Dear Editor,

Clonal cytopenia(s) of undetermined significance (CCUS) is defined by the presence of the somatic mutation(s) of genes associated with myeloid neoplasms, with unexplained cytopenia(s) but without definitive morphologic evidence of myeloid neoplasms. CCUS is a newly described entity with a high probability of progression to myeloid disorders upon follow-up. However, there is a paucity of data on the efficacy of treatment. We hereby describe a single-institution’s experience in managing CCUS patients who required treatment beyond blood transfusions.

This retrospective study was approved by scientific and ethical review boards at our institution. Patients who had CCUS and had received treatment beyond blood transfusions were identified from medical records. CCUS diagnosis was confirmed based on the absence of definitive morphologic evidence of myeloid neoplasms from bone marrow biopsy evaluation combined with evidence of pathogenic myeloid somatic mutation with a variant allele frequency (VAF) of at least 2% using our institution’s next-generation sequencing (NGS) panel (Onco-Heme, Mayo Clinic). Treatment type and the indication were based on the treating physician’s choice. Clinical and laboratory results were collected. Hematologic improvement (HI) was graded based on the MDS International Working Group (IWG) 2006 criteria. Symptomatic improvement (SI) was defined by the subjective improvement of CCUS-related inflammatory symptoms based on the patients’ report. The treatment response rate (RR) was calculated based on HI and SI. Progression was defined by either the occurrence of myeloid neoplasm or worsening cytopenia(s). Dependence on blood transfusion was defined as requiring an average of ≥2 units of packed red blood cells (RBCs) or platelets over 4 weeks or ≥4 units over 8 weeks. The date of treatment initiation was used for calculating overall survival (OS) and progression-free survival (PFS). Stata 14.1 (StataCorp) was used for data analysis.

Between July 2015 and July 2020, 24 patients met the inclusion criteria with a median age of 72 years (range: 24–87). The majority of patients were males (N = 20, 83%). Three (13%) patients had a history of the hematologic condition (one monoclonal B cell lymphocytosis [MBL], one congenital neutropenia, and one acute myeloid leukemia [AML]) in remission). Three (13%) had a history of solid organ malignancy (one bladder cancer, one bronchogenic carcinoma, and one lung cancer). Two (8%) patients had prior radiation therapy, two (8%) had prior chemotherapy, and one (4%) had prior allogeneic stem cell transplantation. Six (25%) patients had inflammatory conditions (one Sweet’s syndrome, one periodic inflammatory purpura, one myopericarditis, and one inflammatory purpura) (Table 1 and Supplemental Table 1). At the time of treatment, median hemoglobin was 8.9 g/dl (range 6.8–13.7), white blood cell 2.9 × 10^9/l (range 1.4–12.3), and platelet 76 × 10^9/l (range 8–407). RBC- and platelet transfusion-dependent rates were 54% and 29%, respectively. We identified 21 different mutations. Most
Table 1  Patient characteristics with CCUS, the treatment regimens and outcomes.

| Case no. | Age (years) | Sex | Clinical features | 1st NGS-mutated gene (VAF%) | 2nd NGS, if available (VAF%) | 3rd NGS, if available (VAF%) | Treatment | Response | Follow-up duration (M) | Outcomes |
|----------|-------------|-----|-------------------|-----------------------------|-------------------------------|-------------------------------|------------|----------|------------------------|----------|
| 1        | 60          | M   | Anemia and thrombocytopenia; Sweet syndrome | TET2 (44%); TET2 (41%) | —                            | —                            | HMA (DAC 5d); | SI       | 6.7                    | Alive    |
| 2        | 71          | M   | Neutropenia; a history of chronic lymphocytic leukemia | IDH1 (33%); SRSF2 (39%) | —                            | —                            | HMA (DAC 3d) | HI       | 1.4                    | Alive    |
| 3        | 74          | M   | Neutropenia; acquired periodic fever syndrome | IDH2 (37%); ZRSR2 (74%) | —                            | —                            | HMA (DAC 3d) | SI       | 2.4                    | Alive    |
| 4        | 71          | M   | Anemia and thrombocytopenia; hypogammaglobulinemia; systemic inflammatory response syndrome | DNMT3A (47%) | DNMT3A (44%) | —                            | HMA (DAC 3d) | HI       | 12.2                   | Alive    |
| 5        | 78          | M   | Pancytopenia | U2AF1 (22%) | —                            | —                            | Steroid; IVG; ESA | Stable   | 53.3                   | Alive    |
| 6        | 70          | M   | Pancytopenia; a history of bladder cancer | TP53 (9%) | —                            | —                            | HMA (DAC 5d) | HI       | 9.6                    | Alive    |
| 7        | 73          | M   | Anemia | SF3B1 (34%); TET2 (8%) | —                            | —                            | SF3B1 (32%); IDH1 (48%) | MDS      | 87.8                   | Alive    |
| 8        | 78          | M   | Pancytopenia | SF3B1 (missing) | —                            | —                            | Vitamin B12; iron | MDS      | 12.1                   | Alive    |
| 9        | 67          | F   | Neutropenia and thrombocytopenia | RUNX1 (missing) | BCO1 (27%); KRAS (23%); U2AF1 (24%) | —                            | TPA       | Initial HI, then AML | 15.5     | Alive    |
| 10       | 78          | M   | Anemia and thrombocytopenia; poly-inflammatory syndrome characterized by vasculitis; bronchogenic carcinoma | KDM6A (8%); TET2 (22%); U2AF1 (18%); ZRSR2 (92%) | —                            | —                            | HMA (DAC 3d) | SI       | 2.3                    | Alive    |
| 11       | 64          | M   | Neutropenia and thrombocytopenia | CUL3 (25.7%); SRSF2 (43.5%); SRSF2 (43%); TET2 (11%); TET2 (4.5%); TET2 (1.5%) | —                            | —                            | Rituximab | Stable   | 1.7                    | Alive    |
| 12       | 81          | M   | Neutropenia and thrombocytopenia | IDH1 (12%); SRSF2 (15%) | —                            | —                            | Steroid; GCSF | HI       | 6.6                    | Alive    |
| 13       | 88          | M   | Anemia | ZRSR2 (34%) | —                            | —                            | Testosterone | Stable   | 48.3                   | Alive    |
| 14       | 74          | M   | Pancytopenia | DDX41 (5%); DNMT3A (7%) | —                            | —                            | GCSF       | Stable   | 3.5                    | Alive    |
| Case no. | Age (years) | Sex | Clinical features | 1st NGS-mutated gene (VAF%) | 2nd NGS, if available (VAF%) | 3rd NGS, if available (VAF%) | Treatment | Response | Follow-up duration (M) | Outcomes |
|---------|-------------|-----|-------------------|----------------------------|-----------------------------|----------------------------|------------|----------|----------------------|----------|
| 15      | 24          | M   | Neutropenia and thrombocytopenia | ASXL1 (32%) | — | — | GCSF; ESA | Initial HI, then progressed with worsening cytopenia (s) | 18.5 | Dead |
| 16      | 76          | M   | Anemia and thrombocytopenia | SRSF2 (32%); TP53 (22%) | TP53 (missing) | — | ESA | Stable | 18.9 | Alive |
| 17      | 69          | F   | Pancytopenia; history of lung cancer | JAK2 (2%); TP53 (16%) | JAK2 (10%); TP53 (13%); U2AF1 (6%) | — | Steroid; cyclosporine; ESA | Worsening cytopenia (s) | 15.4 | Dead |
| 18      | 78          | M   | Pancytopenia | U2AF1 (25%) | ASXL1 (9%); U2AF1 (18%) | — | ESA; testosterone | Stable | 10.7 | Alive |
| 19      | 69          | F   | Anemia and neutropenia | IDH1 (38%); SRSF2 (43%); TP53 (42%) | — | — | SI | 22 | Alive |
| 20      | 75          | M   | Pancytopenia | BCOR (19%); U2AF1 (9%) | — | — | Cyclosporine | Worsening cytopenia (s) | 102 | Dead |
| 21      | 33          | M   | Pancytopenia; recurrent skin, soft tissue infection, diverticulitis; myopericarditis; history of congenital neutropenia | SETBP1 (50%)\(^a\) | — | — | Allogeneic stem cell transplantation | HI | 32.6 | Alive |
| 22      | 58          | F   | Pancytopenia; irritable bowel syndrome; fibromyalgia; and inflammatory purpura without vasculitis | ASXL1 (25%) | — | ASXL1 (27%); SETBP1 (12%); SETBP1 (9%) | HMA (DAC 3d) Initial HI, then MDS | 26.3 | Alive |
| 23      | 77          | M   | Anemia and neutropenia | ASXL1 (missing); U2AF1 (missing) | — | — | HMA (AZA) | MDS | 9 | Alive |
| 24      | 70          | M   | Pancytopenia; AML | BCOR (19%); DNMT3A (19%); PHF6 (15%); RUNX1 (8%); SF3B1 (12%); STAG2 (22%); TET2 (9%) | BCOR (64%); DNMT3A (37%); FLT3 (39%); PHF6 (6%); RUNX1 (32%); SF3B1 (36%); STAG2 (68%); TET2 (32%) | — | HMA (AZA 3d) | AML | 4.7 | Alive |

**NGS** next-generation sequencing, **VAF** variant allele frequency, **MDS** myelodysplasia neoplasm, **AML** acute myeloid leukemia, **SI** symptomatic improvement, **HI** hematologic improvement, **HMA** hypomethylating agents, **ESA** erythropoietin-stimulating agents, **GCSF** granulocyte-stimulating factor, **TPA** thrombopoietin receptor agonist, **IVIG** intravenous immunoglobulin, **DAC 5d** decitabine 5 days regimen, **DAC 3 d** decitabine 3 days regimen, **AZA 3d** azacitidine 3 days regimen, **M** month.

\(^a\)Missing details.
\(^b\)Non-germline mutation.
common mutations occurred in SRSF2 (N = 5, 21%), TET2 (N = 5, 21%), U2AF1 (N = 5, 21%), and TP53 (N = 4, 17%) (Supplemental Fig. 1). This is consistent with previous reports that these genes are commonly seen in clonal hematopoiesis of indeterminate potential or CCUS. Ten (42%) patients had 1 mutation, and 14 (58%) had ≥2 mutations. The median VAF was 25.7% (range 1.5–92%) (Supplemental Table 2). None of the responders had ASXL1 mutation. Figure 1 demonstrates the RR for each treatment. Patients with a single mutation (compared to ≥2 mutations) were significantly more likely to achieve HI (RR 60 vs. 14%, p = 0.03). At the data cutoff, RBC and platelet transfusion-dependent rate were 38% (30% reduction) and 25% (14% reduction), respectively.

Six patients (25%) progressed to myeloid malignancy (four MDS and two AML). Five (21%) had worsening cytopenias. Among the four MDS patients, they had SF3B1, TET2, ASXL1, and U2AF1 mutations at the diagnosis of CCUS. Within the two AML patients, both had RUNX1 mutation (Table 1 and Supplemental Table 3). A recent report from Malcovati et al. suggests that SF3B1 mutation in CCUS represents a distinct MDS entity. All three SF3B1 mutant CCUS patients progressed to myeloid neoplasms in this cohort. Two patients with a prior diagnosis of solid organ malignancies (treated with cytotoxic chemotherapy) had CCUS with TP53 mutation. One patient treated with HMA achieved HI, while the other, treated with steroids, cyclosporine, and ESA, did not respond. The median PFS was 17.1 months (95% CI: 7.1–not reached). The median OS was not reached with an estimated 2-year OS of 73% (Supplemental Figure 3A, B). Three (13%) patients had worsening cytopenias leading to death (one septic shock, two transitioned to hospice care). These three patients’ molecular signature at CCUS diagnosis showed ASXL1, TP53, and U2AF1 mutations, respectively (Table 1 and Supplemental Table 4). The 30- and 60-day mortality was 0%.

Subsequent NGS was available for nine (38%) patients. Six (66.7%) had a clonal evolution (acquiring new mutations). Among these, two progressed to AML (one had new BCOR, KRAS, and U2AF1 mutations, one had new FLT3 mutation); two progressed to MDS (one had new IDH1 mutation, one had new SETBP1 mutation); one had...
worsening cytopenia (new U2AF1 mutation); one had stable disease (new ASXL1 mutation); two had stable disease with no clonal evolution; one responded to HMA treatment with the DNMT3A VAF decreasing from 47 to 44% (Table 1 and Supplemental Table 3). In our cohort, five (83%) clonal evolution co-occurred with the progression of myeloid neoplasm.

In summary, this is the first report of the outcome of treatment in CCUS patients requiring therapies beyond blood transfusion. Our results suggest that patients with CCUS responded to available treatments for myeloid disorders and immune cytopenias. HMA was safe and associated with the highest RR and CCUS-associated inflammatory symptoms in this cohort. Our data also suggest that the mutation profile may impact treatment response. Several limitations exist in our study. First, the retrospective nature of our cohort and the relatively small sample size limit the generalizability of our observations. Second, there were heterogeneities in both patients and therapies. Three patients with pre/co-existing hematological conditions (one MBL, one congenital neutropenia who received stem cell transplantation for pancytopenia, and one AML in remission) were included as they did not fulfill myeloid malignancy criteria. Several patients were diagnosed with inflammatory disorders or malignancies and received prior cytotoxic chemotherapy making them a higher risk CCUS. Although CCUS is associated with high inflammatory status11, it is unclear if the immune-mediated marrow process is the driver of cytopenia or the clonal process itself. The therapies also varied based on the treating physician’s decision and were adopted from myeloid disorder or immune-related cytopenia as no standard of care exists to date. Third, the best treatment response was defined as both hematological and symptomatic responses due to the unique features of this cohort, with inflammatory status present in a quarter of our patients. Last, the MDS IWG criteria were used to assess hematological response for this cohort due to a lack of other criteria. An international multicenter study is ongoing to validate our current findings and determine the clinical outcome for treatment in CCUS. Future studies are warranted to develop response and progression criteria to facilitate and standardize reporting treatment outcomes.

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Author contributions
Z.X. and A.A designed the study, interpreted the data, and wrote the manuscript; Z.X. collected the data and conducted the statistical analysis; A.N.S., N.G., H.B.A., A.T., M.L., M.P., M.S., and A.A cared for the patients and provided patients’ information; A.N., R.H., D.V., P.N., D.J., P.G., and M.E.S. performed the next-generation sequencing; and all authors critically reviewed and approved the manuscript.

Conflict of interest
The authors declare no competing interests.

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