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Clonal Hematopoiesis and Inflammation in the VasculaturE (CHIVE): a prospective, longitudinal cohort and biorepository

Tracking no: ADV-2023-011510R2

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Abstract:
Clonal hematopoiesis (CH) is an age-associated phenomenon leading to increased risk of both hematologic malignancy and non-malignant organ dysfunction. Increasingly available genetic testing has made incidental discovery of CH clinically common, yet evidence-based guidelines and effective management strategies to prevent adverse CH health outcomes are lacking. To address this gap, the prospective CHIVE registry and biorepository was created to identify and monitor individuals at risk, support multidisciplinary CH clinics, and to refine standards of practice for CH risk mitigation. Data from the first 181 patients enrolled in this registry recapitulate the molecular epidemiology of CH from biobank scale retrospective studies, with DNMT3A, TET2, ASXL1, and TP53 as the most commonly mutated genes. CH patients had higher rates of end organ dysfunction, in particular chronic kidney disease (p=0.001). Among patients with CH, variant allele frequency was independently associated with presence of cytopenias (p=0.008) and progression to hematologic malignancy (p=0.010), while other common high-risk CH clone features were not clear. Notably, accumulation of multiple distinct high-risk clone features was also associated with cytopenias (p=0.013) and hematologic malignancy progression (p=0.004), supporting a recently published CH risk score. Surprisingly, ~30% of patients enrolled in CHIVE from CH clinics were adjudicated as not having CHIP, highlighting the need for molecular standards and purpose-built assays in this field. Maintenance of this well-annotated cohort and continued expansion of CHIVE to multiple institutions is underway and will be critical to understand how to thoughtfully care for this patient population.

Conflict of interest: COI declared - see note

COI notes: All unrelated to the present work: M.R.S. reports personal fees from AbbVie, BMS, CTI, Sierra Oncology, Novartis, grants from Astex and Incyte, personal fees and other support from Karyopharm, Ryvu, personal fees from Sierra Oncology, grants and personal fees from Takeda, and TG Therapeutics outside the submitted work. JM has served on advisory boards for Bristol Myers Squibb, AstraZeneca, Myovant, Cytokinetics, Takeda, BeiGene, Kiniksa, Kurome Therapeutics, BitterRoot Bio, Deciphera, Regeneron, Repare Therapeutics, Antev, Daiichi Sankyo, Prelude Therapeutics and Voyager Therapeutics. P.B.F. reports grants from Incyte. A.G.B. is a scientific co-founder and has equity in TenSixteen Bio. All other authors declare that they have no competing interests.

Preprint server: No;

Author contributions and disclosures: M.L.S., J.B.H., and S.O. Collected data, analyzed data, drafted the manuscript. A.D., Z.C., C.S., A.J.S., D.B., T.S., M.B., K.P., A.K., S.S.S., and J.U. Collected data, recruited patients, and revised the manuscript. C.V. Supplied essential analysis of CHIP genes and revised the manuscript. Y.X., M.B., J.M., E.J., P.B.F., D.S., A.G.B., and M.R.S. Conceived of the study, oversaw the development of the study, and revised the manuscript.

Non-author contributions and disclosures: No;

Agreement to Share Publication-Related Data and Data Sharing Statement: The authors will make available the de-identified dataset upon publication

Clinical trial registration information (if any):

Title: *Clonal Hematopoiesis and Inflammation in the VasculaturE* (*CHIVE*): a prospective, longitudinal clonal hematopoiesis cohort and biorepository

Running Title: CHIVE Cohort

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The authors will make available the de-identified dataset upon publication

Abstract Word Count 233/250

Text Word Count 4000/4000

Key Points:

- Development of a genotyped and phenotyped cohort of CH patients is required to establish clinical guidelines and translational research.
- Initial data from a prospective registry and biorepository of patients with CH recapitulates findings derived from retrospective studies.

Abstract:

Clonal hematopoiesis (CH) is an age-associated phenomenon leading to increased risk of both hematologic malignancy and non-malignant organ dysfunction. Increasingly available genetic testing has made incidental discovery of CH clinically common, yet evidence-based guidelines and effective management strategies to prevent adverse CH health outcomes are lacking. To address this gap, the prospective *CHIVE* registry and biorepository was created to identify and monitor individuals at risk, support multidisciplinary CH clinics, and to refine taxonomy and standards of practice for CH risk mitigation. Data from the first 181 patients enrolled in this prospective registry recapitulate the molecular epidemiology of CH from biobank scale retrospective studies, with *DNMT3A*, *TET2*, *ASXL1,* and *TP53* as the most commonly mutated genes. Blood counts across all hematopoietic lineages trended lower in CH patients. Additionally, CH patients had higher rates of end organ dysfunction, in particular chronic kidney disease (p=0.001). Among patients with CH, variant allele frequency was independently associated with presence of cytopenias (p=0.008) and progression to hematologic malignancy (p=0.010), while other common high-risk CH clone features were not clear. Notably, accumulation of multiple distinct high-risk clone features was also associated with cytopenias (p=0.013) and hematologic malignancy progression (p=0.004), supporting a recently published CH risk score. Surprisingly, ~30% of patients enrolled in CHIVE from CH clinics were adjudicated as not having CHIP, highlighting the need for molecular standards and purpose-built assays in this field. Maintenance of this well-annotated cohort and continued expansion of *CHIVE* to multiple institutions is underway and will be critical to understand how to thoughtfully care for this patient population.

Introduction:

Clonal hematopoiesis (CH) is an over-representation of mature blood cells derived from a single, genetically identical clone.[1] CH is highly correlated with increasing age, with 15% of patients over the age of 65 estimated to have CH with a variant allele fraction (VAF) of at least 2%.[1–4] CH is genetically heterogeneous, with most cases resulting from somatically-derived mutations in leukemogenic driver genes within hematopoietic stem and progenitor cells (HSPCs).[3] Variants have been reported from 72 CH driver genes, though more than two-thirds of CH mutations are found in one of three genes: *DNMT3A*, *TET2*, and *ASXL1* ('DTA' mutations).[3,5,6] While CH-associated genes span a diverse set of cellular functions and processes, including epigenetic regulation, transcription, and RNA splicing,[7,8] the resulting effect of a CH driver mutation is enhanced cellular fitness leading to a selective advantage for the clone and subsequent clonal expansion.[1,8,9]

CH presents as clonal hematopoiesis of indeterminate potential (CHIP), an asymptomatic state with normal complete blood counts (CBC). Clonal cytopenia of uncertain significance (CCUS) connotes the presence of a clone and one or more associated cytopenias without a clear identifiable cause and a bone marrow biopsy lacking morphologic myelodysplasia.[10] Numerous studies have demonstrated that CHIP and CCUS both increase potential to progress to hematologic malignancy.[1,8] Thus, CH is considered a premalignant state, and it is estimated that 0.5-1% of CHIP cases transform into an overt hematologic malignancy per year after acquiring additional somatic mutations.[9] By definition, CCUS includes a hematologic phenotype and thus can be more pervasive in patients with multiple mutations, high VAFs, and/or those with non-DTA, myeloid-neoplasm type clones.[11] The concept of high versus low-risk of progression to myeloid neoplasm has been recently evaluated and a risk score calculator developed for the evaluation individuals for CH, though this has not been prospectively validated[12].

In addition to malignancy risk, CH is associated with a high burden of organ dysfunction, and confers a 40% increase in all-cause mortality.[2] Recent reports of CH-associated organ dysfunction include increased risk of stroke and atherosclerotic vascular disease (ASCVD),[13–16] inflammation and autoimmune disease,[17–19] chronic obstructive pulmonary disease,[20] infection,[21] and chronic kidney

disease,[22–24], among others.[25] Moreover, the mechanisms driving CH-associated adverse outcomes, in particular ASCVD, have recently been the subject of intense study.[13,14,16,26] Given the diversity of genes involved in CH, myriad inflammatory and non-inflammatory mechanisms likely exist for all downstream pathologies. As such, the prevailing immune dysregulation hypothesis as it currently exists does not completely reflect the complexity of CH across disease manifestations, and prospective assessment of collected patient samples and variant-specific mutational changes are needed.

Despite ample evidence supporting the association of CH with malignancy risk and end organ dysfunction, clinical guidelines for the identification, management, and surveillance of patients with CH are lacking, and treatment strategies for CH are nascent. Likewise, while recent progress has been made in our mechanistic understanding of CH progression and association with adverse outcomes, our clinical comprehension of factors mediating CH expansion and risk of progression remains poorly understood. This is due, in part, to the retrospective nature of available data and paucity of serial evaluation of CH-related clones. Because CH represents a broad array of genes and clinical outcomes, research infrastructure must be built to study patients in a prospective manner to understand the mechanisms of progression at the level of the individual mutation in variable germline contexts. Large-scale prospective cohorts will allow for the resolution needed to not only codify the natural history of CH but also give insight to possible therapeutic approaches. Ultimately, the development of clinical guidelines to risk-stratify and treat patients with CH is needed to advance healthcare for a significant proportion of our aging population. To begin to address these challenges, we developed *CHIVE (Clonal Hematopoiesis and Inflammation in the VasculaturE)*, a collaborative registry and biorepository, and here, we describe a novel approach to identification and enrollment of patients at risk for CH. We collate an early evaluation of clinical outcomes in CH patients compared to those considered at risk for CH demonstrating the importance of development and maintenance of multidisciplinary, prospective CH clinics and biorepositories.

Methods

Identification and Inclusion of Study Participants

Prospective *CHIVE* participants were identified by outpatient providers across Vanderbilt University Medical Center (VUMC). Patients were eligible for study inclusion if they were either over 40 years age or over 18 years of age with a known risk factor for CH, could give informed consent, and did not have an active hematologic malignancy (**Figure 1**). Our existing CH Clinic (established in 2018) includes patients with idiopathic cytopenia of uncertain significance (ICUS), CCUS, or CH as noted on clinical next generation sequencing (NGS): these patients were included in **Arm A** of *CHIVE* (n=57). By contrast, we also sought out a population *at risk* for CH which liberally included patients over 40 years of age, or 18 years of age with a history of solid tumor, cardiovascular disease, renal disease, rheumatologic disease, or diabetes: **Arm B** (n=122). To identify patients at risk, we collaborated with specialists in hematology/oncology, cardiology, rheumatology, and genetic medicine, given the known associations between CH and cardiovascular disease,[13,14,16] autoimmune disease,[17,19] history of chemotherapy/radiation treatment,[27,28] and common hereditary cancer-related mutations,[29] our initial collaborations were between these subspecialists.

Patients in Arm A were actively and voluntarily enrolled in CHIVE directly from CH Clinic. For those patients in Arm B, enrollment occurred via initial collaborators as noted above, or from direct patient interest in participation in research studies. In the case of the latter, our institution offers patients with interest in research studies to be contacted when new opportunities arise, so, eligible patients with interest in research studies were considered for *CHIVE* if deemed eligible via review of the electronic medical record (EMR). This subset of patients with upcoming appointments at the hospital system were contacted via telephone, introduced to the study, and if meeting criteria, offered the opportunity to voluntarily participate in *CHIVE*.

Once enrolled, the research study was integrated with their regularly scheduled clinical care for all patients. Consensus guidelines are lacking regarding clinical and laboratory monitoring of patients with CH, though experts agree that CH patients with high-risk features (multiple mutations, high VAF, high-risk mutations) should be monitored with greater frequency.[12,30] Accordingly, follow-up samples were collected at 6- to 12-month intervals based on a rubric created by clinical experts in CH (**Supplementary Figure 1**).

Samples were collected from “high-risk” patients every six months, while collection was extended to twelve months for those without CH or with “low-risk” CH. This study was reviewed and approved by the Vanderbilt Institutional Review Board, and enrollment is ongoing.

Development of a Biorepository

After participants gave informed consent, clinical data available from standard clinical care was collected, including demographic data, medical histories, vital signs, laboratory studies, imaging studies, data from bone marrow biopsies, and clinically ordered genotyping. Genetic data included germline testing, clinical next generation sequencing mutation analyses, and chromosome analyses. Additional cardiovascular studies including electrocardiogram data, and echocardiogram data were collected when available. Upon study enrollment, initial research samples from peripheral blood draws were obtained at the time of planned, routine, clinical sample collection. Similarly, aliquots from bone marrow aspirate and biopsies were procured when available.

Blood samples collected at 6- to 12-month intervals were stored within the *CHIVE* biorepository, a dedicated space with capacity to store peripheral blood and bone marrow samples. Specimens were maintained in a liquid nitrogen freezer with a password protected lock, which was housed in a dedicated laboratory space under supervision. Specimens were assigned a de-identified participant number for archiving to prevent subject identification; patient identifiers were separately stored in a REDcap database, accessible only with secure ID and password.

CH Status Ascertainment

A low-cost custom oligonucleotide gene capture panel (Twist Bioscience) covering 95% of all CH gene mutations (**Supplementary Table 1**) was used to perform targeted sequencing of prospective *CHIVE* participants as previously described[31]. Briefly, DNA was extracted from whole blood using Qiagen Mini kits (Cat #27104) according to manufacturer's recommendations. DNA library preparation was performed using a hybrid capture system to selectively amplify genes of interest (Twist Bioscience) prior to sequencing on an Illumina Novaseq 6000 targeting 1000x coverage. Mutect2, a publicly available somatic

variant caller in the Genome Analysis Toolkit (GATK), was used to detect CH variants within the aligned sequencing data using the Tera biocomputing platform (http://Terra.bio).[32] A putative variant list was created and filtered. Variants with total low read depth (<100), low variant allele read depth (<3), or variant allele fraction below the threshold for CHIP (<2%) were removed from the dataset. Manual filtering was performed to remove known sequencing artifacts including variants that appear recurrently in multiple samples and/or across multiple projects but that are not known CHIP hotspots[2,6,33]. We additionally sought to distinguish germline from somatically occurring variants. We performed a binomial test to determine whether the measured allele depth for the variant is statistically different from half of the total allele depth at that site. We removed any variants that were not significant at $p<0.05$. We refer to this sequencing assay henceforth as 'CHIVEseq'.

For analysis, patients from Arm A or Arm B were included in the CH-positive (CH+) group if they met criteria for CH via the CHIVEseq assay at any time during their enrollment in the study (i.e., on initial or follow-up sequencing). Patients with confirmed CH mutations both with cytopenias (CCUS) and without cytopenias (CHIP) were included in the CH+ group. All consented, genotyped patients who were not found to have CH by the CHIVEseq assay at any point in time were included in the CH-negative (CH-) group. Notably, these CH- patients were not true controls because all patients enrolled in *CHIVE* were at higher risk for CH than the general population based on clinical characteristics.

For any cases in which CH status determined via CHIVEseq assay differed from that determined via clinical genotyping, the discrepancy was rectified, and CH status was designated via manual evaluation of genotyping results. Common reasons for such discrepancies included misidentification of a germline variant as CH, VAF below 2%, and mutations found via clinical sequencing were in genetic regions not covered by the CHIVEseq assay. In the latter case these variants were reviewed and classified as CH if they otherwise met criteria used for CHIP determination in whole genome- or exome-based methods[33]

Clinical Management

All patients followed in CH Clinic (Arm A) were referred to collaborating cardio-oncology for individualized risk assessment for cardiovascular disease. Patients in Arm A had blood pressure measurement, physical exam, and basic laboratory testing including CBC with differential, complete metabolic panel (CMP), and inflammatory markers. For patients in Arm B, who were not followed in CH clinic, laboratory and imaging data were recorded and maintained in the *CHIVE* database as available from routine clinical care.

Care for CH patients demands a multidisciplinary approach as input from investigators across clinical specialties enriches patient care. Our CH clinic, like most early CH clinics, emanated in hematology, but as experience grows, it is important to assure that patients with CH or at risk for CH can be initially managed at various points of entry. At our institution, patients can enter *CHIVE* from throughout the medical system, and providers across specialties can initiate evaluation and enroll patients.

Baseline clinical characteristics and laboratory results collected during routine care were extracted from the EMR and organized according to CH status. Laboratories were analyzed if collected within six months of the most recently sequenced sample. If multiple laboratories were collected within this six-month range, the lab values collected nearest in time to the sequencing sample were used for analysis. Laboratory results from hemoglobin A1c (HbA1c) and brain natriuretic peptide (BNP) were analyzed if collected as part of routine care within one year of the most recently sequenced sample. Resting ejection fraction (EF) measurements were collected from a variety of tests of cardiovascular function, including echocardiograms, cardiac magnetic resonance imaging, myocardial perfusion imaging, and left heart catheterization.

Statistical Analysis

Chi-squared tests were performed to determine statistical significance of categorical variables, and two sample t-tests were used to compare the means of continuous variables. If the assumptions of these parametric tests were violated, the nonparametric alternatives were used. To account for possible confounders, a multivariable regression was performed for each statistically significant outcome to assess independent contribution from age, sex, and body mass index (BMI), in addition to CH status.

The largest VAF detected per individual with a CH variant was tested for an association with age using linear regression analysis and Pearson's correlation coefficient. Clonal hematopoiesis risk score were derived from initial blood count and sequencing information to categorize patient prognostic risk as high intermediate or low. A fisher's exact test was used to determine if there was a statistically significant correlation with risk categorization. All analyses were performed using R statistical software (v4.1.1) with a statistical significance alpha value defined as 0.05. For visualization of mutational landscape among CH patients, an UpSet plot was generated using the UpSetR package.[34,35]

Results

Study Population Characteristics

From October 2020 to April 2023, 261 patients were approached for consent to enroll in *CHIVE*. 34 patients did not meet inclusion criteria, 47 declined consent, and one patient voluntarily withdrew from the study. Therefore, genotyping results were obtained from 336 samples from 181 total patients: 99 patients were determined to have at least one CH mutation (CH+) and 82 patients had no CH mutations (CH-; **Figure 1**). Two hundred and forty-six samples were serial samples collected from 89 patients at regular intervals as dictated by the testing rubric (**Supplementary Figure 1**). Of the 181 patients included in the study, 63 were recruited from hematology, 69 from cardiology, 45 from genetics, and 4 from rheumatology (**Supplementary Table 2**).

Males comprised 50.5% of the CH+ patients and 29.3% of the CH- patients ($p = 0.006$) (**Table 1**). Median age of CH+ patients was 71.91 years old and CH- patients is 62.90 ($p < 0.001$), and 74.2% of patients in the CH+ group were ≥ 65 years old compared to 46.3% in the CH- group. The median BMI was similar between groups ($p = 0.256$).

CH Mutation Analysis

The most commonly mutated genes were *DNMT3A* (n=44), *TET2* (n=40), ASXL1 (n=10), and *TP53* (n=10; **Figure 2**). Other genes found to be mutated in this population were *SRSF2, IDH2, SF3B1, PPM1D, JAK2, ASXL2*, *BRCC3, GNB1*, and *GNAS*. The majority of CH+ patients (n=99), had single

mutations in either *DNMT3A* (n = 29) or *TET2* (n=21). Twenty-eight of the 99 total CH patients (28.9%) had multiple mutations (**Figure 2**). VAF ranged from 2.1 – 79.8%. The highest VAF for any gene for each CH+ patient was plotted against age at genotyping revealing a nominal positive correlation between age and VAF (Pearson's correlation coefficient r = 0.18, p=0.079, **Supplementary Figure 2**). Bone marrow aspirates were collected during routine care for 15 patients. Of these, 8 had concordant mutations with similar overall VAFs between bone marrow and peripheral blood. Five samples lacked mutations in either the bone marrow or the peripheral blood. There was sample with a mutation in the peripheral blood with no corresponding mutation in the BM and similarly, one mutation found in a bone marrow aspirate without concordant findings in the peripheral blood. Putative germline variants were also able to be identified within our CHIVE-seq assay. Thirteen patients harbored 17 mutations in CH genes in germline distribution. None were known pathologic variants (**Supplementary Table 3**).

Of note, 57 patients recruited to *CHIVE* were included in Arm A and were suspected to have a CH mutation based on a clinical sequencing panel. However, only 41 of these patients were found to have CH using the CHIVEseq assay. We investigated the 16 discrepant results and identified that all variants reported on in clinical panels did not meet conventional CH criteria (**Table 2**) Most of these (n=12 patients, n=16 variants) were missense variants in CH genes at non-hotspot sites present at a VAF of roughly 50% and failing the binomial test, consistent with germline variants. Two patients had a missense mutation in *CUX1*, a gene not included on the CHIVEseq assay. One individual had a *JAK2* V617F variant at 0.2% VAF, which was below the current 2% VAF threshold for CH diagnosis. One individual had a *SF3B1* K700E variant at a low VAF (3.1%) via clinical sequencing that was not subsequently identified via CHIVEseq, even at VAF below 2%. The same patient had a *DNMT3A* L888Q variant, which does not meet conventional criteria for CH.[13,33] Finally, one patient had a *ASXL1* G646Wfs*12 variant in a homopolymer region: by CHIVEseq, variants within this region at a VAF below 10% are considered artifactual.[33] This patient's *ASXL1* G646Wfs*12 VAF was 6% and therefore not considered to represent CH.

Clinical Findings

The median white blood cell (WBC) count, hemoglobin (Hgb), hematocrit (Hct), and platelets (Plts), and the depth of cytopenias were not statistically significance between CH- patients and CH+ patients (**Table 3**). Creatinine values were significantly elevated in CH+ patients compared to CH- patients (0.98 vs. 0.90, p=0.015; **Table 3**), however multivariable regression analyses revealed that these differences were independently associated with patient BMI but not with CH status (**Supplementary Figure 3**). Finally, 40 of 99 CH+ patients (41.2%) met criteria for chronic kidney disease (CKD) compared to 15 of 82 controls (18.3%) (p=0.002; **Table 3**). In this case, multivariable regression analyses showed these differences were independently associated with both patient age (p=0.012) and CH status (p=0.039; **Supplementary Figure 3**).

Significantly more CH+ patients had a history of coronary artery disease (CAD) compared to CH- patients (55.7% vs. 32.9%, p=0.004). Similarly, CH+ patients in this cohort had increased rates of hypertension diagnosis 79.4% vs. 52.4%, p<0.001) and heart failure diagnosis (22.7% vs. 9.8%, p=0.035) compared to the CH- group (**Table 4**). However, multivariable regression revealed that these differences were not independently associated with CH status in this small cohort.

Overall non-hematologic cancers were prevalent in CHIVE patients due to recruitment from a hereditary cancer clinic. Among CH patients, 30 (30.9%) had other forms of malignancy while non-CH patients totaled 37 (45.1%). Breast cancer was the most frequent non-hematologic cancer in both subgroups **(Supplementary Data Table 4)**.

Patient Outcomes

Of the 99 CH+ patients, six (4.7%) progressed to frank hematologic malignancy over the course of the 2.5-year study. Two of these 6 patients (33%) harbored CH mutations in two or more genes, 4 (67%) had mutations in genes considered to be high risk, and 5 (83.3%) had one or more variants with a VAF greater than 10% (**Table 5**). The average VAF among these eight patients was 35.7% compared to 10.8% in those CH+ patients who have not progressed to malignancy. Interestingly, when also accounting for age, sex, and BMI, CH VAF was independently and significantly associated with a diagnosis of CCUS

(p=0.004) and with progression to hematologic malignancy (p=0.009), while other high-risk clone features, presence of multiple mutations and presence of mutations in high-risk genes, were not independently associated with either CCUS or malignancy progression in this initial cohort. However, the total number of high-risk clone features present was significantly associated with both diagnosis of CCUS (p = 0.009) and progression to hematologic malignancy (p = 0.004, **Supplementary Figure 4**). The Clonal Hematopoiesis Risk Score (CHRS) calculator was used assign prognostic risk categories (low, intermediate, high) across the CHIVE cohort[12]. Fifty patients were determined to be low risk with one patient (2%) progressing to malignancy. Intermediate risk included 32 patients with one (3.1%) progressing to malignancy while 14 patients were high risk and four (28%) progressed to malignancy. CHRS categorization in our cohort was predictive of malignancy progression (Fisher's exact test, p = 0.007).

Eight out of 99 CH+ patients (8.1%) and 2 of 82 CH- patients (2.4%) died over the course of the study period resulting in a trend toward decreased survival in CH+ patients (p = 0.112). The most common causes of death among CH+ patients were malignancy (n = 5) and septic shock (n = 3), while the two CH- patients died of trauma and renal failure (**Supplementary Table 5**).

Discussion

CH is an increasingly recognized condition with broad implications among aging populations. As NGS continues to evolve and become more widely adopted, additional patients will be identified with putative CH, underscoring the need for a greater understanding of the natural history of CH as well as the development of hematopathology and clinical guidelines. Registries, biorepositories, and prospective observational studies with well-described CH patients are critical to meeting this need. The *CHIVE* biorepository is a proof-of-principle, single institution-wide effort to define and catalog CH patients with the intention of building a cohort of patients from which new clinical insights and treatment strategies can be derived. To our knowledge, this is the first prospective CH registry and biorepository with preliminary outcomes to be described.

Analysis of several aspects of our data aligns with previous research from large-scale studies. Patients with CH variants followed expected proportions of known CH genes with *DNMT3A*, *TET2, ASXL1*, and *TP53* as the most prominent. The median age of CH+ patients was a decade older than CH- patients, providing further data supporting CH as an age-associated process. Patients with CH also had higher mortality rates compared to those without CH without a clear categorical trend. CH+ patients in this cohort had decreased blood counts across all hematopoietic lineages as well as increased rates of chronic kidney disease. Finally, accumulation of multiple high-risk features of CH clones was associated with increased rates of CCUS diagnosis and development of hematologic malignancy. Taken together, these findings mirror larger retrospective datasets, demonstrating enhanced risk of CH across a spectrum of pathologies.

Some elements of our data were not congruent with currently published studies. While we observed higher rates of cardiovascular diagnoses in the CH+ group compared to the CH- group, these outcomes were independently associated with other patient factors, such as age, sex, and BMI, and not with CH status. These findings are attributable to a lack of power in our currently small cohort, more stringent definition of CH-driver genes, and/or, additional patient factors that could serve as potential confounders. Notably, our CH- cohort is not a true control group as all patients enrolled were deemed at risk for CH; many of them (29%) were enrolled via cardiology due to known cardiovascular dysfunction. Given the disproportionately high rates of cardiovascular disease in the CH- group compared to the general population, the emergence of a trend towards worse cardiovascular outcomes in the CH+ group suggests an association between CH and cardiovascular disease that may be partially masked within our cohort.

We also identified ‘largest VAF’ as positively associated with CCUS and development of malignancy in our cohort, while other clone features commonly considered high-risk (multiple mutations, specific high-risk mutations) were not. This observation may point to clone features within the “high-risk” category that confer even higher risk. The CHRS categorization of CHIVE patients were highly congruent with malignancy outcomes in this small cohort with 1-2 years of follow up. One third of patients progressed to malignancy who were categorized as high risk whereas only 2% progressed to malignancy who were in

the low-risk category. While there is not enough longitudinal data to prospectively validate this score, it affirms the utility of such a score and represents an easy-to-use tool for clinicians. Continued refinement of this score through the accrual of prospective data will better inform and equip clinicians in serial monitoring and implementation of early prevention strategies, medical therapies, and treatment plan adjustments.

This study is the first to actively recruit patients from outside of hematology, and 56 of the 117 patients recruited from cardiology, genetics, and rheumatology were found to have CH variants not previously identified. It is important to continue to find and recruit these patients to CH clinics to advance clinical and translational research into the natural history of CH, to identify potential high-risk features of clones that may warrant CLIA-approved sequencing and close monitoring, and to guide specialty-specific follow up of these newly identified CH patients.

This study also highlights current challenges associated with increasing rates of clinical use of NGS and subsequent referral practices. While there are specific genes that account for the vast majority of reported CH, there is not a universally accepted list of CH driver genes.[33] Furthermore, the various NGS platforms and panels differ considerably in which genomic regions are sequenced, and if and how variants are reported out.[33] Predictably, these factors cause confusion among health care providers regarding what constitutes a potential pathologic clone, and ultimately lead to referrals to clinics that may not be necessary. In this real-world cohort, 16 patients were referred to CH Clinic and enrolled in *CHIVE* based on mutations identified via a clinical NGS panel, butwho did not have a bona fide CH mutation. Though these patients did not meet current criteria for CH prospective data such, as those from *CHIVE,* can be used to clarify and validate definitions of CH, so following these patients for future analyses is critical. This will serve as a basis for buttressed CH definitions which will provide guidelines for surveillance and referral practices, and be useful in the evolving CH clinical trials.

There are certain limitations in this study that should be addressed and will improve with enrollment from more patients from multiple sites. First, *CHIVE* is a single-center study, with limited ethnic diversity. Our

novel recruitment strategy with inclusion of patients seen in hereditary cancer clinic (25% of cohort) likely contributed to the female skew and limited ethnic diversity of the patients in our cohort,[36] which limits generalizability to other populations. We have opened CHIVE and are beginning enrollment at other sites to address this directly. As a growing biorepository, our study currently lacks sufficient sample size, which limits the ability to discern all but the strongest relationships within the data. For instance, some measures of cardiovascular and renal function demonstrate decreased function in CH patients and will be expected to meet statistical significance with larger sampling. Enrollment in *CHIVE* is ongoing, and we anticipate that our ability to perform complex analyses will increase as we accumulate a more robust data set. The expansion of participant recruitment intra- and inter- institutionally will both increase sample size and further diversify our patient population.

In conclusion, we demonstrate the feasibility of a prospective, observational study of CH patients utilizing a robust referral network to support both clinical care and translational research. A well-genotyped and phenotyped cohort of CH patients will be critical to future translational research efforts and clinical trials – a key function of our study design. Early clinical findings from our cohort recapitulate large scale retrospective datasets where CH patients are at an increased risk of development of hematologic malignancy, end organ damage, and all-cause mortality. Scaling this resource in collaboration with other centers is underway and will enable the development of clinical guidelines and treatment strategies for this increasingly recognized patient population.

Competing Interests Statement

All unrelated to the present work: M.R.S. reports personal fees from AbbVie, BMS, CTI, Sierra Oncology, Novartis, grants from Astex and Incyte, personal fees and other support from Karyopharm, Ryvu, personal fees from Sierra Oncology, grants and personal fees from Takeda, and TG Therapeutics outside the submitted work. JM has served on advisory boards for Bristol Myers Squibb, AstraZeneca, Myovant, Cytokinetics, Takeda, BeiGene, Kiniksa, Kurome Therapeutics, BitterRoot Bio, Deciphera, Regeneron, Repare Therapeutics, Antev, Daiichi Sankyo, Prelude Therapeutics and Voyager Therapeutics. P.B.F.

reports grants from Incyte. A.G.B. is a scientific co-founder and has equity in TenSixteen Bio. All other authors declare that they have no competing interests.

Author Contributions

M.L.S., J.B.H., and S.O. Collected and analyzed data, recruited patients, drafted the original manuscript, and revised the manuscript.

A.D., Z.C., C.S., A.J.S., D.B., T.S., M.B., K.P., A.K., S.S.S., and J.U. Collected data, recruited patients, and revised the manuscript.

C.V. Collected and analyzed data and revised the manuscript. Y.X., M.B., J.M., E.J., P.B.F., D.S., conceived the study, collected and analyzed data, and revised the manuscript.

A.G.B., and M.R.S. Conceived of the study, supervised the study and revised the manuscript.

Figure Legends

Figure 1. A total of 265 patients were approached for consent and 181 were enrolled; 47 patients were excluded because they declined consent. Thirty-six did not meet inclusion criteria. One patient voluntarily withdrew from the study. Of the enrolled patients, 63 were recruited from CH Clinic in the department of hematology (Arm A) and 118 were recruited from elsewhere in the hospital system (Arm B). CH+ patients were defined as individuals with a CH variant present with a VAF of 2% or greater on the CHIVEseq assay (n = 99).

Table 1. Baseline demographic characteristics of CH+ patients compared to CH- patients. Values are presented as counts (percentage) for categorical variables or median (interquartile range) for continuous variables.

Figure 2. UpSet plot of CH-associated genes. Horizontal bars (Set Size) represent the number of individual mutations in each gene present within our cohort of CH+ patients (n = 99). Vertical bars (Intersection Size) represent the number of CH+ patients with a given mutational landscape. Connecting dot plot displays the specific gene or combination of genes that are mutated in each patient group.

Table 2. Patients who were referred with positive clinical CH determination found to be CH- on CHIVEseq assay. Mutation in question, clinical panel, and reasoning for non-CH diagnosis are reported.

Table 3. Selected laboratory values and clinical diagnoses of CH+ patients compared to CH- patients. Values are presented as median (interquartile range) for continuous variables and counts (percentage) for categorical variables. For blood counts, n = 151 (86 CH+ and 65 CH-). For BUN and creatinine, n = 155 (88 CH+ and 67 CH-). For glucose, n = 158 (90 CH+ and 68 CH-). For HbA1c, n = 83 (53 CH+ and 30 CH-). For ESR, n = 31 (19 CH+ and 12 CH-). For CRP, n = 35 (21 CH+ and 14 CH-). For CKD and diabetes mellitus diagnoses, n = 181 (99 CH+ and 82 CH-).

Table 4. Selected measures of cardiovascular health in CH patients compared to those without CH. Values are presented as median (interquartile range) for continuous variables and counts (percentage) for categorical variables. For brain natriuretic peptide, n = 61 (46 CH+ and 15 CH-). For ejection fraction, n = 43 (15 CH+ and 28 CH-). For systolic blood pressure, diastolic blood pressure, CAD diagnosis, hypertension diagnosis, and heart failure diagnosis, n = 181 (99 CH+ and 82 CH-).

Table 5. Clone features of CH patients that developed hematologic malignancy over the course of the study. Clone features considered to be high-risk include total number of mutations, variant allele fraction (VAF), and mutation(s) in high-risk genes.

References

1. Jaiswal S, Ebert BL. Clonal hematopoiesis in human aging and disease. *Science*. 2019;366(6465):eaan4673. doi:10.1126/science.aan4673

2. Jaiswal S, Fontanillas P, Flannick J, et al. Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes. *N Engl J Med*. 2014;371(26):2488-2498. doi:10.1056/NEJMoa1408617

3. Genovese G, Kähler AK, Handsaker RE, et al. Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence. *N Engl J Med*. 2014;371(26):2477-2487. doi:10.1056/NEJMoa1409405

4. Xie M, Lu C, Wang J, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat Med*. 2014;20(12):1472-1478. doi:10.1038/nm.3733

5. Buscarlet M, Provost S, Zada YF, et al. DNMT3A and TET2 dominate clonal hematopoiesis and demonstrate benign phenotypes and different genetic predispositions. *Blood*. 2017;130(6):753-762. doi:10.1182/blood-2017-04-777029

6. Steensma DP, Bejar R, Jaiswal S, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood*. 2015;126(1):9-16. doi:10.1182/blood-2015-03-631747

7. Sperling AS, Gibson CJ, Ebert BL. The genetics of myelodysplastic syndrome: from clonal haematopoiesis to secondary leukaemia. *Nat Rev Cancer*. 2017;17(1):5-19. doi:10.1038/nrc.2016.112

8. Bowman RL, Busque L, Levine RL. Clonal Hematopoiesis and Evolution to Hematopoietic Malignancies. *Cell Stem Cell*. 2018;22(2):157-170. doi:10.1016/j.stem.2018.01.011

9. Valent P, Kern W, Hoermann G, et al. Clonal Hematopoiesis with Oncogenic Potential (CHOP): Separation from CHIP and Roads to AML. *Int J Mol Sci*. 2019;20(3):789. doi:10.3390/ijms20030789

10. DeZern AE, Malcovati L, Ebert BL. CHIP, CCUS, and Other Acronyms: Definition, Implications, and Impact on Practice. *Am Soc Clin Oncol Educ Book*. 2019;(39):400-410. doi:10.1200/EDBK_239083

11. Gallì A, Todisco G, Catamo E, et al. Relationship between clone metrics and clinical outcome in clonal cytopenia. *Blood*. 2021;138(11):965-976. doi:10.1182/blood.2021011323

12. Weeks LD, Niroula A, Neuberg D, et al. Prediction of Risk for Myeloid Malignancy in Clonal Hematopoiesis. *NEJM Evid*. 2023;2(5). doi:10.1056/EVIDoa2200310

13. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease. *N Engl J Med*. 2017;377(2):111-121. doi:10.1056/NEJMoa1701719

14. Fuster JJ, MacLauchlan S, Zuriaga MA, et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. *Science*. 2017;355(6327):842-847. doi:10.1126/science.aag1381

15. Khetarpal SA, Qamar A, Bick AG, et al. Clonal Hematopoiesis of Indeterminate Potential Reshapes Age-Related CVD. *J Am Coll Cardiol*. 2019;74(4):578-586. doi:10.1016/j.jacc.2019.05.045

16. Bick AG, Pirruccello JP, Griffin GK, et al. Genetic Interleukin 6 Signaling Deficiency Attenuates Cardiovascular Risk in Clonal Hematopoiesis. *Circulation*. 2020;141(2):124-131. doi:10.1161/CIRCULATIONAHA.119.044362

17. Bekele DI, Patnaik MM. Autoimmunity, Clonal Hematopoiesis, and Myeloid Neoplasms. *Rheum Dis Clin N Am*. 2020;46(3):429-444. doi:10.1016/j.rdc.2020.03.001

18. Asada S, Kitamura T. Clonal hematopoiesis and associated diseases: A review of recent findings. *Cancer Sci*. 2021;112(10):3962-3971. doi:10.1111/cas.15094

19. Cumbo C, Tarantini F, Zagaria A, et al. Clonal Hematopoiesis at the Crossroads of Inflammatory Bowel Diseases and Hematological Malignancies: A Biological Link? *Front Oncol*. 2022;12:873896. doi:10.3389/fonc.2022.873896

20. Miller PG, Qiao D, Rojas-Quintero J, et al. Association of clonal hematopoiesis with chronic obstructive pulmonary disease. *Blood*. 2022;139(3):357-368. doi:10.1182/blood.2021013531

21. Bolton KL, Koh Y, Foote MB, et al. Clonal hematopoiesis is associated with risk of severe Covid-19. *Nat Commun*. 2021;12(1):5975. doi:10.1038/s41467-021-26138-6

22. Huang Z, Vlasschaert C, Robinson-Cohen C, et al. Emerging evidence on the role of clonal hematopoiesis of indeterminate potential in chronic kidney disease. *Transl Res*. Published online December 2022:S1931524422003176. doi:10.1016/j.trsl.2022.12.009

23. Vlasschaert C, Moran SM, Rauh MJ. The Myeloid-Kidney Interface in Health and Disease. *Clin J Am Soc Nephrol*. 2022;17(2):323-331. doi:10.2215/CJN.04120321

24. Kestenbaum B, Bick AG, Vlasschaert C, et al. Clonal Hematopoiesis of Indeterminate Potential and Kidney Function Decline in the General Population. *Am J Kidney Dis*. Published online October 2022:S0272638622009258. doi:10.1053/j.ajkd.2022.08.014

25. Evans MA, Walsh K. Clonal hematopoiesis, somatic mosaicism, and age-associated disease. *Physiol Rev*. 2023;103(1):649-716. doi:10.1152/physrev.00004.2022

26. Abplanalp WT, Cremer S, John D, et al. Clonal Hematopoiesis–Driver DNMT3A Mutations Alter Immune Cells in Heart Failure. *Circ Res*. 2021;128(2):216-228. doi:10.1161/CIRCRESAHA.120.317104

27. Bolton KL, Gillis NK, Coombs CC, et al. Managing Clonal Hematopoiesis in Patients With Solid Tumors. *J Clin Oncol*. 2019;37(1):7-11. doi:10.1200/JCO.18.00331

28. Coombs CC, Zehir A, Devlin SM, et al. Therapy-Related Clonal Hematopoiesis in Patients with Non-hematologic Cancers Is Common and Associated with Adverse Clinical Outcomes. *Cell Stem Cell*. 2017;21(3):374-382.e4. doi:10.1016/j.stem.2017.07.010

29. Kar SP, Quiros PM, Gu M, et al. Genome-wide analyses of 200,453 individuals yield new insights into the causes and consequences of clonal hematopoiesis. *Nat Genet*. 2022;54(8):1155-1166. doi:10.1038/s41588-022-01121-z

30. Steensma DP, Bolton KL. What To Tell Your Patient With Clonal Hematopoiesis And Why: Insights From Two Specialized Clinics. *Blood*. Published online July 31, 2020:blood.2019004291. doi:10.1182/blood.2019004291

31. Mack T, Vlasschaert C, Von Beck K, et al. *Cost-Effective and Scalable Clonal Hematopoiesis Assay Provides Insight into Clonal Dynamics*. Genetic and Genomic Medicine; 2023. doi:10.1101/2023.11.08.23298270

32. Cibulskis K, Lawrence MS, Carter SL, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat Biotechnol*. 2013;31(3):213-219. doi:10.1038/nbt.2514

33. Vlasschaert C, Mack T, Heimlich JB, et al. *A Practical Approach to Curate Clonal Hematopoiesis of Indeterminate Potential in Human Genetic Datasets*. Hematology; 2022. doi:10.1101/2022.10.21.22281368

34. Lex A, Gehlenborg N, Strobelt H, Vuillemot R, Pfister H. UpSet: Visualization of Intersecting Sets. *IEEE Trans Vis Comput Graph*. 2014;20(12):1983-1992. doi:10.1109/TVCG.2014.2346248

35. Conway JR, Lex A, Gehlenborg N. UpSetR: an R package for the visualization of intersecting sets and their properties. Hancock J, ed. *Bioinformatics*. 2017;33(18):2938-2940. doi:10.1093/bioinformatics/btx364

36. Winston-McPherson GN, Mathias PC, Lockwood CM, Greene DN. Evaluation of Patient Demographics in Clinical Cancer Genomic Testing. *J Appl Lab Med*. 2021;6(1):119-124. doi:10.1093/jalm/jfaa219

Table 1.

Table 1.

	CH - (n=82)	**CH + (n=99)**	**p value**
Sex			
Male	24 (29.3)	50 (50.5)	**0.009**
Female	58 (70.7)	49 (49.5)	
Age			
18-29	3 (3.7)	0 (0.0)	
30-49	14 (17.1)	1 (1.0)	
50-64	27 (32.9)	24 (24.8)	
65+	38 (46.3)	74 (74.2)	
Median +/- IQR	62.9 (51.5 - 72.7)	71.9 (64.0 - 77.5)	**<0.001**
Body Mass Index (BMI)			
<18.5	3 (3.7)	1 (1.0)	
18.5 - 24.9	23 (28.0)	22 (22.7)	
25.0 - 29.9	28 (34.2)	34 (35.1)	
30.0 - 34.9	17 (20.7)	23 (23.7)	
>35	11 (13.4)	17 (17.5)	

Median +/- IQR	27.4 (24.1 - 32.4)	28.9 (25.4 - 32.1)	0.253
Tobacco Use			
Current Smoker	2 (2.4)	4 (4)	0.691
Former Smoker	22 (26.8)	30 (30.3)	0.624

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558 **Table 2.**

Study ID	Clinically Identified Mutation(s)	Clinical Panel	Reason(s)
0006	JAK2 p.V617F (VAF 0.2%)	Tempus (liquid biopsy)	Below 2% VAF threshold
1020	TET2 p.A915P (VAF 45%)	Myeloid Neoplasm NGS Panel	Germline mutation; mutation is outside of TET2 catalytic domain & therefore not considered a CH variant by CHIVEseq
1071	BCOR p.E1081K (VAF > 99%)	Myeloid Neoplasm NGS Panel	Germline mutation; BCOR gene not covered on CHIVEseq assay
1085	CUX1 p.P1080* (VAF 5.18%)	Myeloid Neoplasm NGS Panel	CUX1 gene not covered on CHIVEseq assay
1095	JAK2 p.N691H (VAF 47.87%)	Myeloid Neoplasm NGS Panel	Germline mutation; mutation not considered a CH variant on CHIVEseq assay
1103	TET2 p.C88R (VAF 45.94%) CSF3R	Myeloid Neoplasm NGS Panel	TET2: Germline mutation; mutation is outside of TET2 catalytic domain and therefore not considered a CH variant by CHIVEseq

	p.R698C (VAF 48.92%)		CSF3R: Germline mutation; CSF3R gene not covered on CHIVEseq assay
2001	BCORL p.V881E (VAF 49.52%)	Myeloid Neoplasm NGS Panel	Germline mutation; BCORL gene not covered on CHIVEseq assay
2006	SH2B3 p.L429V (VAF 48.58%)	Myeloid Neoplasm NGS Panel	Germline mutation; SH2B3 gene not covered on CHIVEseq assay
2011	CALR p.E381del (VAF 48.97%) BCOR p.S209L (VAF 99.2%)	Myeloid Neoplasm NGS Panel	CALR: Germline mutation; CALR gene not covered on CHIVEseq assay BCOR Germline mutation; BCOR gene not covered on CHIVEseq assay
2015	JAK2 p.I724T (VAF 48.99%) SMC1A p.I1062M (VAF 50.47%) TET2 p.A289P (VAF 48.99%)	Myeloid Neoplasm NGS Panel	JAK2: Germline mutation; mutation not considered a CH variant by CHIVEseq SMC1A: Germline mutation; SMC1A gene not covered on CHIVEseq assay TET2: Germline mutation; mutation is outside of TET2 catalytic domain and therefore not considered a CH variant by CHIVEseq
2016	CUX1 p.E1373Q (VAF 48.62%) CUX1	Myeloid Neoplasm NGS Panel	Germline mutations; CUX1 gene not covered on CHIVEseq assay

	p.R843K (VAF 50.35%)		
2019	**DNMT3A p.A462V (VAF 57%)**	**Myeloid Neoplasm NGS Panel**	**Germline mutation**
2041	**SF3B1 p.K700E (VAF 3.1%) DNMT3A p.L888Q (VAF 2.1%)**	**NeoTYPE MDS/CMML Profile**	**SF3B1: This site was sequenced but the SF3B1 K700E variant was not identified, including at VAF <2%. DNMT3A: mutation is not considered CH by conventional definitions**
2046	**SH2B3 p.S213R (VAF 47.77%)**	**Myeloid Neoplasm NGS Panel**	**Germline mutation; SH2B3 gene not covered on CHIVEseq assay**
2059	**KDM6A p.R621H (VAF 51%)**	**Advanced NGS Myeloid Report**	**Germline mutation; KDM6A gene not covered on CHIVEseq assay**
2068	**ASXL1 p.G646Wfs*12 (10%)**	**Advanced NGS Myeloid Report**	**Commonly encountered artifact in a homopolymer region of the CHIVEseq assay. Our practice is to categorize these variants as CH when VAF is > 10%. This variant was not considered CH because the VAF was 6%.**

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Table 3.

	CH -	CH +	Unit	p value
Blood Counts				
White Blood Cells	6.8 (5.2-8.4)	6.2 (4.5 8.1)	x1000/mcL	0.455
Hemoglobin	13.3 (12.3-14.6)	12.9 (11.4 - 14.1)	gm.dL	0.136
Hematocrit	42 (37.0 - 44.0)	39.0 (35.0 - 43.0)	%	0.134
Platelet	245.0 (198.0 - 284.0)	203.0 (163.0 - 262.0)	x1000/mcL	0.093
Kidney Function				
BUN	16.0 (12.0 - 21.0)	18.0 (14.0 - 23.0)	mg/dL	0.168
Creatinine	0.9 (0.76 - 1.11)	0.97 (0.84 - 1.29)	mg/dL	**0.015**
CKD Diagnosis, n (%)	15 (18.3)	41 (41.2)		**0.002**
Blood Glucose				
Glucose	97.0 (88.0 - 117.0)	106.0 (91.0 - 121.0)	mg/dL	0.21
HbA1c	6.0 (5.4 - 6.8)	5.8 (5.3 - 6.4)	%	0.45
Diabetes Mellitus Diagnosis, n(%)	22 (26.8)	31 (30.9)		0.522
Inflammatory Markers				
ESR	16.0 (6.0 - 33.0)	20.0 (15.0 - 32.0)	mm/hr	0.67
CRP	3.2 (1.2 - 13.1)	8.2 (2.3 - 37.3)	mg/L	0.183

567 **Table 4.**

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	CH -	CH +	Unit	p value
Cardiovascular Measurements				
Systolic Blood Pressure	125 (117 - 134)	129 (118 - 139)	mmHg	0.249
Diasolic Blood Pressure	75 (68 - 82)	72 (66 - 78)	mmHg	0.177
Coronary Artery Disease Diagnosis, n(%)	27 (32.9)	55 (55.7)		**0.004**
Hypertension Diagnosis, n(%)	43 (52.4)	77 (79.4)		**<0.001**
Clinical Heart Failure Diagnosis, n(%)	8 (9.8)	24 (22.7)		**0.035**
Brain Natriuretic Peptide	79 (47 - 110)	56 (34 - 184)	pg.mL	0.908
Ejection Fraction	60 (55 - 63)	61 (54 - 68)	%	0.424

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570 Table 5.

Patient ID	Number of Mutations	Mutation	Maximum VAF	High Risk Gene	Average VAF	Type of Malignancy
0004	4	TET2 R1516X	0.398	No	0.217	MDS
		TET2 Q695X	0.369	No		
		SRSF2 P95H	0.177	Yes		
		JAK2 V617F	0.02	Yes		
1060	1	SF3B1 R625C	0.241	Yes	0.236	MDS
1073	4	TET2 Q742X	0.422	No	0.237	CMML
		SRSF2 P95R	0.330	Yes		
		TET2 Y1245Lfs*22	0.270	No		
		TET2 N535Kfs*6	0.033	No		
1096	1	TET2 G1288D	0.796	No	0.758	CMML
2026	1	TP53 Y220C	0.676	Yes	0.676	AML

2038	1	TET2 Q749Rfs*15	0.021	No	0.021	MDS

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1060	1	SF3B1 R625C	0.241	Yes	0.236	MDS
1073	4	TET2 Q742X	0.422	No	0.237	CMML
		SRSF2 P95R	0.330	Yes		
		TET2 Y1245Lfs*22	0.270	No		
		TET2 N535Kfs*6	0.033	No		
1096	1	TET2 G1288D	0.796	No	0.758	CMML
2026	1	TP53 Y220C	0.676	Yes	0.676	AML
2038	1	TET2 Q749Rfs*15	0.021	No	0.021	MDS

Figure 1.

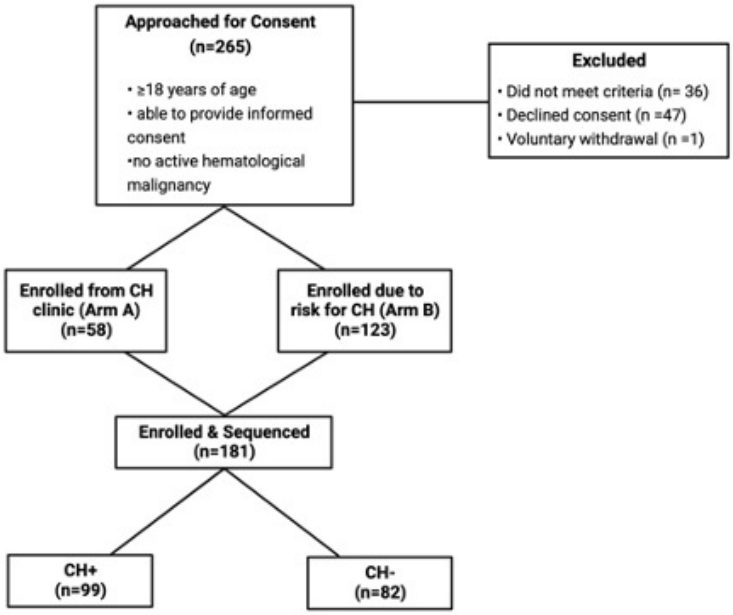

Figure 2.