Clonal Cytopenias of Undetermined Significance: Potential Predictor of Myeloid Malignancies?

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Abstract: The recent identification of the potential for clonal replication in patients with unexplained cytopenias, resulting in myelodysplastic syndrome (MDS) or myeloid malignancies, has opened the way to identifying a new precursor entity: clonal cytopenia of undetermined significance (CCUS). CCUS has come into the spotlight in recent years with the detection of molecular abnormalities in cytogenetic studies, fluorescence in situ hybridization, and next-generation sequencing. Several clinical trials and retrospective studies are underway to examine further the associated mutation profiles, study the progression of CCUS to MDS or myeloid neoplasm, and investigate potential treatment options. In this review, we discuss CCUS-related mutations in genes such as *DNMT3A*, *TET2*, *IDH1/2*, *ASXL1*, *KDM6A*, *PHF6*, *SF3B1*, *SRSF2*, *U2AF1*, *ZRSR2*, *RUNX1*, *BCOR*, *NRAS*, *KRAS*, *KIT*, *PTEN*, *CBL*, *TP53*, and *ATM*. We highlight the most common mutations in CCUS, including those in *DNMT3A*, *TET2*, *ASXL1*, *SRSF2*, and *SF3B1*, and high-risk mutations, including those in *U2AF1*, *ZRSR2*, *SRSF2*, *JAK2*, *RUNX1*, and *TP53*. Cognizance of these mutations can guide surveillance and heighten awareness of the need to screen patients with unexplained cytopenia as a means of primary prevention in the realm of MDS and AML. Knowledge of mutation profiles, prognostic risk factors, treatment, and follow-up strategies is evolving, and prospective studies are warranted.

Keywords
Acute myeloid leukemia, clonal cytopenia, clonal cytopenia of undetermined significance, clonal hematopoiesis, clonal hematopoiesis of indeterminate potential, myelodysplastic syndrome

Background

Clonal cytopenia of undetermined significance (CCUS) is defined as unexplained cytopenia with evidence of clonal cells (variant allele frequency [VAF] ≥2%), no morphologic or cytogenetic evidence of dysplasia (<10%), but the presence of one or more myelodysplastic syndrome (MDS)–related gene mutations (Table 1).[1,2] In studies

Table 1. Classification of Unexplained Cytopenia and Clonality

Unexplained Cytopenia	Characteristic Features				
	Cytopenia	Clonality (VAF), %	Dysplasia, %	BM Blasts, %	Risk for Progression to MDS/AML
Age-related	+	<2	-	<5	None/very low
ICUS	+	<2	<10	<5	Very low
CHIP	–	≥2	<10	<5	Very low
CCUS	+	≥2	<10	<5	Low
MDS, low-risk	+	Usually ≥2	≥10	<5	Low
MDS, high-risk	+	Usually ≥2	≥10	<20	High

AML, acute myeloid leukemia; BM, bone marrow; CCUS, clonal cytopenia of undetermined significance; CHIP, clonal hematopoiesis with indeterminate potential; ICUS, idiopathic cytopenia of undetermined significance; MDS, myelodysplastic syndrome; VAF, variant allele frequency.

of patients with unexplained cytopenias, 8% to 36% of them were identified as having CCUS.[2,3] A myeloid neoplasm is 14 times more likely to develop in patients with CCUS than in patients without clonality (P<.001). Progression to MDS/myeloid neoplasm occurred in 18% of patients with CCUS within 16 months, and progression to myeloid neoplasm occurred in 80% within 5 years.[3,4]

Few terminologies for conditions related to CCUS are defined in the literature. Idiopathic cytopenia of undetermined significance (ICUS) is another category of unexplained cytopenia with minimal or no dysplasia (<10%). ICUS is similar to CCUS, but with minimal or no clonality (VAF <2%) and no known MDS-related gene mutations (Table 1). ICUS seems to resolve over time in most cases, although progression to myeloid neoplasm occurs in approximately 10% of patients. In some cases of ICUS, mutations similar to those seen in CCUS lead to myeloid neoplasms, which provides some evidence of a temporal relationship.[3,5] Clonal hematopoiesis with indeterminate potential (CHIP) is a clonal hematopoietic disorder and is not included in the category of unexplained cytopenia. CHIP is characterized by the presence of clonal mutations (usually 1 detectable mutation), minimal dysplasia (<10% of cells per lineage), clonality (VAF ≥2%), the absence of cytopenias, and few or no bone marrow blasts (<5%; Table 1). CHIP is common in healthy individuals, with a risk for progression to MDS, acute myeloid leukemia (AML), myeloproliferative neoplasms (MPN), or chronic myelomonocytic leukemia (CMML) of approximately 0.5% to 1% per year. CHIP has also been described as a subset of CCUS, in which cytopenias are due to the clonal proliferation of a particular cell line.[5] The paradigm of the clonal evolution of CHIP to myeloid neoplasm is comparable to that of monoclonal gammopathy of undetermined significance (MGUS) to multiple myeloma. That is, both CHIP and MGUS are clonal proliferative disorders without apparent cytopenia and with a low annual risk for progression (0.5%-1%).[6,7] In contrast, CCUS has clonality but lacks a proliferative nature, and it has very high rates of neoplastic transformation (risk for progression within 5 years of 80%).[3]

The true incidence and prevalence of CCUS are unknown. The median age of patients with CCUS (74 years) is similar to that of patients with MDS (75 years); the median age of patients with ICUS is younger (66 years).[2,3] Somatic mutations play a significant role in CCUS, as in other myeloid disorders, such as MDS and AML. Possible environmental factors, such as aging, smoking, inflammatory states, and previous malignancy, are postulated to contribute to the progression of unexplained cytopenias, given the variable levels of progression despite similar mutation profiles.[8-10]

Several pathogenic pathways leading to CCUS have been proposed. One potential pathogenic trigger is the acquisition of one or more driver mutations (*DNMT3A*, *TET2*, *ASXL1*), possibly in the presence of environmental factors (eg, aging, smoking). Over time, more mutations may be acquired, increasing molecular complexity, although the factors contributing to the acquisition of additional mutations are not well understood.[10,11] Another explanation, proposed by Jajosky and colleagues, is the development of dysplasia with progression to MDS with or without the acquisition of new mutations in patients with CCUS. A possibility of CCUS as a part of a spectrum has been proposed; when it was subclassified into CCUS with dysplasia (CCUS-D) and CCUS without dysplasia (CCUS-ND), the rate of progression to MDS was higher in patients with CCUS-D than in those with CCUS-ND.[10] Lastly, it has been proposed that among a small percentage of patients with CHIP, the expansion of hematopoietic clones suppresses other cell lines, leading to cytopenia and CCUS.[5] Thus, the acquisition of additional mutations, dysplasia, progression to MDS, and clonal hematopoiesis can lead to the development of

myeloid neoplasms, illustrating the leukemogenic potential of CCUS (Figure).

Mutations Associated With CCUS

The mutations in CCUS are similar to the MDS-related mutations typically found in the workup of patients with unexplained cytopenias. Myeloid neoplasm is 14 times more likely to develop in patients with CCUS than in patients without clonal disease.[3,12] Mutations in CCUS have effects on DNA methylation (*DNMT3A*, *TET2*, *IDH1/2*), chromatin modification (*ASXL1*, *KDM6A*, *PHF6*), RNA splicing (*SF3B1*, *SRSF2*, *U2AF1*, *ZRSR2*), transcription regulation (*RUNX1*, *BCOR*), signaling (*NRAS*, *KRAS*, *KIT*, *PTEN*, *CBL*), and cell cycle/DNA repair (*TP53*, *ATM*) (Table 2).[4,10,12-14]

DNA Methylation

Mutations in *DNMT3A* and *TET2* occur in approximately 21% and 34% of patients with CCUS, respectively.[10,13,14] Missense mutations in the *DNMT3A* gene block methyltransferase activity, causing the hypermethylation of genes that increase hematopoietic stem cell self-renewal and abrogate differentiation. *DNMT3A* mutations are found in normal aging, CCUS, MDS, and AML, supporting the notion of a common genetic thread among these myeloid disorders. However, the prognostic implication of *DNMT3A* in CCUS remains unclear, with some studies reporting no significantly increased risk for progression to MDS; this finding raises the possibility that mutations in *DNMT3A* may need to act in concert with mutations in other genes.[15,16] It is also possible that the variation in risk for progression is related to *DNMT3A* R882 mutations; their presence has been associated with a poor prognosis in MDS and myeloid neoplasms, whereas *DNMT3A* without R882 mutations has not.[17] Inactivating mutation of *TET2* decreases the enzymatic *TET2* function and downregulates the conversion of methylcytosine to hydroxymethylcytosine, leading to increased hematopoietic stem cell self-renewal and impaired differentiation. *TET2* mutations in CCUS are considered to signal a high risk for progression to myeloid neoplasms.[16] Isocitrate dehydrogenase (IDH) enzymatic site mutations alter the reduction of alpha-ketoglutarate, a vital step in the Krebs cycle, to an oncometabolite, 2-hydroxyglutarate. The oncometabolite activates the hypoxia-inducible factor 1 alpha pathway via prolyl hydrolase inhibition.[18] *IDH1* and *IDH2* mutations occur relatively infrequently in CCUS, in 13% and 2% of cases, respectively. *IDH1* and *IDH2* mutations have been implicated in progression from MDS to AML in prospective studies, although the associated risk for progression in patients with CCUS remains unknown.[10,13,18]

Chromatin Modification

Chromatin modifier mutations such as *ASXL1* mutations are common (12% frequency of occurrence) in CCUS, whereas *KDM6A* and *PHF6* mutations are uncommon. Loss-of-function mutation of *ASXL1* reduces polycomb repressor complex 2 recruitment and decreases trimethylation. *ASXL1* mutations in CCUS are known to signal a high risk for progression.[14,16]

Spliceosome

Among spliceosome gene mutations, *SF3B1* and *SRSF2* mutations are more common than *U2AF1* and *ZRSR2* mutations in CCUS. *SRSF2* mutation occurs more frequently as a co-mutation with *TET2* and *ASXL1* mutations in patients with CCUS. However, all occur more frequently in patients with MDS/AML than in those with CCUS. Spliceosomal mutations of *SF3B1* and *U2AF1* genes and RNA splicing mutations of *ZRSR2* and *SRSF2* genes are relatively common in patients with MDS.[3,10] The differences between the patterns of these epigenetic mutations in CCUS and the patterns in MDS demonstrate that even in a case of mild or unclear dysplasia in an unexplained cytopenia, the presence of specific mutation patterns may help to differentiate MDS from CCUS.[3]

Transcription Regulation

RUNX1 is a transcription regulator gene; *RUNX1* mutation is considered to be among the high-risk CCUS mutations owing to its more frequent association with CCUS with minimal dysplasia and MDS than with CCUS with no dysplasia.[10,14] *BCOR* is an X-chromosome gene, a co-repressor of BCL-6 that interacts with histone deacetylases in cell cycle regulation. It occurs in 5% to 8% of patients with CCUS, a rate similar to that found in patients with MDS. A negative effect of *BCOR* mutation has been detected among patients with MDS, although its prognostic implication in CCUS is unknown.[19] Other mutations, such as *EZH2* and *ETV6* mutations, are rarer among patients with CCUS than in patients with MDS, and they may not play a role in CCUS pathogenesis.[10] *BCOR* mutations more commonly occur as co-mutations with *TET2*, *ASXL1*, and *DNMT3A* mutations among patients with CCUS.[3,20]

Signal Transduction

NRAS and *KRAS* mutations activate aberrant cell proliferation by signaling RAS/RAF/MEK/ERK pathways. *NRAS* and *KRAS* mutations are rare in CCUS (2.8%).[4] *NRAS* and *KRAS* mutations are found in patients with CCUS that progresses to myeloid neoplasm, implicating these mutations in progression.[14,18] Mutations in the receptor tyrosine kinase *KIT* gene are rare (1.4%) in CCUS, with an unknown risk for progression.[4] *JAK2* mutations were

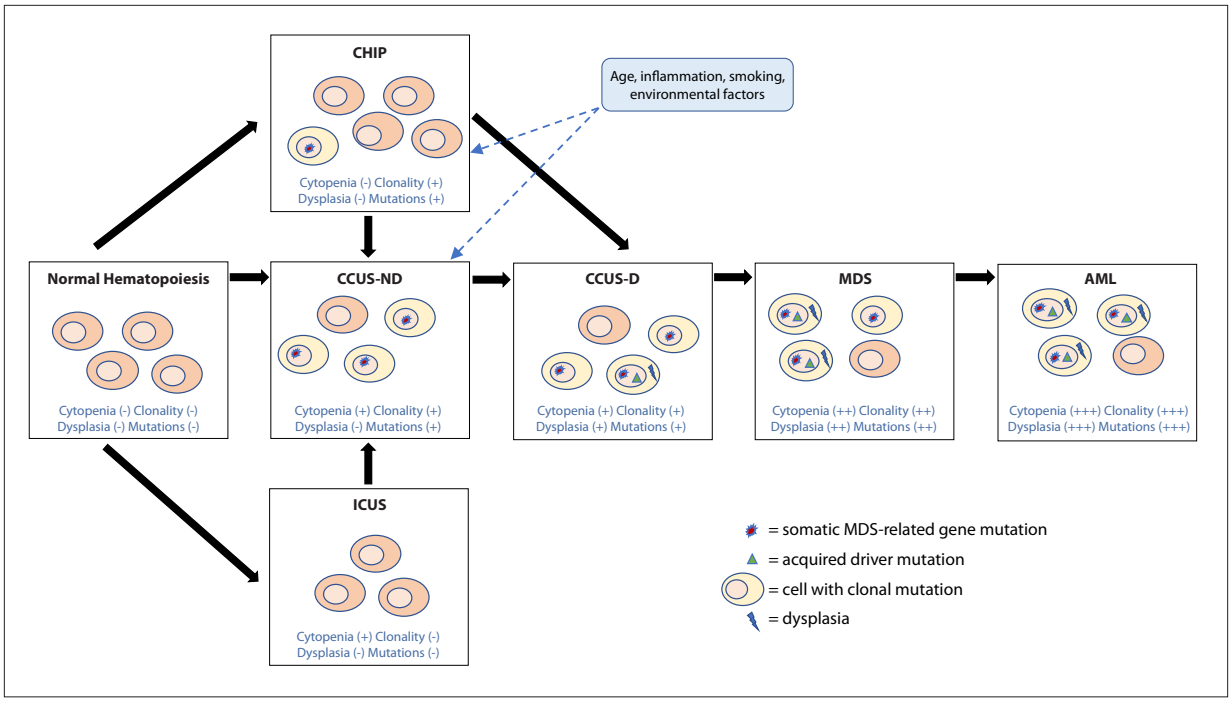

Figure. Proposed pathogenesis of progression from of clonal cytopenia of undetermined significance (CCUS) without dysplasia to CCUS with dysplasia to myelodysplastic syndrome (MDS), including common mutations. Genetic factors, such as MDS-related driver mutations, and environmental triggers, such as age, smoking, and inflammatory processes, may contribute to the clonal expansion of MDS-related mutations, and therefore to the progression of clonal hematopoiesis with indeterminate potential/idiopathic cytopenia of undetermined significance to CCUS once variant allele frequency is at least 2%.

AML, acute myeloid leukemia; CCUS, clonal cytopenia of undetermined significance; CCUS-D, CCUS with dysplasia; CCUS-ND, CCUS without dysplasia; CHIP, clonal hematopoiesis with indeterminate potential; ICUS, idiopathic cytopenia of undetermined significance; MDS, myelodysplastic syndrome.

also rare in CCUS, although their presence in unexplained cytopenia revealed MDS/myelofibrosis/myeloid neoplasms.[3,21] *CBL* mutations impair the negative regulatory effect of cell signaling pathways by inhibiting the ubiquitination of several signaling molecules, including some tyrosine kinases. *CBL* gene mutations are found at a rate of approximately 1% among patients with CCUS. They occur as co-mutations, but the associated risk for progression is unknown.[4,13]

Cell Cycle/DNA Damage Response Mutations

Mutations in the classic tumor suppressor genes *TP53* and *PPM1D* occur most commonly in patients with previous malignancy who have received cytotoxic therapy; they lead to aberrations in cell cycle regulation and DNA damage response/repair pathways. The heterogeneity in *TP53* mutations and variance in P53 protein function have been extensively studied. It is possible that after exposure to cytotoxic therapy (chemotherapy or radiation therapy), either new *TP53*-mutant cells are generated or pre-existing/germline *TP53* mutations become resistant to cytotoxic therapy and are activated, resulting in an alteration of clonal hematopoiesis or the development of a new primary myeloid neoplasm.[22-24] Other extrinsic factors, such as inflammation, autoimmune disease, and infection, may contribute to a bone marrow microenvironment that permits or enhances *TP53* clonal expansion.[21] In one study, 8 patients with previous malignancies treated with cytotoxic therapy were subsequently found to have CCUS with *TP53* mutations, similar to the *TP53* mutation exon sites seen in MDS. Patients with progression showed a transition from normal to complex karyotype and an exponential increase in VAF from 6.9% in CCUS, to 14.4% in MDS, to 41.1% in AML. Therefore, an increase in the VAF of a *TP53* mutation and serial cytogenetic studies may be used to assess disease progression in patients with CCUS and a *TP53* mutation.[9,25] However, the significance of differences in the rates of progression among such patients is unclear.[24] Germline mutations can also be confirmed in biopsy specimens obtained from unaffected tissue, such as skin or hair follicle.[23] *TP53* mutations may carry a

relatively better prognosis among patients without a history of previous cytotoxic therapy than among patients whose myeloid disorder arises in the setting of previous cytotoxic therapy. Overall, *TP53* mutations in CCUS are associated with variable rates of progression and carry a poor prognosis once CCUS has progressed to MDS or AML.

PPM1D, a protein phosphatase, downregulates several DNA damage repair genes of the cell cycle. *PPM1D* mutations are associated with CHIP and solid tumors previously treated with chemotherapy, specifically cisplatin and doxorubicin, and they are associated with a high risk for transformation to therapy-related myeloid neoplasms. Hyperactive *PPM1D* mutations in hematopoietic cells increase their resistance to apoptosis so that they out-compete their normal counterparts, resulting in clonal hematopoiesis. However, this mutation, unlike *TP53* mutations, has not been associated with CCUS.[12,26] Another tumor suppressor gene/cell cycle regulator gene with mutations is *PTEN*. Mutations in *PTEN* occur rarely in CCUS (1.4%), and the risk for progression associated with these mutations is unknown.

Common Mutations in CCUS

The common mutations in CCUS include *DNMT3A*, *TET2*, *ASXL1*, *SRSF2*, and *SF3B1*, which together occur in approximately 70% of patients with CCUS.[8,13-15,27] The so-called DTA mutations—*DNMT3A*, *TET2*, and *ASXL1*—are the most common mutations in CCUS. They are associated with a "typical aging pattern," meaning that they are frequently found in older patients whether they have cytopenias or not.[2,3,12] *TET2* gene mutations occur more frequently than *ASXL1* and *DNMT3A* mutations in patients with CCUS, as they do in those with MDS or AML, whereas *ASXL1* mutations are more common than *TET2* or *DNM3TA* mutations among patients with CHIP.[2,28] Interestingly, these mutation patterns are not present in patients with cytopenias with identified causes, indicating the relevance of the mutations within the spectrum of CCUS, MDS, and AML.

Co-mutation Patterns in CCUS

Whereas CCUS is associated with molecular aberrations in single genes, co-mutations of *TET2*, *ASXL1*, and *DNMT3A* with *RUNX1*, *EZH2*, *CBL*, *BCOR*, *CUX1*, *TP53*, or *IDH1/IDH2* occur more frequently in myeloid neoplasms. The acquisition of new mutations may therefore shed light on progression from CCUS to MDS and/or AML.[3,25]

Other Mutations

Other, less common CCUS mutations include *CUL3*, *DDX41*, *SETBP1*, and *STAG2*. The risk for progression with these mutations is unknown (Table 2).

Prognostic Risk Factors for CCUS Progression

A myeloid neoplasm develops in 80% of patients with CCUS in 5 years and in 95% in 10 years, suggesting that early identification could guide longitudinal clinical and genomic monitoring. In turn, monitoring could lead to interventions that prevent progression. In contrast, the rate of progression among patients with ICUS was 9% at 5 and at 10 years, highlighting the importance of differentiating CCUS from ICUS among patients with unexplained cytopenias.[3] Monitoring for the acquisition of additional mutations, dysplasia, or an increase in the percentage of bone marrow blasts can facilitate the early identification and treatment of myeloid neoplastic disorders.

Nonetheless, not all cases of CCUS progress to MDS or myeloid malignancies, and at present, the specific risk factors for CCUS progression are largely unknown.[1] Despite the presence of mutations, progression occurs in only some patients, indicating that other factors are associated with progression. A higher number of mutations, a VAF higher than 10%, and the acquisition of additional mutations are associated with an elevated risk for progression.[9,16,25] Inflammation has been associated with progression in CHIP and may also cause progression in CCUS.[25,29,30]

Mutation Patterns

The rates of progression to MDS and AML are higher in patients with specific CCUS mutation patterns, especially mutations in *U2AF1*, *ZRSR2*, *SRSF2*, *SF3B1*, *JAK2*, *RUNX1*, and *TP53*, either singly or as co-mutations.[21] Patients with these mutations need to be watched closely, even in the absence of dysplasia, given the high likelihood of progression.[3] In addition, *TP53* mutations rarely occur among patients with CCUS, but an increase in VAF can be associated with faster progression to MDS and can be an important prognostic factor.[9] Mutations in *ASXL1*, *CBL*, *DNMT3A*, *NRAS*, and *RUNX1* may be associated with worsening cytopenias, increased monocytosis, and a heightened risk for progression.[31]

Number of Mutations

A higher number of mutations (>2) elevates the risk for progression. The presence of 2 or more mutations is more frequent with MDS/myeloid neoplasm than with CCUS, which is indicative of an elevated risk for progression among patients with CCUS.[3,14] In particular, the predictive value of co-mutations of *DNMT3A*, *TET2*, or *ASXL1* with *RUNX1*, *EZH2*, *CBL*, *BCOR*, *CUX1*, or *TP53* for

myeloid progression was higher than the predictive value of isolated DTA mutations.[3]

Variant Allele Frequency

A higher VAF for any mutations has been shown generally to confer an increased risk for progression. A VAF of at least 10% was the most accurate predictor of MDS in one study[3]; in another, a VAF of at least 30% was the most accurate predictor.[25] Therefore, a closer follow-up for patients who have CCUS with a mutational VAF higher than 10% would be reasonable.

Environmental Factors

The association between smoking and mutations in *ASXL1*, non-*TET2*, *DNMT3A*, and *RUNX1*, including combinational mutation pattern of *TET2*, *DNMT3A*, and *ASXL1*, has recently been described in patients with CCUS. However, the mutational burden was not different between smokers and nonsmokers.[8,10] In a cohort study evaluating patients with myeloid clonal hematopoiesis, mutations in *ASXL1* were strongly associated with smoking status. The authors hypothesized that this could have been due to the inflammatory environment created by smoking, which may enhance the growth of *ASXL1*-mutated clones.[8]

Management

At the present time, it is not known whether treatment of CCUS improves progression-free survival or overall survival because no specific guidelines for diagnosing and treating CCUS are available. With an 80% risk for progression from CCUS to MDS/AML, we propose some principles for the management of patients with CCUS, with the goals of early detection and treatment of MDS/AML transformation.

Management strategies should strive to achieve a balance among survival advantage, patient quality of life, and cost-effectiveness. Tools and guidelines to stratify patients by risk become necessary for making decisions on how to proceed. CCUS knowledge is scarce, so it is challenging to formulate a generalized plan. It is also important to tailor a plan to each patient individually and incorporate shared decision making. As an example, younger patients may require more frequent monitoring to detect progression earlier.

We present strategies for disease monitoring that are based on our current understanding. When persistent, unexplained cytopenias are detected, baseline testing should be considered and include the following: bone marrow aspiration and biopsy, cytogenetic analysis, fluorescence in situ hybridization (FISH), flow cytometry, and next-generation sequencing (NGS). The diagnosis of CCUS is based on cell clonality with a VAF of 2% of higher, the absence of morphologic or cytogenetic evidence of dysplasia (<10%), and the presence of one or more MDS-related gene mutations. CCUS can be subdivided into 2 categories based on a patient's profile: high-risk CCUS (HR-CCUS) and low-risk CCUS (LR-CCUS). The criteria for HR-CCUS are the following: the presence of 2 or more mutations associated with CCUS; VAF of 10% or higher; the presence of mutations in *U2AF1*, *ZRSR2*, *SRSF2*, *SF3B1*, *JAK2*, *RUNX1*, or *TP53,* either singly or as co-mutations, or the presence of co-mutations in *DNMT3A*, *TET2*, or *ASXL1* with *RUNX1*, *EZH2*, *CBL*, *BCOR*, *CUX1*, or *TP53*. In patients with CCUS, we recommend complete blood cell counts and a peripheral smear once every 3 to 6 months with bone marrow analysis at any time of a significant change in cytopenias. A patient with HR-CCUS can be monitored more frequently with peripheral blood counts if the patient and physician feel that this is necessary.

Symptomatic management with erythroid-stimulating agents should be discussed with patients who have significant anemia that is affecting quality of life. Other potential interventions stem from MDS/AML treatment options and include hypomethylating agents (HMAs) and targeted treatments against mutations in genes such as *IDH1*, *IDH2*, *KRAS*, and *NRAS.* Efficacy is measured by the symptomatic and hematologic response in a median follow-up period of 14.3 months (range, 2.3-59.9).[2,14] However, no conclusive evidence is available for these recommendations, so risks and benefits must be carefully weighed.

HMAs such as azacitidine and decitabine are widely used alone or in combination with the BCL-2 inhibitor venetoclax (Venclexta, AbbVie) to treat AML in the elderly and AML evolving from MDS.[21,32,33] Inhibition of DNA methyltransferase 1 (DNMT-1) with resultant hypomethylation and reversal of the effects of mutations in epigenetic genes affecting DNA methylation and chromatin modification (*DNMT3A*, *TET2*, *IDH1*, *IDH2*, and *ASXL1*) is a presumed HMA mechanism of action in AML. In a retrospective study of 24 patients with CCUS who received treatment other than blood transfusions, most of the responders had received an HMA (response rate, 78%; *P*=.04). HMAs were also the most commonly used treatment. Treatment was not based on a particular type of mutation and the criteria used to determine treatment were not clearly defined, although 60% of patients in this study had epigenetic modifier mutations.[14] In prior studies among MDS patients, treatment with HMA showed a higher response rate when mutant *TET2* was combined with wild-type *ASXL1* than when this combination was not present (60% vs 43%, respectively). No difference was found between the 2 treatments in terms of

Table 2. CCUS-Associated Mutation Types, Frequency, and Likelihood of Progression

Mutation	Type of Gene	Approximate Frequency of Mutation, %	Risk for Progression to MDS
DNMT3A	DNA methylation	13-35[4,10,13,14]	Unclear risk for progression. The authors note that this genetic mutation does not play a direct role in cytopenia.[15,16]
TET2	DNA methylation	21-42[4,10,13,14]	Potentially high risk[14,16]
IDH1	DNA methylation	8.5-13[4,14]	Unknown
IDH2	DNA methylation	2-3[4,10,13]	Unknown
ASXL1	Chromatin modification	8.2-14[4,10,13,14]	High risk[14,16]
KDM6A	Chromatin modification	1.4-4[4,14]	Unknown
PHF6	Chromatin modification	2.8-4[4,14]	Unknown
SRSF2	RNA splicing	14-21[4,10,13,14]	High risk[3]
U2AF1	RNA splicing	4-21[4,10,13,14]	High risk[3,14]
SF3B1	RNA splicing	5.6-16[4,10,13,14]	High risk[2,14] Interestingly, *SF3B1* is associated with a better prognosis and with ring sideroblasts in patients who have MDS.[15]
ZRSR2	RNA splicing	13-14[4,14]	High risk[3]
BCOR	Transcription regulation	5.6-8[4,14]	Unknown
RUNX1	Transcription regulation	1.4-8.2[2,4,10,13]	High risk for progression to MDS and AML[3,14]
JAK2	Signaling	4[14]	High risk among isolated mutations or co-mutations of *SRSF2* and *U2AF1* in myelofibrosis[3]
CBL	Signaling	1.4[4]	Unknown
KRAS	Signaling	2.8[4]	High risk[14]
NRAS	Signaling	2.8[4]	High risk[14,18]
KIT	Signaling	1.4[4]	Unknown
TP53	Cell cycle/ DNA repair	2-17[10,13,14]	Associated with a poor prognosis in MDS and AML[9]
PTEN	Cell cycle/tumor suppressor	1.4[4]	Unknown
CUL3	Ubiquitin-proteasome system	4[14]	Unknown
DDX41	Encodes RNA helicase, tumor suppressor gene	4[14]	Unknown
SETBP1	Encodes SETBP1 protein; binds to DNA to increase gene expression	1.4-4[4,14]	Unknown
STAG2	DNA replication	2-4.2[4,13,14]	Unknown

AML, acute myeloid leukemia; MDS, myelodysplastic syndrome

overall survival rates.[34] Among AML patients, the combination of TET2 and ASXL1 mutations did not correlate with a better response to HMA treatment and further validation is needed in patients with CCUS.[18,32]

Other treatment options in CCUS include growth factors, corticosteroids, thrombopoietin receptor agonists, rituximab, and intravenous immunoglobulin.[14] However, it is challenging to determine which factors affected physician preference and if other diseases were present (eg, immune thrombocytopenia). Targeted therapies against IDH2 (enasidenib; Idhifa, Celgene) and IDH1 (ivosidenib; Tibsovo, Agios) are approved as single agents in relapsed/refractory AML, but not yet in CCUS or MDS. Rigosertib, an oral multikinase inhibitor targeting the RAS pathway, is currently being investigated in patients who have MDS with *KRAS* or *NRAS*

mutations.[33] The anti-CD47 antibody magrolimab is a macrophage immune checkpoint inhibitor that induces tumor phagocytosis and eliminates leukemia stem cells. In combination with azacitidine, magrolimab significantly improves median overall survival and complete response rates among patients with MDS or AML, including those with and those without *TP53* mutations.[35] APR-246 is an experimental agent that targets *TP53* mutations by refolding the aberrant protein, restoring its transcriptional activity.[33] Luspatercept (Reblozyl, Celgene) is an option for patients with mutations involving *SF3B1* and is approved in transfusion-dependent patients who have low-risk MDS with ringed sideroblasts refractory to erythropoiesis-stimulating agents. Allogeneic stem cell transplant is the only potentially curative therapy and may be indicated in patients who have a high-risk mutation profile and may be at risk for progression to MDS.[2] However, because not all cases of CCUS progress to MDS, allogeneic stem cell transplant is not a valid treatment option at this time. Further research and prospective trials in CCUS are warranted to clarify the effectiveness of targeted therapies for patients with CCUS and the criteria for the initiation of treatment.

Patients' mutation profiles may help to predict their response to a given treatment option. All patients with mutations in *IDH1* and most patients with *SF3B1* mutations responded to treatment, suggesting that these mutations may carry a good prognosis.[14] Additionally, patients with a single mutation, as opposed to those with multiple mutations, were more likely to improve with treatment (response rate, 60% vs 14%, respectively; *P*=.03). Again, this finding indicates that co-mutations and multiple mutations are signs of a poor prognosis in CCUS.[14] This study was limited in sample size, and the results require further validation. Even with the targeted treatment options available, prospective studies are warranted to determine if they have any efficacy in preventing CCUS progression.

Conclusion

CCUS is a recently identified precursor condition in the realm of MDS/myeloid neoplasms; the early identification and treatment of CCUS may lead to potentially rewarding outcomes and the prevention of progression to MDS or myeloid neoplasms. The most common mutations in CCUS include those in *DNMT3A*, *TET2*, *ASXL1*, *SRSF2*, and *SF3B1*. The goal of diagnosing a clonal cytopenia as one of the precursor states, such as CCUS, is to monitor patients for early signs of progression to a myeloid neoplasm. Awareness of these mutations can guide surveillance in the primary prevention of MDS and AML. The identification of associated high-risk prognostic factors is essential to achieve this goal. CCUS carries an 80% risk for progression within 5 years, and close follow-up of patients who have unexplained cytopenias with thorough blood, bone marrow, and molecular testing is beneficial. For all patients, we recommend a complete blood cell count and peripheral smear once every 3 to 6 months, as well as a baseline bone marrow analysis that includes cytogenetics, FISH, and NGS. Patients with HR-CCUS can be monitored more frequently. Treatment guidelines are lacking, and the recommendations tend to consist mainly of supportive management. Further studies and trials are needed to determine the effectiveness of therapies for patients with CCUS.

Disclosures

Dr Vobugari, Ms Heuston, and Dr Lai declare no competing interests.

Contributions

NV and CH were involved in the literature search and writing the manuscript. CL formulated the idea; mentored NV and CH; and was involved in writing, review, and revision of the manuscript. All authors reviewed and approved the final manuscript. Dr Judith Karp reviewed the manuscript and provided valuable comments.

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