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The impact of cytotoxic therapy on the risk of progression and death in clonal cytopenia(s) of undetermined significance

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Abstract:
Clonal cytopenia of undetermined significance (CCUS) is defined by a myeloid driver mutation in the context of otherwise unexplained cytopenia. CCUS has an inherent risk of progressing to myeloid neoplasm. However, it is unknown how exposure to previous cytotoxic therapy may impact the risk of progression and survival. We stratified CCUS patients by prior exposure to DNA-damaging therapy. Of 151 patients, 46 (30%) had received cytotoxic therapy and were classified as therapy-related CCUS (t-CCUS), whereas 105 (70%) had de novo CCUS. A lower proportion of t-CCUS had hypercellular marrows (17.8% vs. 44.8%, P=0.002) but had higher median bone marrow blast percentages. After a median follow up of 2.2 years, t-CCUS had significantly shorter PFS (1.8 vs. 6.3 years, HR 2.1, P=0.007) and median OS (3.6 years vs. not reached, HR 2.3, P=0.007) compared to CCUS. Univariable and multivariable time-to-event analyses showed that exposure to cytotoxic therapy independently accounted for inferior PFS and OS. Despite the similarities in clinical presentation between CCUS and t-CCUS, we show that exposure to prior cytotoxic therapies was an independent risk-factor for inferior outcomes. This suggests that t-CCUS represents a unique clinical entity that needs more stringent monitoring or earlier intervention strategies. -

Conflict of interest: COI declared - see note

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Key Point 1: Exposure to prior cytotoxic therapy in CCUS patients independently accounts for inferior PFS and OS.

Key Point 2: CCUS patients who have received cytotoxic therapy have distinct clinical features and can be considered a unique entity called t-CCUS.

Abstract

Clonal cytopenia of undetermined significance (CCUS) is defined by a myeloid driver mutation in the context of otherwise unexplained cytopenia. CCUS has an inherent risk of progressing to myeloid neoplasm. However, it is unknown how exposure to previous cytotoxic therapy may impact the risk of progression and survival. We stratified CCUS patients by prior exposure to DNA-damaging therapy. Of 151 patients, 46 (30%) had received cytotoxic therapy and were classified as therapy-related CCUS (t-CCUS), whereas 105 (70%) had *de novo* CCUS. A lower proportion of t-CCUS had hypercellular marrows (17.8% *vs.* 44.8%, *P*=0.002) but had higher median bone marrow blast percentages. After a median follow up of 2.2 years, t-CCUS had significantly shorter PFS (1.8 *vs*. 6.3 years, HR 2.1, *P*=0.007) and median OS (3.6 years *vs*. not reached, HR 2.3, *P*=0.007) compared to CCUS. Univariable and multivariable time-to-event analyses showed that exposure to cytotoxic therapy independently accounted for inferior PFS and OS. Despite the similarities in clinical presentation between CCUS and t-CCUS, we show that exposure to prior cytotoxic therapies was an independent risk-factor for inferior outcomes. This suggests that t-CCUS represents a unique clinical entity that needs more stringent monitoring or earlier intervention strategies.

Introduction

Improvements in sequencing methods has led to increased recognition of precursor states for myeloid neoplasms. These precursor states fall under the umbrella of clonal hematopoiesis (CH), which is defined as clonal expansion in a myeloid driver mutation originating in hematopoietic stem and progenitor cells[1]. CH inherently carries a risk for progression to myeloid neoplasm[2,3]. Cytotoxic therapies are not only associated with a characteristic genetic landscape, but also shapes the fitness of the clone[3]. However, the proportion of CH patients developing t-MN relatively low and latency is long[4,5].

Clonal cytopenia of undetermined significance (CCUS) is defined as the presence of at least one line of unexplainable cytopenia(s) lasting for ≥4 months, along with a clonal abnormality, without evidence of a *bona fide* myeloid neoplasm[1]. The risk of leukemic progression from CCUS is higher and latency shorter. CCUS, therefore, is considered an intermediary premalignant state between CH and MN. While the impact of therapy on CH and the resultant t-MN development has been studied extensively[3,6], its impact on CCUS remains uncharacterized.

In CCUS, common mutations encountered include *TET2 (23.8%), DNMT3A (13.3%), SRSF2 (10.1%), ASXL1 (8.3%), and U2AF1 (4.6%)*[7]. In a previous single institution study, the median progression free survival (PFS) for patients with CCUS was 17.1 months, with an estimated 2-year overall survival (OS) of 73%[8]. However, the impact of previous DNA-damaging therapies on characteristics and outcomes of CCUS has not been studied.

Therapy-related myeloid neoplasms (t-MN) are aggressive hematologic neoplasms that develop as a complication from exposure to DNA-damaging therapies and carry particularly poor survival. The DNA-damaging agents include cytotoxic chemotherapy used either alone, in combination with radiation as well as immunosuppressive therapies (e.g. Azathioprine)[9]. Although t-MN is rare—its estimated population incidence is 0.62 per 100,000[10]—and its incidence is expected to rise as the population ages and the prevalence of cancer survivors increase. Patients who develop t-MN have an estimated median survival of 15 months or less, as they are more likely than *de novo* acute myeloid leukemia (AML) to carry high-risk karyotypes and *TP53* mutations[11]. While studying t-MN patients, a subset of patients was noted who received DNA-damaging therapies and developed unexplained cytopenia but did not have t-MN. We recently described this group as a clinical entity of therapy-related CCUS (t-CCUS)[11]. t-CCUS had a significantly better survival compared to World Health Organization (WHO) defined t-MN, suggesting that it is a distinct entity from t-MN[11]. The International Consensus Classification (ICC) of Hematological Malignancies adapted 'therapy-related' qualifier to CCUS[12]. In contrast, the 5th edition of the WHO classification of hematological neoplasms did not comment on the status of t-CCUS[1]. This discrepancy suggests lack of consensus, rooted in the lack of relevant data.

Therefore, the aims of this study were to characterize the impact of prior cytotoxic therapy on clinicopathological features and the risk of progression to myeloid neoplasm and death.

Methods

This was a retrospective review of all adult patients diagnosed with CCUS at Mayo Clinic Enterprise (Rochester, Jacksonville, and Scottsdale) between the years 2010 and 2022. CCUS was defined using the International Consensus Criteria[12]: cytopenia was defined as hemoglobin <13g/dL in male and <12g/dL in females, absolute neutrophil count <1.8 $x10^9$/L, and platelet less than 150 $x10^9$/L. All patients had presence of myeloid driver mutation or a non-myelodysplastic syndrome (MDS) defining clonal cytogenetic alteration. MDS-defining cytogenetics were defined per the ICC criteria - i.e., patients with isolated 5q deletion, monosomy 7 or 7q deletion, or complex karyotype were determined to have MDS and excluded from further analysis[12]. Pathology was reviewed (DC) to ensure that none of the pathologic diagnosis of patients collected met the diagnostic criteria for myeloid neoplasm.

In addition, patients were classified as t-CCUS if they received DNA-damaging agents in the form of either cytotoxic chemotherapy (Supplementary Table 1), radiation (including radioligand therapy and field radiation) or a combination of the above prior to CCUS diagnosis.

Demographic and clinical information was extracted at the time of diagnosis, including age, sex, smoking status, blood count, bone marrow blast count and cellularity, type of cytotoxic therapy received, type of therapy after CCUS diagnosis, and next generation sequencing (NGS). NGS was performed on DNA extracted from bone marrow aspirates at Mayo using either a clinical targeted myeloid (NGS HemOnc, versions 1-3, Supplementary Table 2-4) or an expanded gene research-based panel (Supplementary Table 5). Further information regarding the sensitivity of these panels can be found in

the Supplementary Methods. Out of 147 patients with available NGS data, 9 underwent the expanded research panel with the rest being clinical.

The cause of death was determined based on provider documentation from electronic medical record and were found to be generally due to 1) hematologic malignancy; 2) cardiac related; 3) infection or 4) multifactorial or indeterminate causes. Since a combination of the above could contribute to death and there is ambiguity in this determination via provider documentation, hematological malignancy would only be considered the cause of death if the patient progressed to leukemia and chose hospice as a direct result of the new diagnosis or passed away from complications of chemotherapy related to hematologic malignancy. Infection or cardiac was considered cause of death only if the infection or cardiac event was not a direct result of active cancer treatment.

Descriptive and summary statistics for numerical values were calculated using Wilcoxon rank sum test. Fisher exact test was used to compare categorical data between groups. Time to event outcomes were calculated using Cox-proportional hazard. Univariable and multivariable analysis for time to event data was performed using the same. Subset multivariable analysis was based on the most significant findings from initial univariable analysis. PFS was defined as the interval from the diagnosis to progression to myeloid neoplasm or death. OS was defined as the interval from the diagnosis to death from any cause. Statistical analysis was performed using Stata/MP v.16 and survival curves generated from the same.

This study was reviewed and approved by the IRB at Mayo Clinic, Rochester.

Results

Clinical and Pathological Characteristics

Of 151 patients, 105 (70%) met criteria for CCUS and 46 (30%) t-CCUS. For those classified as t-CCUS, 17 patients (37%) received prior cytotoxic chemotherapy, 17 (37%) received combined chemotherapy and radiation, 11 (24%) received radiation therapy alone, and one patient received peptide receptor radionuclide therapy (PRRT). The cancer types for the t-CCUS group are listed in Supplementary Table 6. The median age at diagnosis for the entire cohort was 68 years with the majority being male (60%) and was not different between CCUS and t-CCUS (*P*=0.208). Patients with history of or active tobacco use were comparable between the two cohorts (41.8% *vs*. 44.4%, *P*=0.857). There were no differences between median blood counts at diagnosis between the two groups (**Table 1**). The median red cell distribution width (RDW) at diagnosis was 15% (range 11.7% – 26.7%) for both CCUS and t-CCCUS. CCUS patients were more likely to have hypercellular bone marrows at diagnosis (median 45% *vs*. 17%, *P*=0.002), while t-CCUS patients were more likely to have hypocellular bone marrows at diagnosis (median 20% vs. 5%, p=0.011). t-CCUS was associated with higher bone marrow blasts at diagnosis (median 1% *vs*. 0%, *P*=0.026). There were no differences in number of patients with ringed sideroblasts on their bone marrow biopsy at diagnosis (median 14% and 11% for CCUS and t-CCUS, *P*=0.926). We applied the recently proposed clonal hematopoiesis risk score (CHRS) to our cohort[13], acknowledging that all of our patients already have CCUS which incurs a higher risk. The median CHRS score was 12 (range 8.5-15.5). Majority (90%) of patients were

157 evenly distributed between intermediate- and high-risk CHRS and this was not different
158 between CCUS and t-CCUS (**Table 1**).

159 **Cytogenetic and NGS findings**

160 The median percent of patients with abnormal cytogenetics (not meeting criteria for
161 MDS-defining cytogenetics) at diagnosis were 25% and 24% for CCUS and t-CCUS,
162 respectively (*P*=0.375). There were no significant differences in the number of mutations
163 on NGS, with a median of 2 mutations for CCUS and 1 mutation for t-CCUS (p=0.551).
164 The most common mutations in the entire cohort were *TET2* (37%), *SRSF2* (24%),
165 *ASXL1* (14%), *DNMT3A* (12%), *ZRSR2* (9%), *U2AF1* (8%), and *TP53* (7%) (**Table 2**).
166 The CCUS group had more *SRSF2* mutations (30% *vs*. 9%, *P*=0.003), whereas t-CCUS
167 was disproportionally enriched in *TP53* mutations (20% *vs*. 2%, *P*=0.001, **Figure 1A**).
168 There were no differences in variant allele frequency (VAF) of all cumulative mutations
169 between the two groups (39% *vs*. 38.5% in CCUS and t-CCUS, respectively, *P*=0.858),
170 (**Table 1**). This remained consistent when comparing VAF within each specific gene,
171 including those that were differentially enriched between the two groups
172 (Supplementary table 7). For example, the median VAF of *SRSF2* mutations was 39%
173 in each cohort (*P*=0.775, **Figure 1B**). The median VAF in *TP53* mutations was
174 numerically higher in CCUS compared to t-CCUS; however, the difference was not
175 statistically significant (26% *vs*. 8%, *P*=0.186). This statistical analysis is limited by the
176 fact that only 2 CCUS patients harbored $TP53^{mut}$. Updated myeloid guidelines consider
177 VAF>49% as presumptive evidence of bi-allelic *TP53*[1,9]. All $TP53^{mut}$ patients in our
178 cohort with available cytogenetics (n=10) had diploid cytogenetics and none had
179 VAF>49%.

Outcomes Following CCUS Diagnosis

Management of CCUS and t-CCUS patients. Following CCUS diagnosis, the subsequent treatment was at the treating physician's discretion. Overall, there were no differences in the observed treatment modalities chosen between the two groups. A majority in both groups underwent surveillance only (71% *vs*. 80% in CCUS and t-CCUS, respectively), with the second most common modality being supportive care (growth factor support and/or transfusion, 15% *vs*. 6% in CCUS and t-CCUS, respectively). A small number of patients in both groups were treated with a hypomethylating agent (3.8% vs 4.4% in CCUS and t-CCUS, respectively). Finally, 4 total patients underwent enrollment in NCT03418038, a phase II clinical trial assessing IV ascorbic acid in *TET2* mutated CCUS. One of these 4 patients in the clinical trial did receive prior cytotoxic therapy and was in the t-CCUS group.

Phenotype upon progression and cause of death. 28 of 151 (18.5%, 16/105 CCUS and 12/46 t-CCUS) patients progressed to myeloid neoplasms. MDS was the most common diagnosis upon progression in both cohorts (7.6% CCUS *vs*. 15% t-CCUS), followed by AML and CMML (**Table 1**). 44 (29%) patients died during follow up. The causes of death of were evenly distributed between those listed above, with 6 (23%) malignancy, 4 (15%) cardiac, 8 (30%) infection, 8 (30%) multifactorial in CCUS. In contrast, the cause of death in t-CCUS cohort was 8 (44%) malignancy, 4 (22%) cardiac, 2 (11%) infection, and 4 (22%) multifactorial (P=0.299).

Progression-free and overall survival. The median length of follow up of the study cohort was 2.2 years, with the CCUS group having a significantly longer length of follow up (2.5 *vs*. 1.5 years, P=0.002). CCUS had a significantly longer PFS compared to t-

CCUS, (6.3 *vs*. 1.8 years, HR 2.1, *P*=0.007, **Figure 2A**). Similarly, median OS was longer for CCUS compared to t-CCUS (not reached *vs*. 3.6 years, HR 2.3, *P*=0.007, **Figure 2B**).

We next performed univariable time-to-event analysis (UVA) for PFS and OS including the clinical and genetic variables available at CCUS diagnosis (**Table 3**). The most frequent mutations as listed above were analyzed in addition to groupings by different mutational types such as role in cell signaling, spliceosome factors, or epigenetic regulators[14].The grouping of *DNMT3A, ASXL1,* and *TET2* (*DAT* mutations) was also included, as 62% cases harbored ≥1 of these 3 mutations. Exposure to prior therapy was associated with inferior PFS (HR 2.11, CI 1.22-3.65, *P*=0.007) and inferior OS (HR 2.33, CI 1.27-4.31, *P*=0.007, **Figure 2**)**.** Similarly, higher CHRS score was associated with inferior PFS (HR 1.37, CI 1.18-1.59, *P*<0.001) and OS (HR 1.29, CI 1.1-1.51, *P*=0.002). In addition, lower hemoglobin, lower platelet count, higher RDW, higher maximum VAF, higher CHRS score, *TP53*^{mut} and *SF3B1*^{mut} were associated with inferior PFS. Increased age, lower hemoglobin, lower platelets, higher RDW, the presence of abnormal cytogenetics, and *TP53*^{mut} were associated with inferior OS. Age, sex, and smoking status were not significantly associated with PFS or OS.

Given the limited number of events, multivariable time-to-event analysis (MVA) was limited to the inclusion of 3-5 factors significant in the univariable analysis. Clinical model consisted only of CBC parameters available at diagnosis and a baseline variable of exposure to therapy (**Table 4**). Exposure to prior therapy was independently associated with an inferior PFS (HR 2.01, CI 1.12-3.61, *P*=0.020) and OS (HR 2.13, CI 1.10-4.14, *P*=0.026). Next, we investigated if the inclusion of prior therapy further risk-

stratifies CHRS and noted that prior therapy remained significant for PFS and OS even in the context of CHRS. Finally, we included statistically significant genetic factors identified on UVA to observe that the prior exposure therapy was independently associated with PFS and OS. Interestingly, 'Clinical' models had the highest C-index statistics for PFS and OS (0.751 and 0.792, respectively) among all the models tested.

Analysis of Previous Therapy

In t-CCUS, the median time from the first cytotoxic therapy to diagnosis was 3.2 years (range 1 month – 38 years). The median time from exposure to topoisomerase inhibitor to diagnosis of CCUS was significantly shorter than other types of therapy (1.8 years vs. 5.1 years, *P*=0.006). Nine patients had exposure to topoisomerase inhibitor (8 lymphoma, 1 ovarian cancer). Three (33%) of the 9 topoisomerase exposed patients harbored *TP53*^{mut} CCUS. Exposure to topoisomerase inhibitors—but not other class of therapiess—was associated with inferior PFS (HR 3.37, CI 1.34-8.49, *P*=0.010) and OS (HR 2.89, CI 1.07-7.83, *P*=0.037) (**Supplementary Table 8)**. Finally, when stratified by therapy as topoisomerase *vs*. non-topoisomerase class, topoisomerase II therapies were associated with shorter PFS and OS (**Supplementary Table 9**).

Findings Specific to Patients who Experienced Progression or Death

Fifty-six (37%) of 151 patients experienced either progression to myeloid neoplasm or death (event). This was compared to 95 patients who remained event-free, agnostic of the CCUS or t-CCUS groups (**Supplementary Table 10**). There were no differences in the sex distribution, smoking status, or median age between the event- and the event-free groups. Those experiencing an event presented with a significantly lower

hemoglobin (9.9 *vs*. 11.5, *P*=0.001), higher MCV (102.2 *vs*. 98, *P*=0.042), lower platelet count (92 *vs*. 129, *P*=0.001), and higher RDW (14.3 *vs*. 17.1, *P*=0.001) at diagnosis. No differences were found in bone marrow cellularity, median bone marrow blasts, proportion of cases with abnormal cytogenetics, or number of mutations on NGS. Both the patients who experienced event and the event-free cohort were similarly treated, primarily with surveillance (75% *vs*. 74%). Interestingly, there was a trend towards a higher VAF in event-free cohort compared to the event-cohort (38% *vs*. 43, *P*=0.088).

Discussion

Our aim was to evaluate if the risk of progression and/or death in CCUS are different based on prior exposure to DNA-damaging therapies. Comparing a cohort of consecutive CCUS patients with the largest published cohort of t-CCUS, we show that prior DNA-damaging therapies was indeed an independent risk-factor for inferior PFS and OS.

Clinical and demographic characteristics were generally comparable between the two cohorts, except t-CCUS patients were more likely to present with hypocellular bone marrow aspirates/biopsy along with more bone marrow blasts while remaining less than 5%. Intuitively, one would ascribe hypocellular marrow states with higher degrees of cytopenias in t-CCUS compared to CCUS, however, blood parameters were comparable between the two cohorts.

Previously, we showed that within 2 years of diagnosis, 17% CCUS patients experienced progression to myeloid neoplasms, with transfusion requirement and survival being comparable to that of lower risk MDS[15]. In this study, we curated our database, classifying CCUS patients who had received prior cytotoxic therapy as t-CCUS and found that this subgroup of patients had significantly worse progression free survival and overall survival in comparison to those with de novo CCUS. In contrast, t-CCUS patients had better survival outcomes in comparison to those with t-MN, regardless of the t-MN phenotype at diagnosis[11]. Despite the relative similarity in presentation and management, previous exposure to DNA-damaging agents for CCUS patients was independently associated with inferior PFS and OS, suggesting that prior cytotoxic therapies assign a distinct sub-entity within the larger CCUS umbrella.

We created clinically useful risk-prediction models utilizing data available at the outset and after comprehensive evaluation is completed. In all the models, inclusion of prior cytotoxic therapy as a variable remained independently associated with PFS and OS, highlighting the value of clinical history taking.

While the underlying rationale remains unclear, the mechanisms of the differential survival rates are likely multifactorial. First, patients with t-CCUS were enriched with *TP53*^{mut} compared to *de novo* CCUS, which are traditionally considered to be higher risk for leukemic progression and survival. Moreover, we recently showed that *TP53*^{mut} and *TP53*^{wt} CCUS had comparable PFS and OS[16]. Second, having encountered an additional malignancy and receiving therapy may lead to more pronounced cytopenia and inability or preference for not receiving CCUS-directed therapy. However, such patterns did not emerge as the degree of cytopenia was comparable between the two cohorts. Moreover, within the scope of retrospective analysis, management strategies were not different between CCUS and t-CCUS. Finally, progression of the primary disease may have led to increased mortality in t-CCUS compared to (39.1% *vs*. 24.8%), though the difference was not statistically significant. A larger, preferably prospective study may help answer these questions.

Our retrospective analysis has notable limitations. First, while ours is the largest study comparing outcomes of CCUS with t-CCUS, relatively small numbers limited the number of variables that could be analyzed simultaneously. This is in contrast with CH where the association and impact of cytotoxic therapies on the genetic landscape and clonal evolution is well characterized.[3,4,17]. CCUS diagnosis was made clinically as opposed to uniform sequencing of a large cohort at a uniform timing in larger CH

studies[4,5,18]. Finally, the risk of leukemic transformation is much higher and the latency much shorter following CCUS compared to CH. Second, highlighted above, the management following CCUS diagnosis was at providers' discretion and may have been biased by factors beyond our consideration. In addition, the lack of consensus in MDS-defining cytogenetics and diagnostic criteria may have also impacted management decisions. With regard to the newer classifications by ICC, it is likely that 8 patients from of our cohort may represent the entity now recognized as clonal cytopenia and monocytosis of undetermined significance (CCMUS). This may inherently bias the cohort towards higher risk of progression to MDS/MPN, especially to CMML[12]. Finally, progressive versions of the NGS panels utilized over the accrual period included additional genes and higher sensitivity, which may contribute to underrepresentation of some of the emerging variants. A notable example is *PPM1D*—one of the most common therapy-emergent variants—was not included in our earlier clinical NGS panel and is likely underrepresented in the study.

Acknowledging these limitations, we concluded that t-CCUS has distinct morphological and genetic features compared to CCUS and had a higher risk of progression to myeloid neoplasm and death. Given the overall higher risk of progression and death, more stringent monitoring may be considered for t-CCUS patients. Whether earlier intervention should be considered for t-CCUS patients who do not technically have a hematologic malignancy remains to be determined and is a topic for further investigation.

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Variables [Median or n; range or %]	All patients (*n*=151)	CCUS (*n*=105)	t-CCUS (n=46)	*P*-value
Age in years; median	68 (20-99)	68 (20-99)	67 (24-83)	0.268

Tables

Table 1 - Clinicopathological features of patients with *de novo vs*. therapy-related clonal cytopenia of undetermined significance (CCUS).

(range)				
Sex (Male); *n* (%)	91 (60.3)	67 (63.8)	24 (52.2)	0.208
Active or Previous Tobacco Use; *n* (%)	63 (42.6)	43 (41.8)	20 (44.4)	0.857
Hemoglobin g/dL; median (range)	10.9 (6.7-15.9)	10.6 (6.9-15.9)	11.4 (6.7-14.8)	0.831
MCV fL; median (range)	99.2 (80.4-126.7)	98.4 (80.4-123.2)	101.4 (86-126.7)	0.311
WBC x 10^9/L; median (range)	3.5 (0.8-32)	3.5 (1-32)	3.5 (0.8-13.4)	0.689
ANC x10^9/L; median (range)	1.6 (0.04-19.9)	1.6 (0.04-19.9)	1.7 (0.12-9.2)	0.854
AMC x 10^9/L; median (range)	0.4 (0.01-6.2)	0.4 (0.01-6.2)	0.4 (0.03-3.6)	0.681
Platelets x 10^9/L; median(range)	114 (15-595)	119 (15-595)	105 (26-222)	0.101
RDW-CV (%); median (range)	15 (11.7-26.7)	15 (11.7-26.7)	15 (12-24.4)	0.923
Bone marrow cellularity; *n* (%) **1. Hypercellular** **2. Hypercellular with atypia** **3. Hypocellular** **4. Normocellular** **5. Unknown**	 55 (36.4) 29 (19.2) 14 (9.3) 50 (33.1) 3 (1.9)	 47 (44.8) 21 (20) 5 (4.8) 30 (28.6) 2 (1.9)	 8 (17.4) 8 (17.4) 9 (19.6) 20 (43.5) 1 (2.2)	 **0.002** 0.824 **0.011** 0.089 -
Bone marrow blasts %; median (range)	0 (0-5)*	0 (0-4)	1 (0-5)	**0.026**
Abnormal cytogenetics; n (%)	37 (24.5)	26 (24.8)	11 (23.9)	0.375
Presence of ringed sideroblasts; n (%)	26 (13.3)	15 (14.3)	5 (10.9)	0.926
Number of mutations; median (range)	2 (0-5)	2 (0-4)	1 (0-5)	0.551
VAF (%); median (range)	39 (5-92)	39 (5-82)	38.5 (6-92)	0.858
Management of disease; *n* (%) **1. Surveillance only** **2. Hypomethylating agent** **3. Supportive care (GCSF, ESA, TPO, transfusions)** **4. Immunosuppressive agent** **5. Bone Marrow**	 112 (74.2) 6 (4) 23 (15.2) 6 (4) 0 (0) 4 (2.7)	 75 (71.4) 4 (3.8) 17 (16.2) 6 (5.7) 0 (0) 3 (2.9)	 37 (80.4) 2 (4.4) 6 (13) 0 (0) 0 (0) 1 (2.2)	 0.544

Transplantation **6. Vitamin C Clinical Trial**				
Progression to myeloid neoplasm; n (%) **1. CMML** **2. MDS** **3. AML** **4. BPDCN**	28 (17.9) 6 (4) 15 (9.9) 6 (4) 1 (0.6)	16 (15.2) 4 (3.8) 8 (7.6) 3 (2.9) 1 (1)	12 (26.1) 2 (4.3) 7 (15.2) 3 (6.5) 0 (0)	0.171 0.491
Follow up in years; median (range)	2.2 (0.05-12.6)	2.5 (0.05-12.6)	1.5 (0.1-5.9)	**0.002**
Total Deaths; *n* (%) **1. Malignancy related** **2. Cardiac related** **3. Infection related** **4. Other/multifactorial**	44 (29.1) 14 (31.8) 8 (18.2) 10 (22.7) 12 (27.3)	26 (24.8) 6 (23.1) 4 (15.4) 8 (30.1) 8 (30.1)	18 (39.1) 8 (44.4) 4 (22.2) 2 (11.1) 4 (22.2)	 0.299
CHRS Score; median (range)	12 (8.5-15.5)	12 (8.5-15.5)	12 (8.5-15.5)	0.574
CHRS Risk Group; n (%) **1. Low** **2. Intermediate** **3. High**	 16 (10.6) 70 (46.4) 65 (43.1)	 9 (8.6) 50 (47.6) 46 (43.8)	 7 (15.2) 20 (43.5) 19 (41.3)	 0.478

dL – deciliter, MCV – mean corpuscular volume , ANC – absolute neutrophil count, AMC – absolute monocyte count, RDW-CV – red cell distribution width-coefficient of variation , G-CSF – granulocyte-colony stimulating factor, ESA – erythropoietin stimulating agents, TPO - thrombopoietin receptor agaonists, CMML – chronic myelomonocytic leukemia, MDS – myelodysplastic syndrome, AML – acute myeloid leukemia, BPDCN – blastic plasmacytoid dendritic cell neoplasm, CHRS – clonal hematopoiesis risk scoreNGS – next generation sequencing.
*One patient with blasts of 5% had received G-CSF treatment prior to bone marrow biopsy

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459 **Table 2** – Next generation sequencing results of *de novo vs*. therapy-related clonal
460 cytopenia of undetermined significance (CCUS)

Next generation sequencing analysis, n (%)	**All patients (*n*=151)**	**CCUS (*n*=105)**	**t-CCUS (n=46)**	***P*-value**
1. Epigenetic regulators				
TET2	56 (37.1)	38 (36.2)	18 (39.1)	0.855
IDH1	9 (6)	7 (6.7)	2 (4.4)	0.723
IDH2	3 (2)	3 (2.9)	0 (0)	0.553
DNMT3A	18 (11.9)	11 (10.5)	7 (15.2)	0.422
2. Chromatin regulators				
ASXL1	21 (13.9)	16 (15.2)	5 (10.9)	0.612
3. Spliceosome factors				
SRSF2	36 (23.8)	32 (30.4)	4 (8.7)	**0.003**
SF3B1	4 (2.6)	4 (3.8)	0 (0)	0.314
U2AF1	12 (8)	9 (8.6)	3 (6.5)	1.000
ZRSR2	13 (8.6)	11 (10.5)	2 (4.4)	0.346
4. Transcription factors				
RUNX1	6 (4)	3 (2.9)	3 (6.5)	0.369
5. Cell signaling				
KRAS	3 (2)	3 (2.9)	0 (0)	0.553
NRAS	3 (2)	1 (1)	2 (4.4)	0.220
CBL	4 (2.7)	4 (3.8)	0 (0)	0.314
JAK2	3 (2)	2 (1.9)	1 (2.2)	1.000
KIT	1 (0.6)	0 (0)	1 (2.2)	0.305
MPL	2 (1.3)	0 (0)	2 (4.3)	0.091
NOTCH1	1 (0.7)	0 (0)	1 (2.2)	0.305
WT1	2 (1.3)	2 (1.9)	0 (0)	1.000
6. Tumor suppressor genes				
TP53	11 (7.3)	2 (1.9)	9 (19.6)	**<0.001**
7. Others				
SETBP1	2 (1.3)	2 (1.9)	0 (0)	1.000
ATM	3 (2)	2 (1.9)	1 (2.2)	1.000
BCOR	5 (3.3)	3 (2.9)	2 (4.4)	0.641
STAG2	3 (2)	3 (2.9)	0 (0)	0.553
CHEK2	2 (1.3)	1 (1)	1 (2.2)	0.518
PHF6	1 (0.6)	0 (0)	1 (2.2)	0.305
PTEN	1 (0.6)	1 (1)	0 (0)	1.000
PPM1D	2 (1.3)	0 (0)	2 (4.4)	0.091
EZH2	2 (1.3)	1 (1)	1 (2.2)	0.518
ITK	2 (1.3)	1 (1)	1 (2.2)	0.518

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462 **Table 3 -** Univariable analysis for progression-free and overall survival in patients with
463 clonal cytopenia of undetermined significance (CCUS)

Variables	Progression-free survival		Overall survival	
	Hazard Ratio (95% CI)	*P*-value	Hazard Ratio (95% CI)	*P*-value
Exposure to Therapy	2.11 (1.22 – 3.63)	**0.007**	2.33 (1.27 – 4.31)	**0.007**
Age	1.02 (1.00 – 1.04)	0.077	1.03 (1.01 – 1.10)	**0.046**
Sex (Male)	1.28 (0.73 – 2.24)	0.395	1.07 (0.57 – 2.00)	0.833
Active or Previous Tobacco Use	1.10 (0.65 – 1.87)	0.728	0.95 (0.52 – 1.74)	0.876
HGB	0.78 (0.69 – 0.89)	**<0.001**	0.74 (0.63 – 0.86)	**<0.001**
WBC	1.00 (0.93 – 1.08)	0.936	1.03 (0.96 – 1.11)	0.420
PLT	0.99 (0.99 – 0.99)	**0.022**	0.99 (0.99 – 0.99)	**0.023**
ANC	1.02 0.92 – 1.12)	0.754	1.06 (0.96 – 1.18)	0.221
AMC	1.10 (0.73 – 1.65)	0.667	1.25 (0.84 – 1.87)	0.264
RDW	1.18 (1.10 – 1.26)	**<0.001**	1.20 (1.11 – 1.29)	**<0.001**
MCV	1.03 (1.00 – 1.06)	**0.022**	1.03 (0.99 – 1.06)	0.059
Abnormal Cytogenetics	1.55 (0.88 – 2.73)	0.132	1.93 (1.04 – 3.59)	**0.040**
Bone marrow blasts	1.15 (0.91 – 1.45)	0.236	1.24 (0.96 – 1.59)	0.098
Number of mutations	1.15 (0.90 – 1.48)	0.259	1.18 (0.90 – 1.56)	0.237
CHRS Score	1.37 (1.18 – 1.59)	**<0.001**	1.29 (1.10 – 1.51)	**0.002**
Maximum VAF	0.98 (0.97 – 0.99)	**0.043**	0.99 (0.97 – 1.00)	0.144
***TET2* mutated**	0.97 (0.56 – 1.68)	0.923	0.87 (0.46 – 1.61)	0.649
***SRSF2* mutated**	1.25 (0.68 – 2.28)	0.478	1.23 (0.62 – 2.43)	0.559
***ASXL1* mutated**	1.02 (0.48 – 2.15)	0.966	1.17 (0.52 – 2.63)	0.704
***DNMT3A* mutated**	1.07 (0.48 – 2.37)	0.868	1.16 (0.49 – 2.76)	0.730
***ZRSR2* mutated**	1.19 (0.51 – 2.79)	0.684	0.89 (0.32 – 2.49)	0.820
***U2AF1* mutated**	2.06 (0.97 – 4.39)	0.061	2.15 (0.95 – 4.83)	0.065
***TP53* mutated**	2.66 (1.14 – 6.24)	**0.024**	3.29 (1.28 – 8.43)	**0.013**
***SF3B1* mutated**	3.86 (1.2 – 12.50)	**0.024**	0.73 (0.10 – 5.31)	0.755
***RUNX1* mutated**	1.46 (0.45 – 4.67)	0.526	1.94 (0.60 – 6.30)	0.268
DAT* mutation	0.88 (0.52 – 1.49)	0.633	0.78 (0.43 – 1.41)	0.412
Spliceosome factor mutation	1.68 (0.99 – 2.84)	0.053	1.18 (0.65 – 2.15)	0.585
Epigenetic regulator mutation	0.90 (0.53 – 1.53)	0.702	0.85 (0.47 – 1.53)	0.588
Cell signaling mutation	0.77 (0.33 – 1.80)	0.550	0.83 (0.33 – 2.11)	0.695

* DAT mutations – *DNMT3A, ASXL1, TET2*
Abbreviations: HGB – hemoglobin, WBC – white blood cell count, PLT – platelets, ANC – absolute neutrophil count, AMC – absolute monocyte count, MCV – mean

corpouscular volume, RDW – red cell distribution width, CHRS – clonal hematopoiesis risk score, VAF – variant allele frequency

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Table 4 - Multivariable analysis for progression-free (PFS) and overall survival (OS) in patients with clonal cytopenia of undetermined significance (CCUS)

Model	Variable	Hazard Ratio (95% CI)	*P*-value	C-index
Progression-free survival				
Clinical	Exposure to Therapy	1.98 (1.10 – 3.56)	**0.023**	0.751
	Lower HGB	1.11 (0.93 – 1.33)	0.250	
	Lower PLT	1.01 (1.01 – 1.01)	**0.014**	
	Higher RDW	1.19 (1.07 – 1.31)	**<0.001**	
	Higher MCV	1.01 (0.98 – 1.05)	0.463	
Modified CHRS	Exposure to Therapy	2.34 (1.35 – 4.05)	**0.002**	0.688
	Higher CHRS Score	1.38 (1.19 – 1.60)	**<0.001**	
Genetics Only	Exposure to therapy	2.08 (1.17 3.70)	**0.013**	0.632
	TP53 mutated	2.03 (0.84 – 4.95)	0.118	
	SF3B1 mutated	5.31 (1.60 – 17.77)	**0.006**	
Overall survival				
Clinical	Exposure to Therapy	2.13 (1.10 – 4.14)	**0.026**	0.792
	Lower HGB	1.21 (1.01 – 1.47)	**0.042**	
	Lower PLT	1.01 (1.01 – 1.01)	**0.015**	
	Higher RDW	1.19 (1.07 – 1.32)	**0.001**	
Modified CHRS	Exposure to Therapy	2.28 (1.20 – 4.31)	**0.012**	0.662
	Abnormal cyto.	2.20 (1.17 – 4.13)	**0.014**	
	Higher CHRS Score	1.29 (1.10 – 1.53)	**0.002**	
Genetics Only	Exposure to Therapy	2.02 (1.05 – 3.85)	**0.034**	0.607
	TP53 mutated	2.03 (0.59 – 6.99)	0.264	
	Abnormal cyto.	2.07 (1.09 – 3.93)	**0.006**	

Abbreviations: HGB – hemoglobin, PLT – platelet, RDW – red cell distribution width, MCV – mean corpuscular volume, CHRS – clonal hematopoiesis risk score, cyto. - cytogenetics

Figure Legends

Figure 1. (A) Genetic characteristics and (B) variance allele frequency (VAF) for select genes) in clonal cytopenia of undetermined significance (CCUS) patients stratified by the receipt of prior cytotoxic therapies.

Figure 2. Prior cytotoxic therapies is associated with inferior (A) progression-free and (B) overall survival in patients with clonal cytopenia of undetermined significance (CCUS)

Figure 3. Forest plot of multivariable analysis for (A) progression-free (PFS) and (B) overall survival in clonal cytopenia of undetermined significance (CCUS).

Figure 1

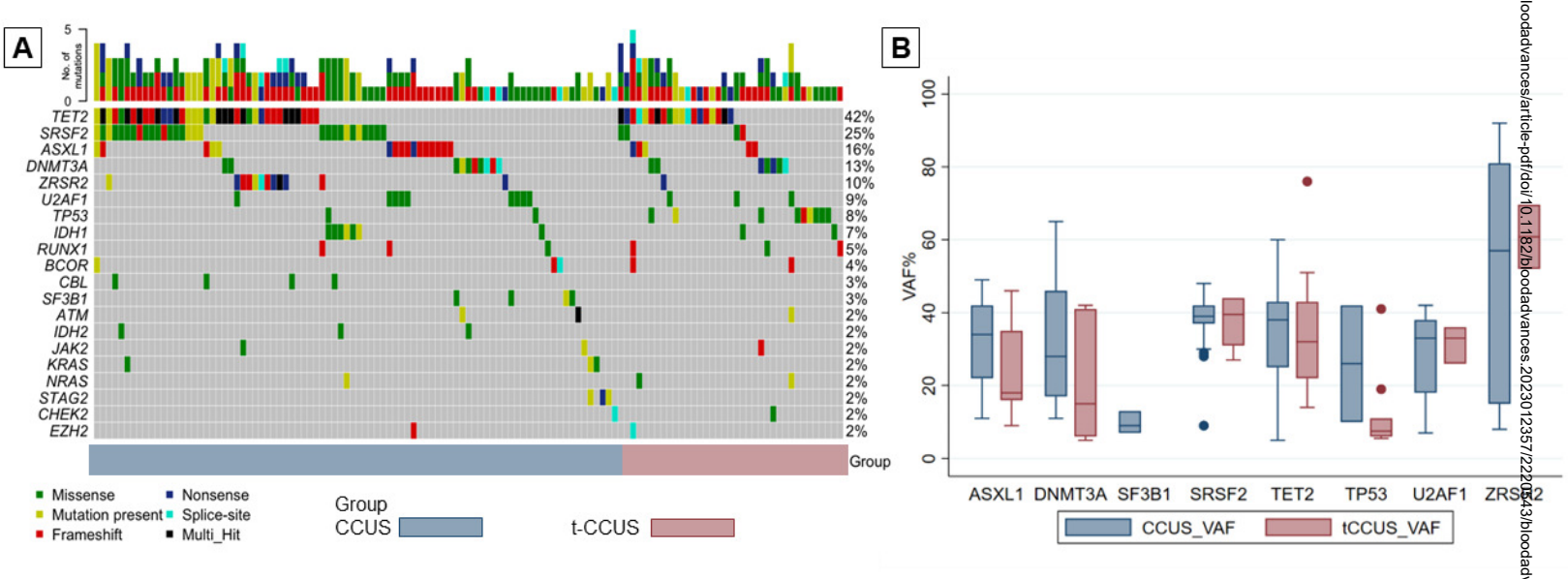
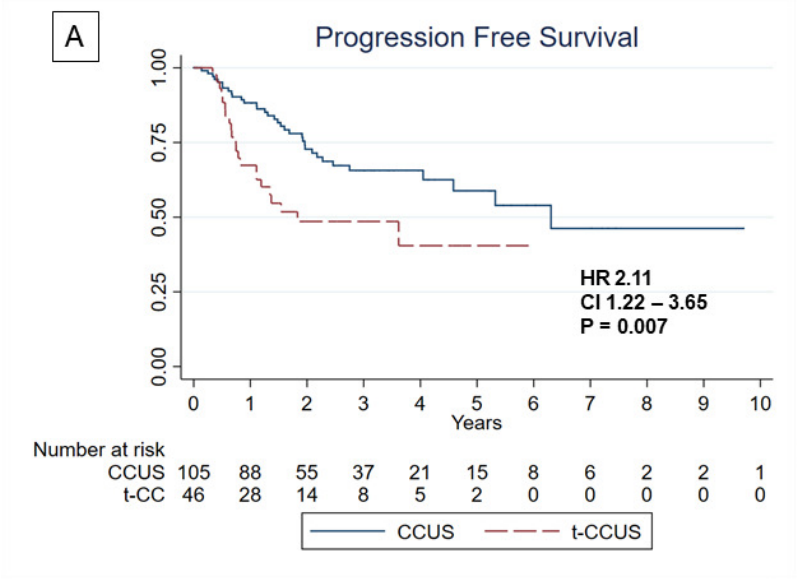


Figure 2



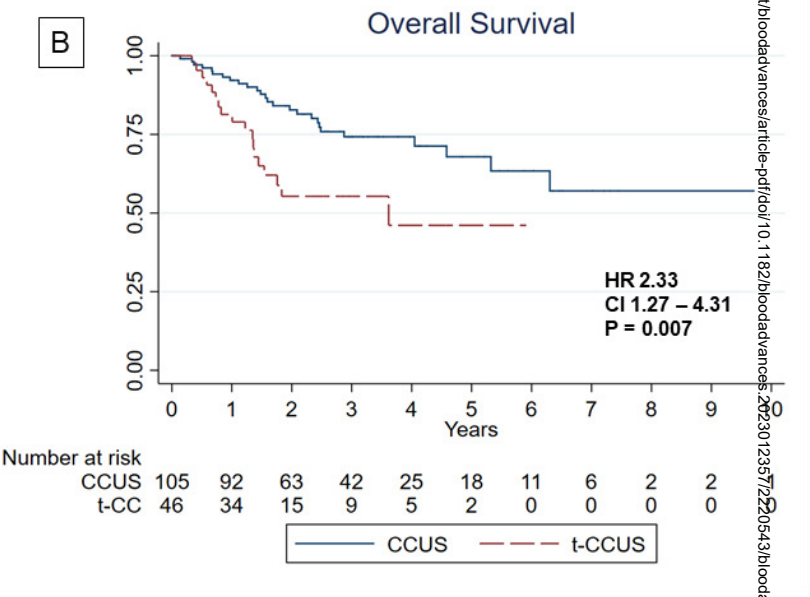


Figure 3