloproliferative neoplasms. Am J Hematol. 2018;93(2):277-285.
106. Hunter AM, Newman H, Dezern AE, et al. Integrated human and murine clinical study establishes clinical efficacy of ruxolitinib in chronic myelomonocytic leukemia. Clin Cancer Res. 2021;27(22):6095-6105.
107. Padron E, Tinsley-Vance SM, DeZern AE, et al. Efficacy and safety of ruxolitinib for treatment of symptomatic chronic myelomonocytic leukemia (CMML): results of a multicenter phase II clinical trial. Blood. 2022;140(Supplement 1):1101-1103.
108. Borthakur G, Popplewell L, Boyiadzis M, et al. Activity of the oral mitogen-activated protein kinase kinase Inhibitor trametinib in RAS-mutant relapsed or refractory myeloid malignancies. Cancer. 2016;122(12):1871-1879.
109. Feldman EJ, Cortes J, DeAngelo DJ, et al. On the use of lonafarnib in myelodysplastic syndrome and chronic myelomonocytic leukemia. Leukemia. 2008;22(9):1707-1711.
110. Ravoet C, Mineur P, Robin V, et al. Farnesyl transferase inhibitor (lonafarnib) in patients with myelodysplastic syndrome or secondary acute myeloid leukaemia: a phase II study. Ann Hematol. 2008;87(11):881-885.
111. Buckstein R, Kerbel R, Cheung M, et al. Lenalidomide and metronomic melphalan for CMML and higher risk MDS: a phase 2 clinical study with biomarkers of angiogenesis. Leuk Res. 2014;38(7):756-763.
112. Kenealy M, Patton N, Filshie R, et al. Results of a phase II study of thalidomide and azacitidine in patients with clinically advanced myelodysplastic syndromes (MDS), chronic myelomonocytic leukemia (CMML) and low blast count acute myeloid leukemia (AML). Leuk Lymphoma. 2017;58(2):298-307.
113. Burgstaller S, Stauder R, Kuehr T, et al. A phase I study of lenalidomide in patients with chronic myelomonocytic leukemia (CMML) - AGMT_CMML-1. Leuk Lymphoma. 2018;59(5):1121-1126.
114. Sekeres MA, Othus M, List AF, et al. Randomized phase II study of azacitidine alone or in combination with lenalidomide or with vorinostat in higher-risk myelodysplastic syndromes and chronic myelomonocytic leukemia: North American Intergroup Study SWOG S1117. J Clin Oncol. 2017;35(24):2745-2753.
115. Adès L, Duployez N, Guerci-Bresler A, et al. A randomised phase II study of azacitidine (AZA) alone or with lenalidomide (LEN), valproic acid (VPA) or idarubicin (IDA) in higher-risk MDS or low blast AML: GFM's "pick a winner" trial, with the impact of somatic mutations. Br J Haematol. 2022;198(3):535-544.
116. Attar EC, Amrein PC, Fraser JW, et al. Phase I dose escalation study of bortezomib in combination with lenalidomide in patients with myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Leuk Res. 2013;37(9):1016-1020.
117. Sekeres MA, Watts J, Radinoff A, et al. Randomized phase 2 trial of pevonedistat plus azacitidine versus azacitidine for higher-risk MDS/CMML or low-blast AML. Leukemia. 2021;35(7):2119-2124.
118. Short NJ, Muftuoglu M, Ong F, et al. A phase 1/2 study of azacitidine, venetoclax and pevonedistat in newly diagnosed secondary AML and in MDS or CMML after failure of hypomethylating agents. J Hematol Oncol. 2023;16(1):73.
119. Patnaik MM, Ali H, Wang ES, et al. Tagraxofusp (SL-401) in patients with chronic myelomonocytic leukemia (CMML): updated results of an ongoing phase 1/2 trial. Blood. 2021;138(Supplement 1):538.
120. Patnaik MM, Sallman DA, Mangaonkar AA, et al. Phase 1 study of lenzilumab, a recombinant anti-human GM-CSF antibody, for chronic myelomonocytic leukemia. Blood. 2020;136(7):909-913.
121. Hiwase D, Ross DM, Lane SW, et al. Lenzilumab in addition to azacitidine improves complete response rates in chronic myelomonocytic leukemia. Blood. 2023;142(Supplement 1):1847.
122. Brunner AM, Esteve J, Porkka K, et al. Phase Ib study of sabatolimab (MBG453), a novel immunotherapy targeting TIM-3 antibody, in combination with decitabine or azacitidine in high- or very high-risk myelodysplastic syndromes. Am J Hematol. 2024;99(2):E32-E36.
123. Saliba AN, Litzow MR, Gangat N, et al. Outcomes of venetoclax-

based therapy in chronic phase and blast transformed chronic myelomonocytic leukemia. Am J Hematol. 2021;96(11):E433-E436.
124. Montalban-Bravo G, Hammond D, DiNardo CD, et al. Activity of venetoclax-based therapy in chronic myelomonocytic leukemia. Leukemia. 2021;35(5):1494-1499.
125. Tremblay D, Csizmar CM, DiNardo CD, et al. Venetoclax (VEN) improves response rates but not survival in patients with chronic myelomonocytic leukemia (CMML) treated with hypomethylating agents (HMA): a multicenter, propensity score analysis. Blood. 2023;142(Supplement 1):321.