Title

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Authors

Xie, Zhuoer

Komrokji, Rami S

Al-Ali, Najla

et al.

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2021 L Street NW, Suite 900,
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Phone: 202-776-0544 | Fax 202-776-0545
editorial@hematology.org

Risk Prediction for Clonal Cytopenia: Multicenter Real-World Evidence

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Zhuoer Xie (Moffitt Cancer Center, United States) Rami Komrokji (H. Lee Moffitt Cancer Center, United States) Najla Al-Ali (H. Lee Moffitt Cancer Center, United States) Alexandra Regelson (Mayo Clinic, United States) Susan Geyer (Mayo Clinic, United States) Anand Patel (University of Chicago, United States) Caner Saygin (Section of Hematology/Oncology, The University of Chicago, Chicago, IL, United States) Amer Zeidan (Yale University, United States) Jan Bewersdorf (Yale School of MedicineUniversity, United States) Lourdes Mendez (Yale University School of Medicine, United States) Ashwin Kishtagari (Vanderbilt University Medical Center, United States) Joshua Zeidner (University of North Carolina, Lineberger Comprehensive Cancer Center, United States) Catherine Coombs (University of California Irvine, United States) Yazan Madanat (UT Southwestern Medical Center, United States) Stephen Chung (University of Texas Southwestern, United States) Talha Badar (Mayo Clinic, United States) James Foran (Mayo Clinic Florida, United States) Pinkal Desai (Weill Cornell Medicine, United States) Charlton Tsai (Weill Cornell Medicine, United States) Elizabeth Griffiths (Roswell Park Comprehensive Cancer Center, United States) Monzr Al Malki (City of Hope National Medical Center, United States) Idoroenyi Amanam (City of Hope National Medical Center, United States) Catherine Lai (University of Pennsylvania, United States) H. Joachim Deeg (Fred Hutchinson Cancer Center, United States) Lionel Ades (Hopital Saint Louis, France) Cecilia Arana-Yi (Mayo Clinic, United States) Afaf Osman (The University of Utah, United States) Shira Dinner (Northwestern University Feinberg School of Medicine and the Robert H. Lurie Comprehensive Cancer Center of Northwestern University, United States) Yasmin Abaza (Northwestern University, United States) Justin Taylor (University of Miami Sylvester Comprehensive Cancer Center, United States) Namrata Chandhok (University of Miami, Sylvester Comprehensive Cancer Center, United States) Deborah Soong (University of Miami Sylvester Comprehensive Cancer Center, United States) Andrew Brunner (Massachusetts General Hospital, United States) Hetty Carraway (Cleveland Clinic, United States) Abhay Singh (,) Chiara Elena (IRCCS Fondazione Policlinico San Matteo, Italy) Jacqueline Ferrari (University of Pavia & Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy, Italy) Anna Galli (Fondazione IRCCS Policlinico San Matteo, Italy) Sara Pozzi (University of Pavia, Pavia, Italy, Italy) Eric Padron (H. Lee Moffitt Cancer Center, United States) Mrinal Patnaik (Mayo Clinic, United States) Luca Malcovati (University of Pavia, Italy) Michael Savona (Vanderbilt University School of Medicine, United States) Aref Al-Kali (Mayo Clinic, United States)

Abstract:
Clonal cytopenia of undetermined significance (CCUS) represents a distinct disease entity characterized by myeloid-related somatic mutations with a variant allele fraction of {greater than or equal to}2% in individuals with unexplained cytopenia(s) but without a myeloid neoplasm (MN). Notably, CCUS carries a risk of progressing to MN, particularly in cases featuring high-risk mutations. Understanding CCUS requires dedicated studies to elucidate its risk factors and natural history. Our analysis of 357 CCUS patients investigated the interplay between clonality, cytopenia, and prognosis. Multivariate analysis identified 3 key adverse prognostic factors: the presence of splicing mutation(s) (score = 2 points), platelet count <100×109/L (score = 2.5), and {greater than or equal to}2 mutations (score = 3). Variable scores were based on the coefficients from the Cox proportional hazards model. This led to the development of the Clonal Cytopenia Risk Score (CCRS), which stratified patients into low- (score <2.5 points), intermediate- (score 2.5-<5), and high-risk (score {greater than or equal to}5) groups. The CCRS effectively predicted 2-year cumulative incidence of MN for low- (6.4%), intermediate- (14.1%), and high- (37.2%) risk groups, respectively, by Gray's test (P <.0001). We further validated the CCRS by applying it to an independent CCUS cohort of 104 patients, demonstrating a c-index of 0.64 (P▮=.005) in stratifying the cumulative incidence of MN. Our study underscores the importance of integrating clinical and molecular data to assess the risk of CCUS progression, making the CCRS a valuable tool that is practical and easily calculable. These findings are clinically relevant, shaping the management strategies for CCUS and informing future clinical trial designs.

Conflict of interest: COI declared - see note

COI notes: Komrokji: BMS: Honoraria, Membership on an entity's Board of Directors or advisory committees; CTI biopharma: Honoraria, Membership on an entity's Board of Directors or advisory committees, Speakers Bureau; Servio: Honoraria, Membership on an entity's Board of Directors or advisory committees, Speakers Bureau; PharmaEssentia: Honoraria, Other, Speakers Bureau; Novartis: Honoraria, Membership on an entity's Board of Directors or advisory committees; Abbvie: Honoraria, Membership on an entity's Board of Directors or advisory committees; Geron: Honoraria, Membership on an entity's Board of Directors or advisory committees; Taiho: Honoraria, Membership on an entity's Board of Directors or advisory committees; Jazz: Honoraria, Membership on an entity's Board of Directors or advisory committees, Speakers Bureau. Anand Patel: research funding from Pfizer, Kronos Bio; honoraria from BMS and AbbVie Elizabeth A. Griffiths has received honoraria for advisory board membership from AbbVie, Alexion Pharmaceuticals, Apellis, Celgene/BMS, CTI Biopharma, Genentech, Novartis, Picnic Health, Takeda Oncology, Taiho Oncology. EAG has received research funding from Astex Pharmaceuticals, AstraZeneca Rare Disease, Alexion Pharmaceuticals, Apellis Pharmaceuticals, Blueprint Medicines, Genentech Inc, and honoraria for CME activities from Physicians Educational Resource, MediComWorldwide, American Society of Hematology, AAMDS International Foundation. Hetty E. Carraway has received honoraria for advisory board memberships from AbbVie, Celgene/BMS, Genentech, Jazz, Novartis and Daiichi. HEC has received research funding from Celgene. HEC has served on speakers bureau for BMS, Jazz, Novartis and Stemline. HEC has served on data safety monitoring board for ASTEX, AbbVie and Takeda as well as Syndax. Andrew Brunner received consulting or advisory board honoraria from Novartis, Acceleron, Agios, Abbvie, Takeda, Celgene/BMS, Keros Therapeutics, Taiho, Gilead. Andrew Brunner has research support from the NIH SPORE in Myeloid Malignancies, and from the Edward P. Evans Foundation. Amer M. Zeidan received research funding (institutional) from Celgene/BMS, Abbvie, Astex, Pfizer, Medimmune/AstraZeneca, Boehringer-Ingelheim, Cardiff oncology, Incyte, Takeda, Novartis, Aprea, and ADC Therapeutics. AMZ participated in advisory boards, and/or had a consultancy with and received honoraria from AbbVie, Otsuka, Pfizer, Celgene/BMS, Jazz, Incyte, Agios, Boehringer-Ingelheim, Novartis, Acceleron, Astellas, Daiichi Sankyo, Cardinal Health, Taiho, Seattle Genetics, BeyondSpring, Cardiff Oncology, Takeda, Ionis, Amgen, Janssen, Epizyme, Syndax, Gilead, Kura, Chiesi, ALX Oncology, BioCryst, Notable, Orum, and Tyme. AMZ served on clinical trial committees for Novartis, Abbvie, Gilead, BioCryst, Abbvie, ALX Oncology, Geron and Celgene/BMS. Padron: Stemline: Honoraria; Taiho: Honoraria; Blueprint: Honoraria; BMS: Research Funding; Incyte: Research Funding; Kura: Research Funding; Syntrix Pharmaceuticals: Research Funding. Yazan F. Madanat: received honoraria/consulting fees from BluePrint Medicines, GERON, OncLive and MD Education. YFM participated in advisory boards and received honoraria from Sierra Oncology, Stemline Therapeutics, Blueprint Medicines, Morphosys, Taiho Oncology, Rigel Pharmaceuticals and Novartis. YFM received travel reimbursement from Blueprint Medicines, MD Education, and Morphosys. Joshua F. Zeidner received honoraria from advisory boards from AbbVie, Bristol Myers Squibb, Daiichi Sankyo, Genentech, Gilead, Immunogen, Servier, Shattuck Labs; Consultancy from AbbVie, Foghorn, Gilead, Sellas, Servier; Research Funding from AbbVie, Arog, Astex, Gilead, Jazz, Loxo, Merck, Newave, Shattuck Labs, Stemline, Sumitomo Dainippon Pharma, Takeda. Abhay Singh: Research funding and on the advisory board for Rigel Pharmaceuticals Coombs received consulting or advisory board honoraria from AbbVie, AstraZeneca, Beigene, Genentech, MEI Pharma, TG Therapeutics, Janssen, Novartis, Mingsight, Octapharma, Lilly/LOXO, serves on independent review committee for Octapharma, serves on steering committees for AbbVie, Lilly/LOXO, has equity in CTI Biopharma and Bluebird Bio, serves on speaker's bureau for AbbVie, Genentech, Beigene, AstraZeneca, and has received research support (to institution) from AbbVie and Lilly/LOXO. All other authors have no relevant conflicts of interest to disclose.

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Risk Prediction for Clonal Cytopenia: Multicenter Real-World Evidence

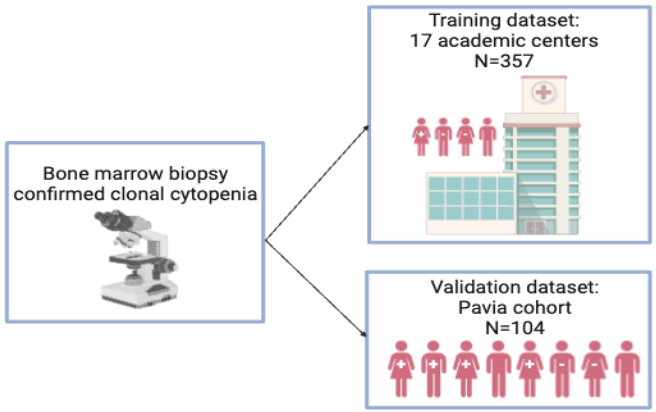
Context of Research

- **Clonal cytopenia of undetermined significance (CCUS)** is characterized by myeloid-related somatic mutations with a VAF of ≥2% in individuals with unexplained cytopenia(s) lacking diagnostic criteria for a myeloid neoplasm

- CCUS carries a risk of progressing to an overt myeloid neoplasm over time. The objective of this study was to develop a **dedicated and refined risk stratification tool** tailored for CCUS patients

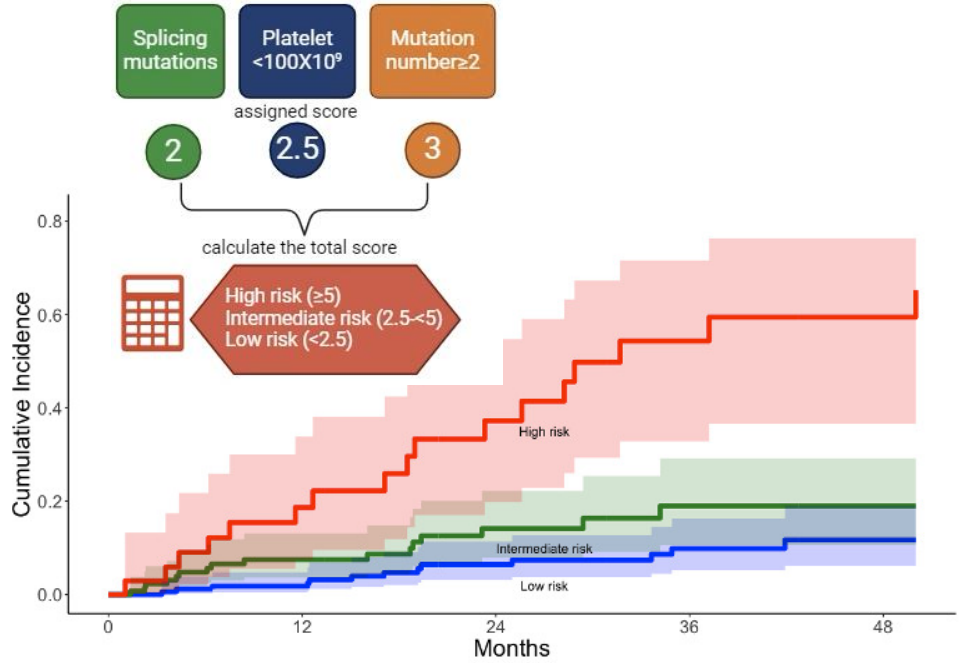
Methods

- CCUS consortium study initiated in 2020



Main Findings

- Ad hoc statistical analyses led to the development of the **Clonal Cytopenia Risk Score (CCRS),** which stratified patients into low-, intermediate-, and high-risk groups



- The predictive performance of the CCRS was **validated using an independent CCUS cohort** from the University of Pavia

Conclusions: A 3-parameter CCRS model was developed for patients diagnosed with CCUS. The CCRS provides a precise assessment of the risk of progression to overt myeloid neoplasm, which is crucial for patient management and clinical trial eligibility.

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blood Visual Abstract

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Zhuoer Xie[1,2], Rami Komrokji[1], Najla Al Ali[1], Alexis Smith[3], Susan Geyer[3], Anand Patel[4], Caner Saygin[4], Amer M. Zeidan[5], Jan Philipp Bewersdorf[6], Lourdes Mendez[5], Ashwin Kishtagari[7], Joshua F. Zeidner[8], Catherine C. Coombs[8], Yazan F. Madanat[9], Stephen Chung, MD[9], Talha Badar[10], James Foran[10], Pinkal Desai[11], Charlton Tsai[11], Elizabeth A. Griffiths[12], Monzr M. Al Malki[13], Idoroenyi Amanam[13], Catherine Lai[14], H. Joachim Deeg[15], Lionel Ades[16], Cecilia Arana Yi[17], Afaf EG Osman[18], Shira Dinner[19], Yasmin Abaza[19], Justin Taylor[20], Namrata Chandhok[20], Deborah Soong[20], Andrew M. Brunner[21], Hetty E. Carraway[22], Abhay Singh[22], Chiara Elena[23], Jacqueline Ferrari[23, 24], Anna Gallì[23], Sara Pozzi[24], Eric Padron[1], Mrinal M. Patnaik[2], Luca Malcovati[23,24], Michael R. Savona[7], Aref Al-Kali[2]

Author affiliations:

[1]Department of Malignant Hematology, H. Lee Moffitt Cancer Center, Tampa, FL, USA; [2]Division of Hematology, Mayo Clinic, Rochester, MN, USA; [3]Department of Biostatistics, Mayo Clinic, Rochester, MN, USA; [4]Leukemia Program, University of Chicago Comprehensive Cancer Center, Chicago, IL, USA; [5]Division of Hematology, Departments of Internal Medicine, Yale University School of Medicine and Yale Cancer Center, New Haven, CT, USA; [6]Department of Medicine, Leukemia Service, Memorial Sloan Kettering Cancer Center, New York, NY, USA; [7]Division of Hematology/Oncology, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA; [8] Division of Hematology, University of North Carolina, Lineberger Comprehensive Cancer Center, Chapel Hill, NC, USA; [9]Division of Hematology, The University of Texas Southwestern Medical Center, TX, USA;[10]Division of Hematology, Mayo Clinic Florida, FL, USA; [11]Division of Hematology and Oncology, Weill Cornell Medicine and The New York-Presbyterian Hospital, NY, USA; [12]Leukemia Service, Department of Medicine, Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA; [13]Department of Hematology and Hematopoietic Cell Transplantation, City of Hope National Medical Center, CA, USA; [14]Division of Hematology, The University of Pennsylvania Perelman Center for Advanced Medicine, Philadelphia, PA, USA; [15]Division of Hematology, Fred Hutchinson Cancer Center, Seattle, WA, USA; [16]Department of Hematology, Hospital Saint-Louis, Paris, France; [17]Division of Hematology, Mayo Clinic Arizona, AZ, USA; [18]Division of Hematology and Hematologic Malignancies, Department of Internal Medicine, University of Utah Salt Lake City, UT, USA; [19]Division of Hematology, Robert H. Lurie Comprehensive Cancer Center, Northwestern University, IL, USA; [20]Division of Hematology, Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL, USA; [21]Leukemia Program, Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA, USA; [22]Department of Hematology and Medical Oncology, Taussig Cancer Institute, Leukemia Program Cleveland, OH, USA; [23]Department of Hematology Oncology, Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy; [24]Department of Molecular Medicine, University of Pavia, Italy & Department of Hematology Oncology, Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy.

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Corresponding Author:
Dr. Zhuoer Xie, MD, MSCR
Assistant Member
Department of Malignant Hematology, H. Lee Moffitt Cancer Center, Tampa, FL, USA
Tel: 813-745-4294, Email: zhuoer.xie@moffitt.org

Dr. Aref Al-Kali, MD
Associate Professor of Medicine, Acute Leukemia and Myeloid Neoplasms Group, Division of Hematology, Mayo Clinic, Rochester, MN 55905,
Tel: 507 294 5096, Email: alkali.aref@mayo.edu

Abbreviations:

AML: acute myeloid leukemia
CH: clonal hematopoiesis
CHIP: clonal hematopoiesis of indeterminate potential
CCUS: clonal cytopenias of undetermined significance
CCRS: clonal cytopenia risk score
CHRS: clonal hematopoiesis risk score
CI: confidence interval
CMML: chronic myelomonocytic leukemia
CMUS: Clonal monocytosis of undetermined significance
CCMUS: clonal cytopenia and monocytosis of undetermined significance
CCVD: cardio-cerebrovascular disease
c-CCUS: patients with a history of cancer
DTA: DNMT3A, TET2, ASXL1
DDR: DNA damage response and repair
Hem: hematological disorders.
MDS: myelodysplastic syndromes
MT: Mutation
NGS: next-generation sequencing
OS: overall survival
LFS: leukemia-free survival
Onc: oncological diseases
t-CCUS: patients received prior cancer therapy.
t-MN: treatment-related myeloid neoplasm
VAF: variant allele fraction

Key Points:

1. A 3-parameter Clonal Cytopenia Risk Score (CCRS) model was devised specifically for patients diagnosed with clonal cytopenia.
2. The CCRS offers precise CCUS risk assessment for patient management and clinical trial eligibility.

Abstract

Clonal cytopenia of undetermined significance (CCUS) represents a distinct disease entity characterized by myeloid-related somatic mutations with a variant allele fraction of ≥2% in individuals with unexplained cytopenia(s) but without a myeloid neoplasm (MN). Notably, CCUS carries a risk of progressing to MN, particularly in cases featuring high-risk mutations. Understanding CCUS requires dedicated studies to elucidate its risk factors and natural history. Our analysis of 357 CCUS patients investigated the interplay between clonality, cytopenia, and prognosis. Multivariate analysis identified 3 key adverse prognostic factors: the presence of splicing mutation(s) (score = 2 points), platelet count $<100\times10^9$/L (score = 2.5), and ≥2 mutations (score = 3). Variable scores were based on the coefficients from the Cox proportional hazards model. This led to the development of the Clonal Cytopenia Risk Score (CCRS), which stratified patients into low- (score <2.5 points), intermediate- (score 2.5-<5), and high-risk (score ≥5) groups. The CCRS effectively predicted 2-year cumulative incidence of MN for low- (6.4%), intermediate- (14.1%), and high- (37.2%) risk groups, respectively, by Gray's test (*P* <.0001). We further validated the CCRS by applying it to an independent CCUS cohort of 104 patients, demonstrating a c-index of 0.64 (*P* =.005) in stratifying the cumulative incidence of MN. Our study underscores the importance of integrating clinical and molecular data to assess the risk of CCUS progression, making the CCRS a valuable tool that is practical and easily calculable. These findings are clinically relevant, shaping the management strategies for CCUS and informing future clinical trial designs.

Introduction

In the fifth edition of the World Health Organization (WHO) Classification of Haematolymphoid Tumours, clonal hematopoiesis (CH) of indeterminate potential (CHIP) was formally defined by the presence of a myeloid-associated somatic mutation in the blood or bone marrow, with a variant allele fraction (VAF) of ≥2% among individuals without a myeloid neoplasm (MN) diagnosis or unexplained cytopenia. If the patient has unexplained cytopenia(s), the condition is then diagnosed as clonal cytopenia of undetermined significance (CCUS).[1] In addition, if the patient has an absolute monocyte count (AMC) ≥0.5×10^9/L, monocytes comprising ≥10% of white blood cell (WBC) differential, and no morphologic findings of chronic myelomonocytic leukemia (CMML) in the bone marrow, CHIP and CCUS are further defined as clonal monocytosis of undetermined significance (CMUS) and clonal cytopenia and monocytosis of undetermined significance (CCMUS), respectively. These classifications are based on the International Consortium Consensus Classification of Myeloid Neoplasms and Acute Leukemias.[2]

CHIP is recognized as an early precursor state for hematologic malignancy, with a low absolute risk of MN transformation (transformation occurs in 0.5%-1% of cases annually).[3,4] However, CHIP is linked to an increased risk of various comorbidities, most notably cardio-cerebrovascular diseases (CCVD).[5–7] In contrast, CCUS presents a 10-fold higher likelihood of progressing to MN.[8] Specific mutation patterns (including splicing mutations; co-mutations involving *DNMT3A*, *TET2*, or *ASXL1* [DTA]; the number of mutations; and VAF) play a crucial role in both diagnosing MN and predicting disease progression.[8,9]

Recent advancements in CH research have led to the development of two risk stratification models, both of which leverage data from population-based studies using the UK Biobank. These models, the Clonal Hematopoiesis Risk Score (CHRS) and the MN-predict model, aim to enhance risk assessment in this context. [10,11] The CHRS model incorporates as the presence of CCUS, mutation patterns, patient age, red blood cell indices, and other factors. Individuals with a CHRS ≥12.5 were identified as high-risk ($P < .001$), with a cumulative 5-year incidence of MN progression of 24%. In contrast, those with a CHRS between 10 and 12 (intermediate risk) exhibited a 5-year MN progression rate of 2.7%, and

individuals with CHRS <9.5 (low risk) had a notably lower rate of 0.23%.[10] The MN-predict model uses genotype, phenotype, and biochemistry data to provide year-by-year probabilities (up to 15 years) for MN transformation.[11]

Recently, an increasing number of cases of CCUS have been identified via routine use of next-generation sequencing for cytopenia assessment.[8,12–17] It is imperative to understand the risk factors and natural history of CCUS to effectively address its various clinical challenges. Notably, there is currently a lack of established standards of care for CCUS, low response rates for existing therapies, and a pressing need to identify high-risk patients for enrollment in clinical trials aimed at effectively managing disease progression.

In this study conducted by the CCUS consortium, our objectives were to delineate the clinical and molecular characteristics of CCUS, examine the associations between clonality and cytopenias, and ascertain the prognostic significance of clonal cytopenia through both gene-specific and functional pathway analyses. Because of the limited data available regarding CCUS treatment,[18] we also discuss treatment approaches and outcomes for a subgroup of patients who underwent therapy. This research resulted in the development of the Clonal Cytopenia Risk Score (CCRS) model for CCUS, a clinically relevant tool for patient risk stratification. The predictive performance of the CCRS was further validated using an independent CCUS cohort from the University of Pavia.

Methods

Patients

Written informed consent was obtained from all individual participants included in the study. The research protocol was approved by the medical ethical committee of the Mayo Clinic. The study is carried out in accordance with the Declaration of Helsinki. This CCUS consortium study was initiated in 2020 as a collaborative effort among 17 academic centers across the United States and Europe (Table S1). The primary objective was to collect real-world data from patients with clonal cytopenias. Inclusion

criteria included adult age (≥18 years) and a bone marrow biopsy that did not meet diagnostic criteria for MN. Patients with cytopenia with a cytogenetic abnormality were included and their disease was categorized as CCUS unless a myelodysplastic syndrome– (MDS-) defining cytogenetic abnormality was present (in accordance with the 2016 WHO Classification of Haematolymphoid Tumours definitions), in which case the patient was excluded.[1,19] Patients must not have received any prior therapy for cytopenia at the time of CCUS diagnosis. Of our total cohort, a subgroup of 71 patients subsequently received treatments for cytopenia at the discretion of the treating physician.

Definitions

In our study, clonal cytopenia was defined as the presence of cytopenia(s), including anemia (hemoglobin [HgB] <13 g/dL for males and <12 g/dL for females), leukopenia (absolute neutrophil count [ANC] $<1.8\times10^9$/L), and thrombocytopenia (platelets [PLT] $<150\times10^9$/L), that were accompanied by either MN-associated somatic mutation(s), non–MN-defining chromosomal abnormalities, or a combination of both. Additionally, we assessed patients experiencing HgB<10 g/dL, PLT$<100\times10^9$/L, and/or ANC$<1\times10^9$/L. Dependence on blood transfusion was defined as requiring an average of ≥2 units of packed red blood cells (RBC) or PLTs over 4 weeks or ≥4 units over 8 weeks. Response rates (RR) for patients who received treatment were determined using the 2006 International Working Group response criteria for MDS.[20] Overall survival (OS) was calculated from the date of CCUS diagnosis to the date of death from any cause, and leukemia-free survival (LFS) was calculated from the time from CCUS diagnosis to disease progression into MDS, CMML, or acute myeloid leukemia (AML).

Mutational data

Details of the mutational analysis and next-generation sequencing panels used across institutions are provided in the supplementary material (Gene Panel). If the VAF was ≥2%, a somatic pathogenic variant call was counted in the analysis. A total of 63 unique somatic genes were identified. We

evaluated each gene as well as genes grouped into functional pathways, including splicing, epigenetic regulation, transcriptional regulation, and signaling pathways (Table S2).

Statistical analyses

Continuous variables were presented as median values (interquartile range [IQR]/range) and categorical variables as frequency values (percentages). Differences in the distribution of continuous variables between categories were compared by either the Mann–Whitney or the Kruskal–Wallis tests. Categorical variables were compared using chi-square or Fisher exact tests. Data were censored at the time patients were last known to be alive. The median point estimate and 95% confidence interval (CI) for follow-up time, OS, and LFS were estimated using the Kaplan–Meier method. Stepwise Cox proportional hazard analyses were conducted to evaluate the prognostic impact of diagnostic variables on OS and LFS in univariate and multivariable analyses. Optimal VAF cut-off points were determined using recursive partitioning algorithms to assess the variable's relation to LFS.

An LFS predictive model was constructed by incorporating significant covariates identified in the multivariable analysis. The weight of each covariate was determined based on the coefficients derived from the Cox proportional hazard model. For clinical practice purposes, a modified model was developed by substituting continuous factors with optimal cut-off points. The cumulative incidence of MNs across the 3 risk groups was assessed using Gray's test. To validate the predictive efficacy of the current model, we used an external cohort that included 104 patients with CCUS (as proven through bone marrow biopsy) diagnosed at the University of Pavia. The model was validated using a receiver operating characteristic (ROC) curve, with the area under the ROC curve (AUC) serving as a comprehensive metric for summarizing the model's performance. All *P* values were two-sided, and statistical computations were conducted using R version 4.0.1.

Results

Baseline characteristics

A total of 357 patients with CCUS were enrolled over 2 years. The median age of the cohort was 70 years (range, 19-94 years), with 126 patients (35%) being female. The most common comorbidity was a prior history of hematological or oncological diseases (n=133 [37%]), followed by CCVD (n=115 [32%]) and inflammatory diseases (n=44 [12%]) (Table S3).

Cytopenia

One-third of the patients (n=120 [34%]) had HgB <10 g/dL; among these patients, 30 (25%) were RBC transfusion–dependent. One hundred and thirty patients (37%) had PLT <100×10^9/L, and 24 (7%) had ANC<1×10^9/L. Additionally, 97 patients (27%) met the diagnostic criteria for CCMUS.

Somatic mutations and chromosomal alterations

Within the cohort, 156 patients (44%) had only one mutation, whereas 162 (45%) had ≥2 mutations. Additionally, 39 (11%) patients' diseases were categorized as CCUS based solely on cytogenetic abnormalities. In total, 592 variants were identified (Table S2), with the most prevalent mutations found in *TET2* (n=141 [24%]), *DNMT3A* (n=77 [13%]), *SRSF2* (n=61 [10.3%]), *ASXL1* (n=49 [8.3%]), and *U2AF1* (n=27 [4.7%]) (Figure 1). The median VAF was 31.7% (range, 3%-99.7%). Among the 79 patients with cytogenetic abnormalities, - Y was the most frequent (n=21 [26.6%]) abnormality, followed by trisomy 8 (n=16 [20.3%]) (Table S3).

The correlation between "clonality" and "cytopenia" in patients with clonal cytopenia

Among patients with HgB <10 g/dL, including those who were dependent on RBC transfusions, mutations involving *DTA* were the most frequent. In contrast, the most prevalent mutations among patients with PLT <100×10^9/L and ANC <1×10^9/L were *TET2*, *SRSF2*, and *ASXL1* (Figure S1). Overall, there was a trend showing that VAF correlated negatively with PLT (r = –0.1; *P* =.09) but positively with AMC (r = 0.11; *P* =.07). Detailed associations between VAF and cytopenia are provided in Figure S2.

Comparisons of subgroups of interest

CCMUS

Patients diagnosed with CCMUS (n=97) tended to be older than those with non-CCMUS cases (median age, 66.7 vs 70.3; $P = .02$) and exhibited a male predominance (74% vs. 61%; $P = .045$). In addition, they presented with higher WBC (median, 5.0×10^9/L vs 3.4×10^9/L; $P < .0001$) and ANC (median, 2.7 x10^9/L vs 1.8×10^9/L; $P = .03$). However, no significant differences were observed in HgB levels (median, 11.3 vs 10.9 g/dL; $P = .08$) or PLT (median, 120×10^9/L vs 122×10^9/L; $P = .70$).

Among patients with CCMUS, 7 (7%) exhibited only cytogenetic abnormalities without any somatic mutations. The median VAF for these cases was 42.8% (range, 2.8%- 99%). Of the 162 genetic variants identified, the most frequent mutations were in *TET2* (n=62 [38%]), *SRSF2* (n=28 [17%]), and *ASXL1* (n=18 [11%]). Figure S3 shows the gene frequencies for patients with CCMUS vs those without. CCMUS was associated with inferior OS (hazard ratio [HR], 1.7 [95% CI, 1.04-2.82]; $P = .03$) and LFS (HR, 1.8 [95% CI, 1.0-3.2]; $P = .05$) (Figure 2A and 3A). Seventeen patients (18%) experienced disease progression, with 9 developing CMML and 8 developing MDS.

History of hematologic or oncologic diseases

Within the subset (n=133) of patients with a history of hematologic or oncologic diseases, 67 (50%) had solid tumors, 71 (53%) had hematologic disorders other than MN, and 6 (5%) had both. Patients with CCUS and solid tumors were older than those with CCUS but no solid tumors (median, 74 vs 67 years; $P < .001$). In contrast, patients with CCUS and hematologic disorders were younger than those without such disorders (median, 67 vs 70.5 years; $P = .016$) and had lower HgB levels (median, 10.4 vs 11.3 g/dL; $P = .03$) and PLT counts (median, 98×10^9/L vs 127×10^9/L; $P = .03$) (Table S4). Mutation analysis revealed that *TET2* mutations were the most frequent in patients with CCUS and solid tumors (36%; median VAF, 9.5%), whereas *DNMT3A* mutations predominated among those with CCUS and hematologic disorders (28%; median VAF, 9.5%). In terms of survival outcomes, patients with co-existing non-myeloid hematological disorders exhibited similar LFS (HR, 0.56 [95% CI, 0.24-1.31];

P =.18) and OS (HR, 1.13 [95% CI, 0.63-2.01]; *P* =.68) as those without such disorders. However, though patients with solid tumors showed similar LFS (HR, 0.73 [95% CI, 0.33-1.64]; *P* =.45), they had significantly worse OS (HR, 1.93 [95% CI, 1.14-3.27]; *P* =.01) (Table S5 and Fig S4-5).

Sixty-six (50%) patients who had a history of other malignant tumors or non-myeloid hematological disorders had received prior therapy, such as chemotherapy, radiation therapy, or both. These patients were categorized as having treatment-related CCUS (t-CCUS). For all patients with t-CCUS, a diagnosis of t-MN was excluded, as no evidence of MN was found in their bone marrow. When comparing t-CCUS to non–t-CCUS subgroups, patients with t-CCUS were older (72 vs 66.7 years; *P* =.003) but showed no differences in HgB, PLT, ANC, or mutation count. The frequency of *TP53* mutations (n=1 [2%]) was low, and no *PPM1D* mutations were identified. However, cytogenetic abnormalities were more common in the t-CCUS group than in the non–t-CCUS group (n=22 [33.3%] vs 58 [20%]; *P* =.02). Patients with t-CCUS experienced inferior OS compared to those who had never received prior therapy (HR, 2.35 [95% CI, 1.41-3.92]; *P* =.001). However, there were no significant differences in LFS between the t-CCUS and non–t-CCUS subgroups (HR, 0.79 [95% CI, 0.35-1.77]; *P* =.057). (Figure S6).

Cardio-cerebrovascular disease

Compared to patients without CCVD, patients with CCVD demonstrated a tendency towards lower HgB levels (median, 9.8 vs 11.6 g/dL; *P* =.0002), higher ANC (median, 2.3×10^9/L vs 1.8×10^9/L; *P* =.04), higher AMC (median, 0.5×10^9/L vs 0.4×10^9/L; *P* =.005), and inferior OS (HR 2.50 [95% CI, 1.54-4.05]; *P* =.0002). However, there was no significant difference in LFS or mutational patterns between the two groups (Figure S7).

Inflammatory diseases

Between patients with and without a history of inflammatory disease, there were no significant differences in clinical or molecular features in our analyses (Figure S8).

Prognostic factors for outcomes

The median follow-up duration was 27.3 (range, 0-191.4) months, during which 47 patients (13%) experienced disease progression to MN; among these patients, 30 (64%) experienced progression to MDS, 15 (32%) to CMML, and 2 (4%) to AML. Sixty-six patients (18%) died from various causes. The estimated 2-year OS was 85.4% (95% CI, 81.4%-89.7%), and the 2-year LFS was 87.4% (95% CI, 83.4%-91.5%) (Figure S9). Notably, the 2-year LFS for patients with cytogenetic abnormalities but without somatic mutations was 83.7% (95% CI, 71.2%-98.4%). Among patients with disease progression, the median time to progression was 17.1 months (range, 1-51.6 months).

Leukemia-free survival

In the univariable analyses, PLT <100×10^9/L (HR, 2.81; [95% CI, 1.56-5.06]; *P* <.001) was associated with shorter LFS (Figure 2B), whereas HgB<10 g/dL (HR, 0.67; [95% CI, 0.34-1.32]; *P* =.4) and ANC <1×10^9/L (HR, 1.34; [95% CI, 0.48-3.76]; *P* =.57) showed no significant associations. The presence of ≥2 mutations was significantly associated with shorter LFS (HR, 3.74; [95% CI, 2.0-7.01]; *P* <.0001) (Figure 2C).

In the functional pathway analyses, mutations in splicing pathways were associated with shorter LFS (HR, 3.61; [95% CI, 2.0-6.49]; *P* <.001) (Figure 2D), whereas mutations in the epigenetic regulator (HR, 1.81, [95% CI, 0.94-3.50]; *P* =.08), transcriptional (1.07, [95% CI, 0.48-2.38]; *P* =.88), and signaling (HR, 1.51, [95% CI, 0.6-3.83]; *P* =.2) pathways were not. In the analysis of individual genes, *TET2* (HR, 3.29; [95% CI, 1.82-5.86]; *P* <.001), *SRSF2* (HR, 3.81; [95% CI, 2.13-6.83], *P* =.001), and *ZRSR2* (HR, 3.19; [95% CI, 1.43-7.12]; *P* =.002) were associated with shorter LFS. *DNMT3A* mutations were associated with a lower risk of disease transformation than the absence of a *DNMT3A* mutation (HR, 0.18 [95% CI, 0.04-0.76]; *P* =.02). The impact of individual genes on LFS is shown in Figure S10A.

In multivariable analyses, PLT<100×10^9/L (HR, 2.49, 95% CI: 1.38-4.50, *P* =.003), splicing pathway mutations (HR, 2.13, [95% CI, 1.10-4.10]; *P* =.02), and having ≥2 mutations (HR, 2.57 [95% CI, 1.28-5.15]; *P* =.008) retained their significance in predicting LFS (Figure 2E and 2F).

Overall survival

In the univariable analyses, HgB <10 g/dL was associated with inferior OS (HR, 2.63; [95% CI, 1.62-4.27], *P* <.001) (Figure 3B), whereas PLT <100×10^9/L (HR, 1.34 [95% CI, 0.82-2.17]; *P* =.24) and ANC <1×10^9/L (HR, 1.01 [95% CI, 0.41-2.52]; *P* =.98) were not associated with OS. Having ≥2 mutations was associated with inferior OS (HR, 1.9; [95% CI, 1.1-3.3]; *P* =.02) (Figure 3C). Additionally, older age was associated with a lower OS rate (HR, 1.03; [95% CI, 1.01-1.06]; *P* =.003).

In functional pathway analyses, mutations in signaling pathways were associated with inferior OS (HR, 2.47 [95% CI, 1.26-4.85]; *P* =.009) (Figure 3D), whereas mutations in splicing (HR, 1.59 [95% CI, 0.97-2.59]; *P* =.06), epigenetic regulator (HR, 0.93 [95% CI, 0.57-1.54]; *P* =.79), and transcriptional pathways (HR, 1.1 [95% CI, 0.56-2.16]; *P* =.78) were not associated with OS. In the analysis of individual genes, *ASXL1* (HR, 2.5 [95% CI, 1.42-4.39]; *P* =.001) and *SRSF2* (HR, 2.31 [95% CI, 1.37-3.91]; *P* =.01) mutations were associated with inferior OS; *DNMT3A* (HR, 0.70 [95% CI, 0.35-1.42]; *P* =.51) and *TET2* (HR, 0.99 [95% CI, 0.59-1.66]; *P* =.96) mutations were not associated with OS. The impact of individual genes on OS is shown in Figure S10B*.*

In multivariable analyses adjusted for anemia, mutations in signaling pathway (HR, 2.32 [95% CI, 1.18-4.56]; *P* =.01) and having ≥2 mutations (HR, 2.24 [95% CI, 1.36-3.68]; *P* =.001) were independent risk factors for OS (Figures 3E and 3F).

Correlation between VAF and LFS or OS

We used a probability-based recursive partitioning algorithm to stratify our data according to the likelihood of MN incidence and identified an optimal VAF cut-off point of 22%. However, this cutoff did not correlate with OS as shown in Figures 4A and 4B.

Clonal cytopenia risk scoring system

Given that PLT <100 x10^9/L, having ≥2 mutations, and the presence of splicing pathway mutations were identified as significant risk factors for LFS, they were selected as diagnostic variables to construct a model for LFS prediction named the Clonal Cytopenia Risk Scoring (CCRS) system. The weighted score for each factor is detailed in Figure 5A. Patients were categorized into 3 groups based on their CCRS score: low risk (score <2.5), intermediate risk (score 2.5-<5), and high risk (score ≥5). The 2-year cumulative incidence of MN progression was 6.4% (95% CI, 3-11.4%) for low-risk, 14.1% (95% CI, 7.9-22.2%) for intermediate-risk, and 37.2% (95% CI,19.8-54.7%) for high-risk groups by Gray's test (Figures 5B& 5C).

To assess the predictive performance, we validated the model using an independent cohort (n=104). The baseline characteristics of the Pavia CCUS cohort are summarized in Table S5. The median follow-up duration for this cohort was 4.2 years (range, 0.5-15.1 years). According to the CCRS model, 46 (44%) patients were low-risk, 26 (25%) were intermediate-risk, and 32 (31%) were high-risk. Overall, the CCRS model significantly stratified LFS (p=.005) in this validation cohort, accompanied by a progressive increase of HRs (intermediate vs low-risk (HR, 1.6 [95% CI, 0.55-4.62]; *P* =.39); high vs low-risk (HR, 3.57 [95% CI, 1.56-8.18]; *P* =.003) (Figure 6). The ROC analysis revealed a c-index of 0.64 (95% CI, 0.54-0.73, p=0.005).

Notably, a predictive model with the variables of VAF ≥22%, PLT <100 x10^9/L, and having ≥2 mutations significantly stratified our data for LFS. However, upon validation, the predictive performance using these 3 variables was not superior to using the combination of PLT count, mutation number, and

presence of splicing mutations. Furthermore, considering the inherent variation in VAF measurement, it was excluded from the predictive model.

Treatment outcomes

Our cohort included 71 patients who subsequently received various treatments for cytopenia, including 28 individuals who received more than one treatment. Recognizing that cytopenia treatments are not known to alter the natural history of the disease, we included these patients in our study to avoid biases in developing prognostic models. Growth factors (n=56 [79%]) were commonly utilized as a treatment, although only 32% of patients experienced improved cytopenia, 14% had worsening cytopenia, and 7% initially responded before their cytopenia worsened. Vitamin supplementation (n=28 [39%]; response rate [RR], 31%), immunosuppressive therapy (n=17 [24%]; RR, 47%), and steroids (n=9 [13%]; RR, 29%) were also used but demonstrated only modest improvements. A subset of patients received decitabine (n=4 [6%]) or azacitidine (n=5 [7%]), with one patient experiencing a disease response in each group (Figure S11).

Discussion

We conducted a comprehensive analysis of real-world data gathered from 357 patients with clonal cytopenia across 17 academic centers. Our findings reveal a significant correlation between clonality and cytopenia, shedding light on the interplay between these factors and their impact on disease risk. Leveraging these insights, we developed the CCRS, a dedicated and refined risk stratification tool tailored for patients with CCUS. This innovative model surpasses the capabilities of existing risk stratification systems, particularly suited for academic settings given the characteristics of our cohort. We propose the integration of the CCRS into clinical practice and its incorporation into the design of future clinical trials.

Based on our CCUS-specific cohort, this streamlined CCRS model integrates only 3 parameters: PLT<100×10^9/L, having ≥2 mutations, and the presence of a splicing mutation. The CCRS model

categorizes patients into three distinct risk strata, each of which is associated with significantly different progression risks. This model was then validated using an external CCUS cohort and showed that CCRS demonstrated a robust ability to effectively stratify the population, excelling in identifying high-risk patients.

In summary, the simplified CCRS presents the potential for straightforward integration into clinical practice, aiding healthcare providers in consultations. Furthermore, as clinical trials are developed to evaluate high-risk CCUS, our model can serve as a crucial tool for identifying trial-eligible patients, filling a notable gap in the field.

Notably, VAF ≥22% appeared to signify an increased risk of progression, and integrating this VAF threshold with PLT count and mutation number ≥2 provided additional stratification of our data for LFS. However, upon validation, this combination's predictive performance did not surpass that of PLT count, mutation number, and the presence of splicing mutations. This outcome likely resulted from a considerable number of patients in the Pavia cohort having high VAF and a high number of mutations but still maintaining PLT>100×10^9/L. Additionally, because of the inherent variation in VAF measurement (e.g., measurement of VAF may be influenced by fluctuations in WBC when measured from peripheral blood), it was excluded from the final predictive model. Integrating VAF into risk-predictive models requires further validation.

Our additional objective was to further elucidate the significance of the term 'clonal cytopenia of undetermined significance'. Previous investigations have indicated that patients with an isolated *DNMT3A* mutation are less likely to experience disease progression, whereas those with splicing and MN-like mutations are more susceptible to progression;[9,10] our findings align with these observations. Notably, *SRSF2* and *U2AF1* emerged as highly mutated genes in our cohort. Further, we identified a correlation between mutational VAF and blood counts across multiple genes, as well as the predictive value of specific mutation pathways and their impact on cytopenias and outcomes. These findings underscore the value of integrating both clinical and molecular information to enhance the precision of

CCUS prognostication. In addition, HgB<10 g/dL was linked to reduced OS. Given that anemia is recognized as an independent risk factor for mortality in MN [21] and improvement in HgB is a key criterion in the MDS response criteria for treated patients,[22] our data lays the foundation for shaping future CCUS trials, particularly in refining clinical trial inclusion criteria and establishing CCUS-specific response criteria, such as hematological improvement.

Two previous studies have presented outcomes for patients with CMUS, but there are currently no available data on CCMUS.[12,13] The present study contributes to the existing knowledge by conducting an analysis of CCMUS, revealing that clinical and molecular patterns were similar between CCMUS and CMML.[23] Patients with CCMUS experienced inferior LFS and OS compared to those in the non-CCMUS group, underscoring the importance of recognizing CCMUS as a precursor entity.

We further reported the impact of extrinsic factors on clonal structure. Mutation patterns are context-dependent, with various selection pressures and microenvironments influencing clone composition and propagation. Factors such as prior cancer therapies or myelosuppressive stress play a crucial role in shaping the clonal landscape; in particular, CH arising after cancer therapy is strongly associated with mutations in DNA damage response (DDR) genes, such as *TP53* and *PPM1D*.[24–27] In our cohort, we observed distinct mutation patterns between solid tumors and hematologic diseases that were potentially influenced by age bias, as patients with solid tumors tended to be older. This aligns with a prior study of the natural history of CH, in which age was a significant factor in *TET2* clone growth and the prevalence of *TET2* mutations was higher at older ages, eventually exceeding the prevalence of *DNMT3A* mutations.[28] Though we did not identify an enrichment of DDR mutations in patients with t-CCUS, we did observe a higher prevalence of cytogenetic abnormalities (33%), which was consistent with prior findings.[29] Given the profound oncogenic potential and adverse outcomes associated with t-CCUS, early diagnosis is crucial and proactive measures are necessary.

Finally, we sought to address the distinct challenge of managing CCUS, with no current standard of care established.[7,18,30–32] In our study, the response rates to existing therapies were reported to be

modest. The pressing unmet need for effective treatment in this context underscores the need to develop innovative therapeutic strategies aimed at delaying or preventing progression and/or alleviating cytopenias in patients with CCUS.

One notable characteristic of the current cohort is that all CCUS cases were sourced from academic centers and diagnoses were confirmed through bone marrow biopsy. Given the referral patterns of academic centers, this cohort may potentially represent a high-risk population, as patients may have been referred to these centers due to severe cytopenia while seeking healthcare in the community setting. Notably, this study constitutes one of the largest CCUS cohorts to date, distinguishing it from prior studies that encompassed patients with CHIP, idiopathic cytopenia, or myeloid malignancies.[8–10] As a result, the newly developed CCRS model can effectively identify CCUS patients who are at the highest risk for disease progression. Patients identified as high risk should undergo closer monitoring and be prioritized for enrollment in clinical trials.

Our study is subject to several limitations. First, it was a retrospective analysis and subject to all related limitations. Second, while all CCUS diagnoses were confirmed through bone marrow biopsy, the absence of a central review for biopsy slides introduces a potential limitation; additionally, there may be variability in how hematopathologists evaluate morphologic dysplasia in bone marrow. Third, the lack of uniformity in sequencing platforms across institutions and the inability to confirm germline mutations in some cases are additional constraints. Fourth, our study population is solely comprised of patients receiving care at academic centers, potentially indicating a more advanced disease stage. Fifth, the relatively short follow-up duration in this study raises the possibility of lead time bias, emphasizing the need for future studies with extended follow-up periods.

Conclusion

We systemically investigated the clinical and laboratory characteristics of individuals with clonal cytopenias. A 3-parameter CCRS model was devised specifically for patients diagnosed with CCUS.

The implementation of the CCRS presents significant clinical relevance, offering precise risk stratification that can guide patient management and assist in eligibility assessment for forthcoming clinical trials, formulation of response criteria, and furthering research to address the pressing unmet need for novel therapeutics to treat CCUS.

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Data availability: For original data, please contact Dr. Zhuoer Xie, email: zhuoer.xie@Moffitt.org

Author contributions: ZX and AA designed the study, contributed cases, and wrote the manuscript. ZX, AS, and SG performed the statistical analysis. CE, JF, AG, SP, and LM provided the independent external cohort to validate our study model. All other authors contributed to the data collection, reviewed, and provided edits to subsequent versions of the manuscript.

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Conflicts of Interest:

Komrokji: *BMS:* Honoraria, Membership on an entity's Board of Directors or advisory committees; *CTI biopharma:* Honoraria, Membership on an entity's Board of Directors or advisory committees, Speakers Bureau; *Servio:* Honoraria, Membership on an entity's Board of Directors or advisory committees, Speakers Bureau; *PharmaEssentia:* Honoraria, Other, Speakers Bureau; *Novartis:* Honoraria, Membership on an entity's Board of Directors or advisory committees; *Abbvie:* Honoraria, Membership on an entity's Board of Directors or advisory committees; *Geron:* Honoraria, Membership on an entity's Board of Directors or advisory committees; *Taiho:* Honoraria, Membership on an entity's Board of Directors or advisory committees; *Jazz:* Honoraria, Membership on an entity's Board of Directors or advisory committees, Speakers Bureau.

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Prior Publications:

Part of the data was presented in poster form at the American Society of Hematology Annual Conferences 2021 and 2022.

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Table 1. Baseline characteristics for the entire cohort.

Characteristic	Entire group (n = 357)
Age, y, median (range)	70 (19-94)
Sex, no. (%)	
Female	126 (36)
Male	231 (63.9)
Body mass index, median (range)	26.9 (16.8-60.2)
Smoking history, no. (%)	
Current	18 (5.0)
Former	142 (39.8)
Never	173 (48.5)
Unknown	24 (6.7)
ECOG performance score, no. (%)	
0	120 (33.6)
1	156 (43.8)
2	30 (8.4)
3	3 (0.8)
Missing	48 (13.5)
Lab, median (IQR)	
Hemoglobin, g/dl, median (IQR)	11 (9.4-12.7)
Patients with hemoglobin <10 g/dl; no. (%)	120 (33.6)
Mean Corpuscular Volume, fL, median (range)	95.4 (90-102.4)
Platelets, $\times 10^9$/L, median (IQR)	121 (78-198)
Patients with platelets <100×10^9/L, no. (%)	133 (37.3)
White blood cells, $\times 10^9$/L, median (IQR)	3.60 (2.60-5.54)
Absolute neutrophil count, $\times 10^9$/L, median (IQR)	1.91 (1.10-3.30)
Patients with absolute neutrophil count <1×10^9/L, no. (%)	25 (7.0)

Absolute monocyte count, $\times 10^9$/L, median (IQR)	0.4 (0.28-0.57)
Patients with CCMUS, no. (%)	97 (27.2)
Total no. of mutational variants	592
Variant allele fraction, median (range)	31.7% (3%-99.7%)
Mutations per patient, no. (%)	
0	39 (10.9)
1	156 (43.7)
2	86 (24.1)
>2	76 (21.3)
Median number of mutations	1
Patients with cytogenetic abnormalities, no. (%)	79 (22.1)

Abbreviations: CCMUS, clonal cytopenia and monocytosis of undetermined significance; ECOG, Eastern Cooperative Oncology Group.

1

Figure Legends

Figure 1. Mutational spectrum for patients with clonal cytopenia.

Figure 2. Leukemia-free survival (N = 357). LFS is stratified by **A.** CCMUS; **B.** platelets < vs ≥ $100X10^9$/L; **C**. having <2 vs. ≥2 mutations; **D.** having a splicing pathway mutation; **E.** Multivariable analysis including the variables of platelets < vs ≥ $100X10^9$/L and < vs. ≥2 mutations (solid lines indicate having ≥2 mutations, dotted lines indicate MT<2, blue indicates platelets ≥$100X10^9$/L, and red indicates platelets<$100X10^9$/L); and **F.** multivariable analysis including the variables of platelet count, having ≥2 mutations, and having a splicing pathway mutation (dotted lines indicate not having splicing mutations, solid lines indicate having splicing mutation, blue indicate platelets <$100X10^9$/L and MT<2, green indicate PLT< $100X10^9$/L and MT≥2, yellow indicate PLT≥$100X10^9$/L and MT<2, and red indicate platelets ≥$100X10^9$/L and MT≥2). **Abbreviations**: CCMUS, clonal monocytosis of undetermined significance; MT, mutation; PLT, platelets.

Figure 3. Overall survival (N = 357). OS is stratified by **A.** CCMUS; **B.** HgB< vs ≥10 g/dL; **C.** having ≥2 mutations; **D.** having a signaling pathway mutation; **E.** multivariable analysis including the variables of HgB <10 g/dL and having a signaling pathway mutation (solid lines indicate having a signaling pathway mutation, dotted lines indicate not having a signaling pathway mutation, blue indicates HgB ≥10 g/dL, and red indicates HgB <10 g/dL); **F.** multivariable analysis including the variables of HgB <10 g/dL and ≥2 mutations (solid lines indicate having ≥2 mutations, dotted lines indicate having <2 mutations, blue indicates HgB ≥10 g/dL, and red indicates HgB <10 g/dL); **Abbreviations**: CCMUS, clonal cytopenia and monocytosis of undetermined significance; HgB, hemoglobin.

Figure 4. Variant allele fraction cut off 22% predicts (A) LFS but not (B) OS. **Abbreviations**: LFS, progression-free survival; OS, overall survival; VAF, variant allele fraction.

Figure 5. Clonal cytopenia scoring system (CCRS). Prognostic models for **A.** multivariate analysis parameters and assigned score for leukemia-free survival; **B.** The 2-year cumulative incidence of

myeloid neoplasm progression based on CCRS: 6.4% (95% CI: 3-11.4) for low-risk, 14.1% (7.9-22.2) for intermediate-risk, and 37.2% (19.8-54.7) for high-risk groups by Gray's test. **C.** The number of patients within each category, cumulative incidence for leukemia progression, and 2-year cumulative incidence. **Abbreviations**: PLT: platelet.

Figure 6. The CCRS model significantly stratified leukemia-free survival in the Pavia cohort (p=0.005). Using low-risk group as a reference group, the hazard ratio (HR) for intermediate vs low risk is: HR, 1.6, [95% CI, 0.55-4.62]; *P* =.39 and high vs low risk is: HR, 3.57, [95% CI, 1.56-8.18]; *P* =.003)

Figure 1

Figure 1. Mutational spectrum for patients with clonal cytopenia.

Figure 2

Figure 2. Leukemia-free survival (N = 357). LFS is stratified by **A.** CCMUS; **B.** platelets < vs ≥ 100X10^9/L; **C**. having <2 vs. ≥2 mutations; **D.** having a splicing pathway mutation; **E.** Multivariable analysis including the variables of platelets < vs ≥ 100X10^9/L and < vs. ≥2 mutations (solid lines indicate having ≥2 mutations, dotted lines indicate MT<2, blue indicates platelets ≥100X10^9/L, and red indicates platelets<100X10^9/L); and **F.** multivariable analysis including the variables of platelet count, having ≥2 mutations, and having a splicing pathway mutation (dotted lines indicate not having splicing mutations, solid lines indicate having splicing mutation, blue indicate platelets <100X10^9/L and MT<2, green indicate PLT< 100X10^9/L and MT≥2, yellow indicate PLT≥100X10^9/L and MT<2, and red indicate platelets ≥100X10^9/L and MT≥2). **Abbreviations**: CCMUS, clonal monocytosis of undetermined significance; MT, mutation; PLT, platelets.

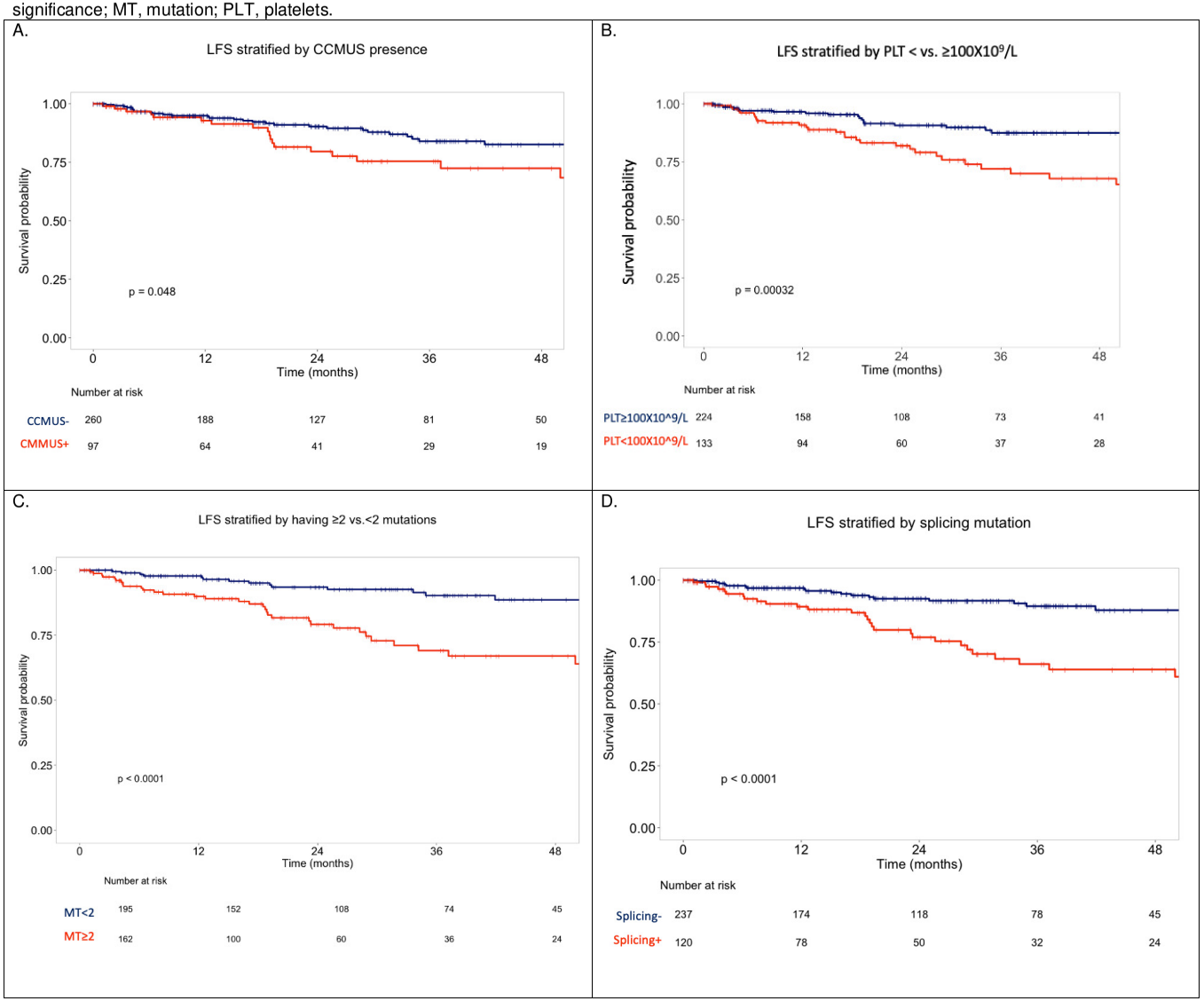


Figure 2

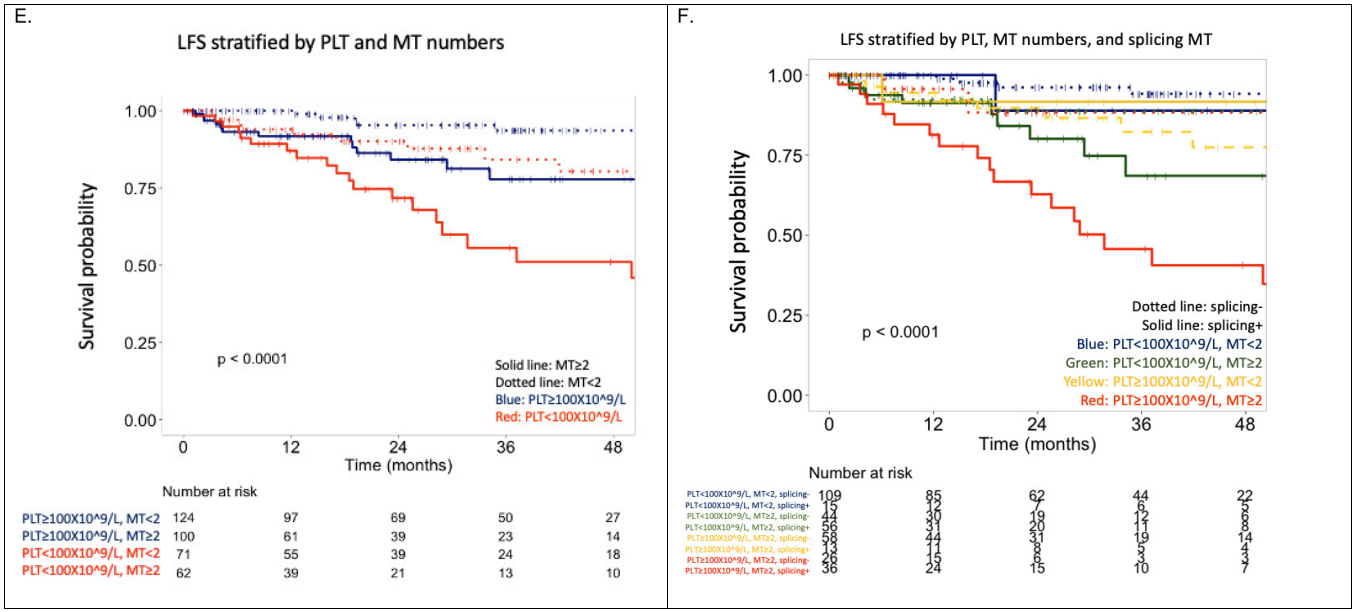


Figure 3

Figure 3. Overall survival (N = 357). OS is stratified by **A.** CCMUS; **B.** HgB< vs ≥10 g/dL; **C.** having ≥2 mutations; **D.** having a signaling pathway mutation; **E.** multivariable analysis including the variables of HgB <10 g/dL and having a signaling pathway mutation (solid lines indicate having a signaling pathway mutation, dotted lines indicate not having a signaling pathway mutation, blue indicates HgB ≥10 g/dL, and red indicates HgB <10 g/dL); **F.** multivariable analysis including the variables of HgB <10 g/dL and ≥2 mutations (solid lines indicate having ≥2 mutations, dotted lines indicate having <2 mutations, blue indicates HgB ≥10 g/dL, and red indicates HgB <10 g/dL); **Abbreviations**: CCMUS, clonal cytopenia and monocytosis of undetermined significance; HgB, hemoglobin.

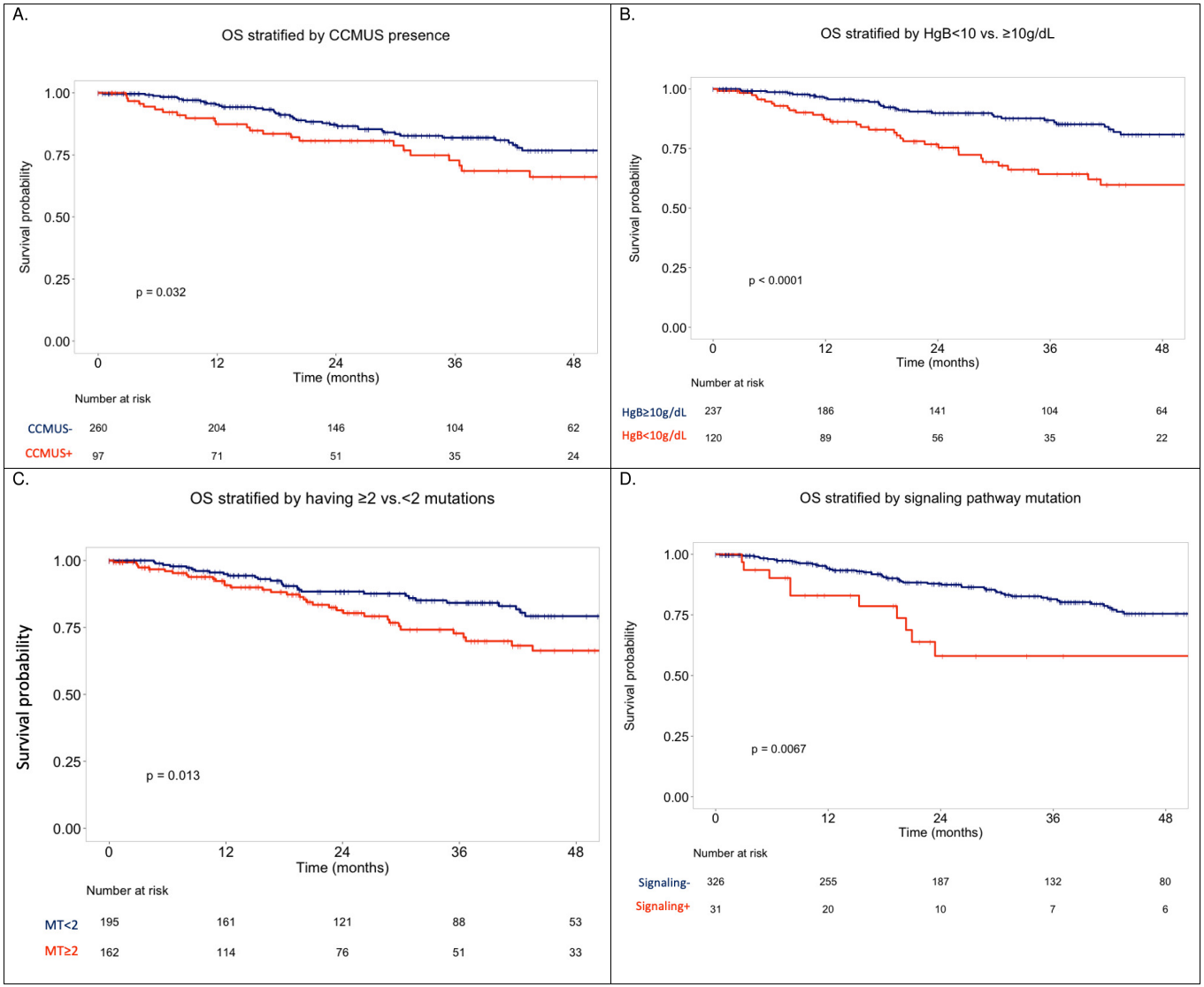
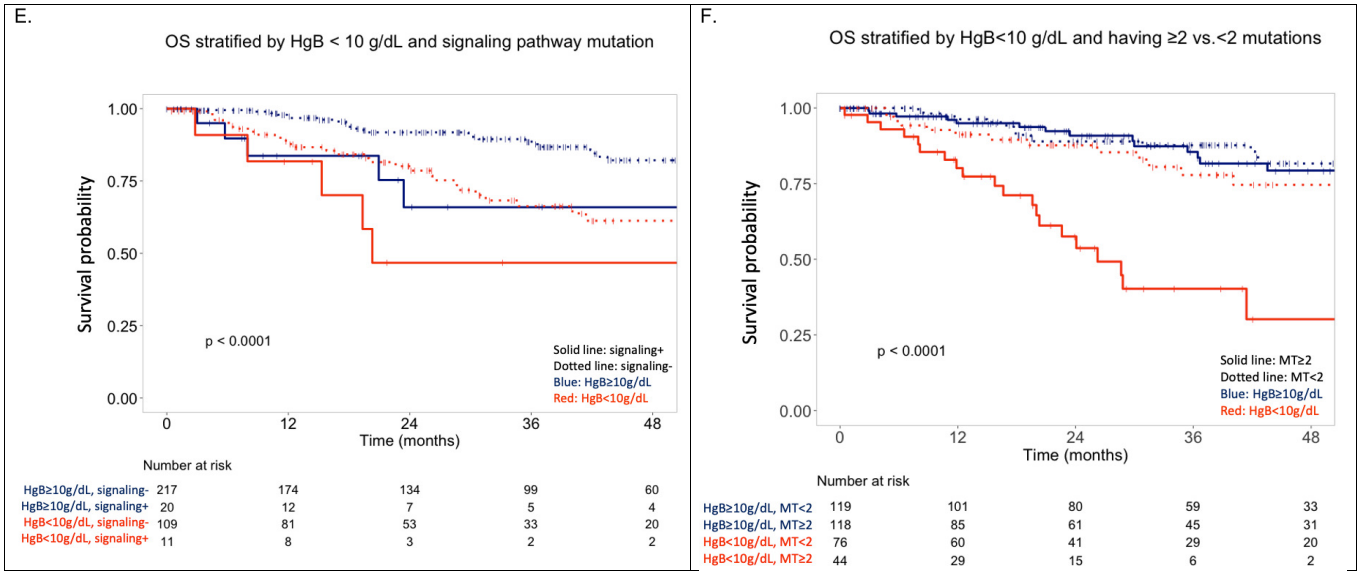


Figure 3



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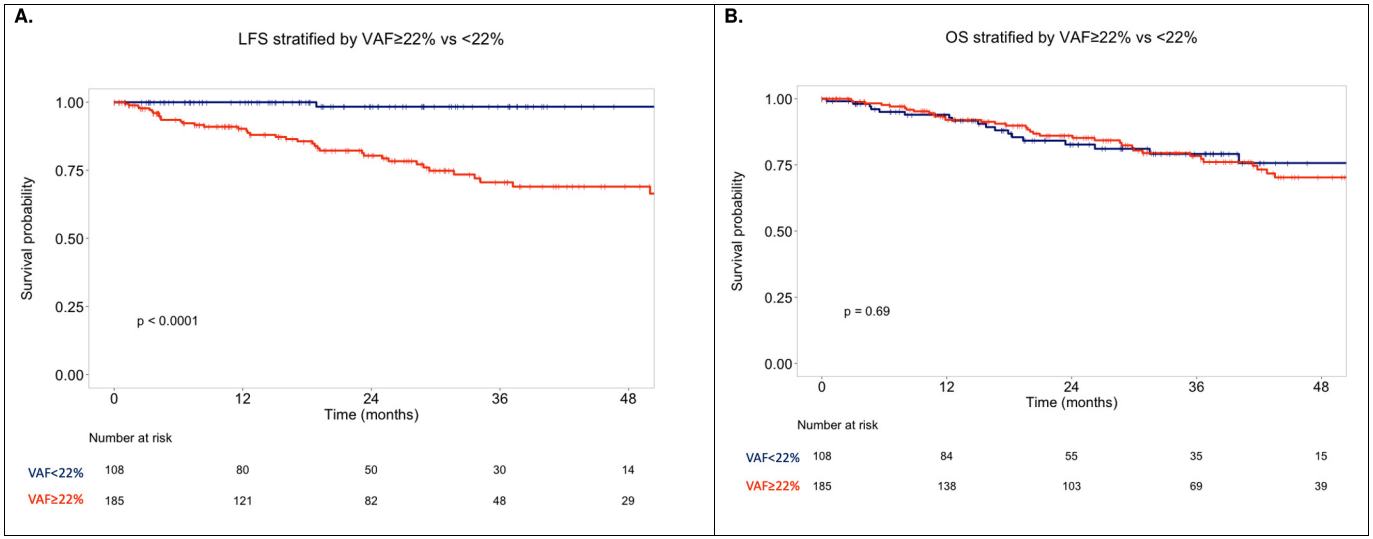

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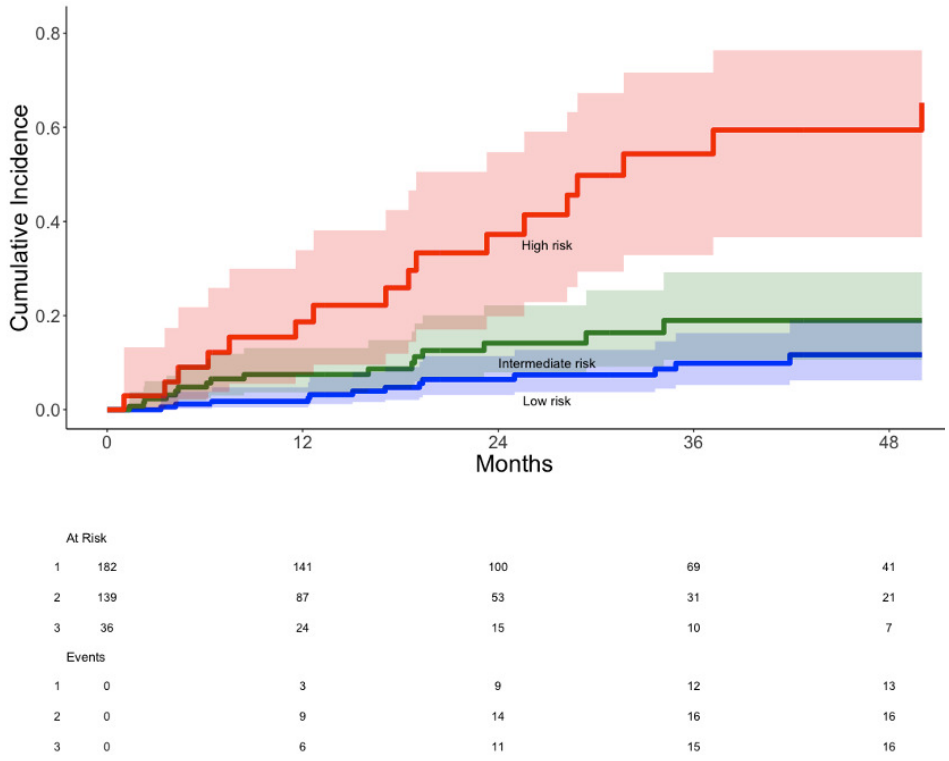
A.

Adverse factor	HR (95% CI)	P value	Assigned score
Splicing mutations	2.13 (1.10-4.10)	0.02	2
PLT<100X10^9/L	2.49 (1.38-4.50)	0.003	2.5
Mutation number≥2	2.57 (1.28-5.15)	0.008	3

B.

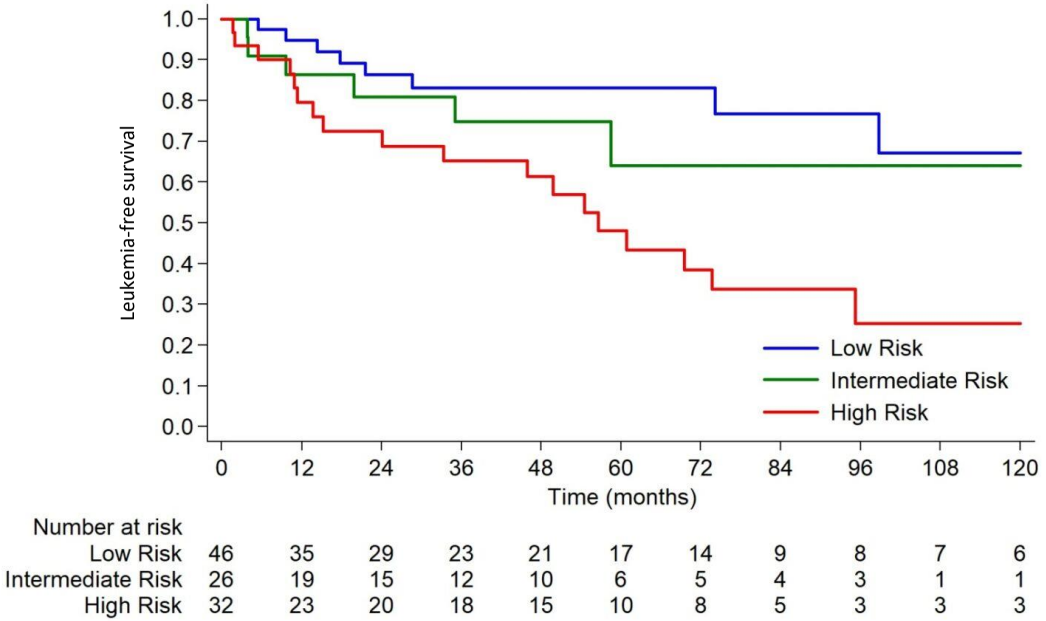


C.

Assigned score	Total (%)	Cumulative events	2-year Cumulative incidence (%, 95% CI)
High risk (≥5)	36	18	37.2% (19.8-54.7)
Intermediate risk (2.5-<5)	139	16	14.1% (7.9-22.2)
Low risk (<2.5)	182	13	6.4% (3-11.4)

Figure 6

Figure 6. The CCRS model significantly stratified leukemia-free survival in the Pavia cohort (p=0.005). Using low-risk group as a reference group, the hazard ratio (HR) for intermediate vs low risk is: HR, 1.6, [95% CI, 0.55-4.62]; *P* =.39 and high vs low risk is: HR, 3.57, [95% CI, 1.56-8.18]; *P* =.003)



Number at risk											
Low Risk	46	35	29	23	21	17	14	9	8	7	6
Intermediate Risk	26	19	15	12	10	6	5	4	3	1	1
High Risk	32	23	20	18	15	10	8	5	3	3	3