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.

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

Keywords
Acute myeloid leukemia, clonal cytopenia, clonal cytopenia of undetermined significance, clonal hematopoiesis, clonal hematopoiesis of indeterminate potential, myelodysplastic syndrome
of patients with unexplained cytopenias, 8% to 36% of them were identified as having CCUS.\textsuperscript{2,3} A myeloid neoplasm is 14 times more likely to develop in patients with CCUS than in patients without clonality (\textit{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.\textsuperscript{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.\textsuperscript{5,5} 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.\textsuperscript{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%).\textsuperscript{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%).\textsuperscript{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).\textsuperscript{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.\textsuperscript{8-10}

Several pathogenic pathways leading to CCUS have been proposed. One potential pathogenic trigger is the acquisition of one or more driver mutations (\textit{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{3} Thus, the acquisition of additional mutations, dysplasia, progression to MDS, and clonal hematopoiesis can lead to the development of

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**Table 1. Classification of Unexplained Cytopenia and Clonality**

<table>
<thead>
<tr>
<th>Unexplained Cytopenia</th>
<th>Characteristic Features</th>
<th>Risk for Progression to MDS/AML</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cytopenia</td>
<td>Clonality (VAF), %</td>
</tr>
<tr>
<td>Age-related</td>
<td>+</td>
<td>&lt;2</td>
</tr>
<tr>
<td>ICUS</td>
<td>+</td>
<td>&lt;2</td>
</tr>
<tr>
<td>CHIP</td>
<td>–</td>
<td>≥2</td>
</tr>
<tr>
<td>CCUS</td>
<td>+</td>
<td>≥2</td>
</tr>
<tr>
<td>MDS, low-risk</td>
<td>+</td>
<td>Usually ≥2</td>
</tr>
<tr>
<td>MDS, high-risk</td>
<td>+</td>
<td>Usually ≥2</td>
</tr>
</tbody>
</table>

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.
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, KRA, KIT, PTEN, CBL), and cell cycle/DNA repair (TP53, ATM) (Table 2).\(^3,10,12-14\)

**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 hydroxylase 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 KRA5 mutations activate aberrant cell proliferation by signaling RAS/RAF/MEK/ERK pathways. NRAS and KRA5 mutations are rare in CCUS (2.8%).\(^4\) NRAS and KRA5 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
also rare in CCUS, although their presence in unexplained cytopenia revealed MDS/myelofibrosis/myeloid neoplasms. 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.

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. Other extrinsic factors, such as inflammation, autoimmune disease, and infection, may contribute to a bone marrow microenvironment that permits or enhances TP53 clonal expansion. 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. However, the significance of differences in the rates of progression among such patients is unclear. Germline mutations can also be confirmed in biopsy specimens obtained from unaffected tissue, such as skin or hair follicle. 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. 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. 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. Two 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 DNMT3A mutations among patients with CHIP. 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.

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. 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. 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. Inflammation has been associated with progression in CHIP and may also cause progression in CCUS.

Mutation Patterns

The rates of progression to MDS and AML are higher in patients with specific CCUS mutation patterns, especially mutations in U2AF1, ZRS2, SRSF2, SF3B1, JAK2, RUNX1, and TP53, either singly or as co-mutations. Patients with these mutations need to be watched closely, even in the absence of dysplasia, given the high likelihood of progression. 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. Mutations in ASXL1, CBL, DNMT3A, NRAS, and RUNX1 may be associated with worsening cytopenias, increased monocytosis, and a heightened risk for progression.

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. 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; 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
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. Other treatment options in CCUS include growth factors, corticosteroids, thrombopoietin receptor agonists, rituximab, and intravenous immunoglobulin. 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.

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

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Type of Gene</th>
<th>Approximate Frequency of Mutation, %</th>
<th>Risk for Progression to MDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNMT3A</td>
<td>DNA methylation</td>
<td>13-35</td>
<td>Unclear risk for progression. The authors note that this genetic mutation does not play a direct role in cytopenia.15,16</td>
</tr>
<tr>
<td>TET2</td>
<td>DNA methylation</td>
<td>21-42</td>
<td>Potentially high risk14,16</td>
</tr>
<tr>
<td>IDH1</td>
<td>DNA methylation</td>
<td>8.5-13</td>
<td>Unknown</td>
</tr>
<tr>
<td>IDH2</td>
<td>DNA methylation</td>
<td>2-3</td>
<td>Unknown</td>
</tr>
<tr>
<td>ASXL1</td>
<td>Chromatin modification</td>
<td>8.2-14</td>
<td>High risk14,16</td>
</tr>
<tr>
<td>KDM6A</td>
<td>Chromatin modification</td>
<td>1.4-4</td>
<td>Unknown</td>
</tr>
<tr>
<td>PHF6</td>
<td>Chromatin modification</td>
<td>2.8-4</td>
<td>Unknown</td>
</tr>
<tr>
<td>SRSF2</td>
<td>RNA splicing</td>
<td>14-21</td>
<td>High risk3</td>
</tr>
<tr>
<td>U2AF1</td>
<td>RNA splicing</td>
<td>4-21</td>
<td>High risk3,14</td>
</tr>
<tr>
<td>SF3B1</td>
<td>RNA splicing</td>
<td>5.6-15</td>
<td>High risk3,14 Interestingly, SF3B1 is associated with a better prognosis and with ring sideroblasts in patients who have MDS.15</td>
</tr>
<tr>
<td>ZRSR2</td>
<td>RNA splicing</td>
<td>13-14</td>
<td>High risk3</td>
</tr>
<tr>
<td>BCOR</td>
<td>Transcription regulation</td>
<td>5.6-8</td>
<td>Unknown</td>
</tr>
<tr>
<td>RUNX1</td>
<td>Transcription regulation</td>
<td>1.4-8,2,4,10,13</td>
<td>High risk for progression to MDS and AML3,14</td>
</tr>
<tr>
<td>JAK2</td>
<td>Signaling</td>
<td>4</td>
<td>High risk among isolated mutations or co-mutations of SRSF2 and U2AF1 in myelofibrosis3</td>
</tr>
<tr>
<td>CBL</td>
<td>Signaling</td>
<td>1.4</td>
<td>Unknown</td>
</tr>
<tr>
<td>KRAS</td>
<td>Signaling</td>
<td>2.8</td>
<td>High risk14</td>
</tr>
<tr>
<td>NRAS</td>
<td>Signaling</td>
<td>2.8</td>
<td>High risk14,18</td>
</tr>
<tr>
<td>KIT</td>
<td>Signaling</td>
<td>1.4</td>
<td>Unknown</td>
</tr>
<tr>
<td>TP53</td>
<td>Cell cycle/DNA repair</td>
<td>2-17</td>
<td>Associated with a poor prognosis in MDS and AML9</td>
</tr>
<tr>
<td>PTEN</td>
<td>Cell cycle/tumor suppressor</td>
<td>1.4</td>
<td>Unknown</td>
</tr>
<tr>
<td>CUL3</td>
<td>Ubiquitin-proteasome system</td>
<td>4</td>
<td>Unknown</td>
</tr>
<tr>
<td>DDX41</td>
<td>Encodes RNA helicase, tumor suppressor gene</td>
<td>4</td>
<td>Unknown</td>
</tr>
<tr>
<td>SETBP1</td>
<td>Encodes SETBP1 protein; binds to DNA to increase gene expression</td>
<td>1.4-4</td>
<td>Unknown</td>
</tr>
<tr>
<td>STAG2</td>
<td>DNA replication</td>
<td>2-4</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

AML, acute myeloid leukemia; MDS, myelodysplastic syndrome
mutations. 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. APR-246 is an experimental agent that targets TP53 mutations by refolding the aberrant protein, restoring its transcriptional activity. 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. 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. 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. 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 cytogentic, 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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