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Clonal hematopoiesis and its progression to myeloid neoplasms: insights into risk, biology, and therapeutic strategies

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Running Head

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Abstract

Clonal hematopoiesis (CH) is defined by the clonal expansion of hematopoietic stem and progenitor cells harboring somatic mutations that confer a fitness advantage. CH is common with advancing age and becomes nearly ubiquitous in middle age. Although typically asymptomatic, CH is associated with an increased risk of hematologic malignancies particularly myeloid neoplasms (MN), diverse non-malignant conditions, and all-cause mortality. Over the past decade, research has provided major insights into the origins of CH. In addition to aging, CH is promoted by environmental exposures, inherited genetic predisposition, and acquired conditions. Large-scale population and longitudinal sequencing studies have identified determinants of clonal behavior. Characterization of the natural history of CH has enabled the development of risk stratification models to identify individuals with CH at high risk for progression to MN, thereby providing a rationale for selecting patient populations best suited for therapeutic intervention trials. Emerging strategies include targeting mutation-specific vulnerabilities, modulating inflammatory pathways, reducing genotoxic therapy-induced clonal selection, and repurposing agents with efficacy in MN. In this review, we summarize current knowledge of the risk factors underlying CH development, highlight recent advances in understanding the determinants of clonal behavior including progression to MN, and discuss emerging therapeutic approaches for preventing malignant transformation and clinical trial design considerations.

Introduction

Clonal hematopoiesis (CH) is characterized by the clonal expansion of hematopoietic stem and progenitor cells (HSPCs). These clones arise through the acquisition of somatic DNA alterations, which confer a selective advantage and enable clonal outgrowth. The process of normal stem cells acquiring somatic mutations that modify fitness is pervasive across human tissues and increases with advancing age.[1–3] A variety of DNA alterations can drive CH including point mutations, insertions-deletions (indels), and large-scale chromosomal changes (gains, losses, and copy-neutral loss of heterozygosity); the latter of which are termed mosaic chromosomal alterations (mCA).[4,5] Most CH is caused by point mutations in a small number of genes that are recurrently mutated in myeloid neoplasms (MN).[6–8] This includes genes involved in epigenetic modification (*DNMT3A*, *TET2*, *ASXL1*), DNA damage response (DDR; *TP53* and *PPM1D*), RNA splicing (*SRSF2*, *SF3B1*, *U2AF1*), and signal transduction (*JAK2*). CH is ubiquitous with aging and while typically clinically silent, is associated with an increased risk of progression to hematologic malignancies including MN, as well as a variety of non-malignant adverse health outcomes which collectively result in increased all-cause mortality.[6,7] CH is a premalignant state, analogous to monoclonal gammopathy of undetermined significance (MGUS), that offers a model for understanding the early stages of carcinogenesis in humans. Importantly, CH provides unique opportunities to study clonal dynamics and somatic mutation acquisition in humans since the peripheral blood allows for non-invasive sampling of the entire hematopoietic stem cell pool which is not possible for other organ systems. This review will summarize current insights into the causes of CH, discuss the factors that drive progression of myeloid CH to MN, and highlight potential therapeutic approaches to prevent progression to MN. It will focus primarily on myeloid CH (commonly referred to as simply CH) driven by somatic mutations, as our understanding of how mCA—which typically involve multiple genes— contribute to progression to MN remains limited. Because the molecular mechanisms underlying many common myeloid CH mutations have been thoroughly reviewed recently,[9] this review will briefly summarize the most relevant pathways to contextualize the clinically important features of CH.

Key CH Concepts and Nomenclature

The nosology of CH was comprehensively summarized in a recent excellent review by Weeks and Ebert.[10] Herein, we summarize the key concepts and definitions related to CH nosology that are fundamental to understanding the role of CH in hematological malignancies. Humans have an estimated 20,000 to 200,000 HSPCs that contribute to hematopoiesis.[11] With clonal expansion, the proportion of peripheral blood cells arising from a single HSPC increases and can be quantified by measuring the variant allele fraction (VAF) of the somatic alterations harbored by the clone. CH mutations with a higher VAF are associated with an increased risk of hematologic malignancies and other adverse health outcomes. A minimum peripheral blood VAF threshold of 2% was used to define CH in early studies, a cutoff based on the sequencing error rate of traditional (non-error corrected) Illumina exome sequencing data.[6,7] This definition ultimately led to the term clonal hematopoiesis of indeterminate potential (CHIP), which is defined as CH with a somatic mutation in a MN driver gene with a VAF ≥2% in an individual

without hematological cancer or blood count abnormalities. While CH can now be detected at a VAF of ≥0.001 (0.1%) using error-corrected sequencing methodologies,[12–15] our understanding of the biological relevance of alterations with VAF <2%, termed micro-CH by some,[16] is limited even today.

CH can be driven by diverse alterations with specific genetic drivers influencing both CH biology and outcomes. For example, CH driven by alterations in known myeloid driver genes (which is referred to as myeloid CH) is associated more strongly with an increased risk of MN.[6,7] Conversely, CH driven by alterations in lymphoid drivers (referred to as lymphoid CH) is more strongly associated with risk of lymphoid neoplasms.[17] Myeloid CH is clearly more common than lymphoid CH largely due to highly recurrent mutations in *DMNT3A*, *TET2*, and *ASXL1*.[17–19] The distinction between these classes of CH is complicated by the fact that somatic mutations in many genes can drive both myeloid and lymphoid neoplasms (e.g. *TP53, TET2, RUNX1, SF3B1, IDH2*).[20] We found that 6.2% of individuals in the UK Biobank had somatic mutations in hematological malignancy driver genes, with 4.6% occurring in myeloid only, 0.4% in lymphoid only, and 1.5% in both myeloid and lymphoid genes.[21] Like hematologic cancer, multiple classes of somatic events can drive CH. This includes single nucleotide variants, indels, and large-scale copy number events including mCAs. Attributing mCAs, which typically involve multiple genes, to a binary myeloid versus lymphoid classification is challenging. In aggregate, the prevalence of autosomal mCAs was lower than somatic CH mutations (3.2 versus 6.2%) among individuals in the UK Biobank.[21] Furthermore, estimating the true prevalence of somatic copy number events is challenging, as the sensitivity for detecting even moderately sized copy number alterations is substantially lower than for single nucleotide variants at comparable sequencing depth and remains limited when using single nucleotide polymorphism arrays. The extent to which complex re-arrangements and fusion events (which are common drivers of hematologic cancer) might also drive CH is unclear due to difficulty in detecting these classes of events at a low VAF.

The evolution of myeloid CH to MN is summarized in Figure 1. Numerous features have been identified that contribute to the risk of CH progression (e.g. high-risk mutations, increased mutation number and VAF, ineffective erythropoiesis, cytopenias) and have been used to develop MN prediction models.[22,23] These will be covered in detail later in this review. Myeloid CH with a persistent explained cytopenia and without dysplastic features meeting criteria for myelodysplasia syndrome (MDS) is termed clonal cytopenia of undetermined significance (CCUS).[24,25] Individuals with CCUS have a significantly elevated risk of progression to MN compared to those with CH without cytopenias or idiopathic cytopenia of undetermined significance.[26]

It is common to detect CH without identifying a somatic mutation or chromosomal alteration in a recognized hematological malignancy driver gene.[6,11,27] Using distinct analytical approaches and large cohorts, Genovese et al[6] and Zink et al[27] found that 39% and 59% of CH cases respectively lacked a known driver. Importantly, CH without a known driver carried a risk of hematological malignancy comparable to CH with an established driver.[6,27] Mitchell et al[11] further confirmed the commonness of CH without a driver using an orthogonal approach—whole

genome sequencing of 3579 of single cell-derived HSPC colonies from 10 donors across the human lifespan. Similar observations have been made in MDS, where ~6% of patients lack genomic alterations in established MDS driver genes.[28] Potential explanations for CH with unknown drivers include the presence of novel driver genes (which would likely be relatively uncommon), mutations in non-coding regions (e.g. enhancers), mutations difficult to detect at a low VAF (e.g. complex rearrangements), genetic drift (i.e. random chance), and technical artifacts. Together, these findings highlight the need to better understand the biological underpinnings of CH lacking a known driver and its relationship with the progressive hematopoietic oligoclonality that is pervasive with aging.

Causes of CH

The modern concept of carcinogenesis, originally developed through the seminal work of Carl Nordling, Peter Armitage, and Richard Doll in the 1950s,[29,30] posits that cancer arises through the stepwise acquisition of genetic alterations in cells over time. Elucidation of the molecular events underlying carcinogenesis was first demonstrated in colon cancer by Fearon and Vogelstein in 1990 through analysis of biopsies obtained during colonoscopy, with publication of the "Vogelgram" model outlining specific genetic alterations associated with progression of normal colonic epithelium to adenoma and eventual carcinoma.[31] Subsequent studies using massively parallel sequencing have since characterized the genomic landscape of numerous cancer types, providing insight into the genetic alterations driving specific tumor subtypes.[32] The discovery of CH provided critical insights into the earliest stages of hematological malignancies in humans through analysis of easily accessible peripheral blood samples. Furthermore, the presence of preexisting large biobanks with linked genetic and longitudinal health data, such as the UK Biobank,[33] has fueled the rapid acquisition of knowledge on factors that influence the initiation and progression of hematological malignancies, particularly MN.

Intrinsic Factors

Age is the strongest risk factor for the development of CH,[6,7] reflecting the fact that age is the principal driver of somatic mutation accumulation across human tissues.[1,34,35] The rate of somatic mutation acquisition is enhanced in highly proliferative tissues such as the human bone marrow, which produces approximately 200 billion red blood cells and 100 billion neutrophils to maintain blood homeostasis.[36,37] With each cell division, somatic mutations are inevitably introduced despite the high fidelity of human DNA replication due to the inherent challenge of correctly copying a 3.2 billion base pair genome. Somatic mutations can serve as a biological clock, marking the passage of time and the accumulation of genetic changes within HSPCs. By age 60 years CH is common, with approximately 10% of individuals having CH detectable at a VAF ≥2% and 75% at a VAF ≥0.1%.[38] The somatic mutations that drive CH can occur early in life, including in utero, and typically take decades to clinically manifest.[39,40]

While the overall prevalence of CH is similar between males and females, sex influences the frequency of CH driven by alterations in specific genes. Males are more likely to have mutations in splicing factors genes (e.g. *SRSF2*, *SF3B1*, *U2AF1*) and *ASXL1*; and less likely to have

DNMT3A mutations.[41–43] Additionally, males are more prone to mosaic loss of the Y chromosome than females are to mosaic loss of the X chromosome (mLOX; 20 versus 5% in the UK Biobank), and do not develop mLOX.[44] These sex-based differences persist after controlling for basic potential confounders (e.g. smoking rates). The CH mutational spectrum observed in males is over-represented for genes that confer an elevated risk of MN, consistent with the increased incidence of hematological malignancies observed in males across ages[45,46] Our understanding of the mechanisms underlying the sex-based differences in CH and hematological malignancies are poorly understood. Potential contributors to increased hematological malignancy risk in males include differential carcinogen exposure, presence of tumor suppressor genes on the X chromosome that can escape X-inactivation[47], and hormonal impacts on HSPC biology and leukemic potential.[48] These data collectively highlight an important and understudied biological role of sex on MN pathogenesis beyond differential environmental exposures, which warrants further investigation.

Environmental Exposures

While smoking is moderately associated with multiple individual CH genes including *SRSF2*, *SF3B1*, and *DNMT3A*; it is strongly associated with *ASXL1* mutations.[41,49] The mechanism underlying the association between *ASXL1* mutations and smoking is not understood. Most *ASXL1* mutations in CH and MN are caused by heterozygous truncating (frameshift indels and nonsense) mutations in the C-terminus, which allows for escape from nonsense-mediated decay.[50] Mutational signature analysis can identify characteristic patterns of somatic mutations induced by distinct mutational processes (e.g. tobacco carcinogens, UV light) through characterization of base substitution subtypes and their trinucleotide context (bases 5' and 3' of the mutated base).[51] It has been used to show that smoking is associated with increased base substitutions and indels in tissues with and without direct exposure to tobacco smoke.[52] However, the trinucleotide context of CH drivers occurring in smokers and non-smokers is largely similar and predominantly driven by the age-related mutation signatures (e.g. SBS1).[53] It is unclear if carcinogens in tobacco preferentially induce *ASXL1* mutations, or whether smoking simply confers a fitness advantage to HSPCs with *ASXL1* mutations.

Genotoxic therapies, including cytotoxic chemotherapy and radiation therapy are associated with CH. These therapies induce DNA damage in HSPCs and create a selection pressure for clones with genetic alterations in DDR genes including *TP53*, *PPM1D*, and *CHEK2*.[49] This process is likely mostly driven by selection for preexisting clones with DDR mutations, rather than generation of *de novo* mutations by genotoxic therapy, but distinguishing between these two possibilities is challenging.[54] However, some CH mutations may be induced by genotoxic therapy. A study of CH in long-term survivors of pediatric cancers (median follow up 32 years since diagnosis) identified recurrent *STAT3* mutations only among individuals with a history of Hodgkin lymphoma.[55] Single cell whole genome sequencing on peripheral blood from three individuals with *STAT3* mutations revealed that *STAT3*-mutant hemopoietic cells (and not wild-type cells) contained a ~3.5 fold increased mutation burden, and were strongly enriched for the mutational signature COSMIC SBS25, which occurs due to exposure to procarbazine, an alkylating drug used in Hodgkin lymphoma.[55] Cytotoxic chemotherapy regimens containing

topoisomerase II inhibitors, platinum agents, alkylating agents, and bleomycin are most strongly associated with DDR CH,[49,55] consistent with the known risk of these agents for secondary MN. Higher radiation therapy doses are associated with increased risk of CH, with a stronger effect observed for DDR CH.[56] DDR CH was significantly associated with specific anatomic sites of radiation therapy delivery including the head and neck, pelvis, brain, and thorax.[56] Interestingly, these sites account for ~65% of active bone marrow tissue in adults,[57] suggesting that radiation-induced CH expansion may be linked to the total dose applied to the hematopoietic compartment.

Genetic Predisposition

Inherited genetic variation has a well-established role in predisposing individuals to CH and modifying clonal behavior. Inherited bone marrow failure syndromes (IBMFSs) and familial acute myeloid leukemia and/or myelodysplastic syndromes (AML/MDS) are caused by rare pathogenic germline variants in genes with critical roles in hematopoiesis and have been identified as strong contributors to inherited CH risk at early ages, in addition to their well-established roles in MN predisposition.[58] While the pathophysiology of IBMFSs is variable, these syndromes are united by ineffective hematopoiesis due to impaired HSPC function, which creates a unique selection pressure for somatic clones with a relative fitness advantage to relieve the germline fitness constraints of the specific IBMFS. In these settings, CH arises through two principal mechanisms: (1) somatic normalization – adaptive mutations that compensate for the defect by reversion (germline defect is directly corrected) or compensation (germline defect is indirectly corrected), while leaving tumor-suppressor pathways intact; and (2) somatic transformation – maladaptive mutations that subvert tumor-suppressor pathways, conferring an elevated risk of malignant transformation.[58] For example, in Shwachman-Diamond syndrome, which involves defects in ribosomal assembly due to loss-of-function mutations in *SBDS*, somatic normalization results from alterations that partially restore ribosome function through *EIF6* disruption (via inactivating mutations or deletion 20q) or partially correct the *SBDS* mutation (via isochromosome 7q).[59] In contrast, somatic transformation occurs as a consequence of *TP53* inactivation, resulting in uncoupling of ribosomal stress from activation of cellular senescence pathways (without correction of the ribosome function defect) and ultimately increased risk of transformation to MN. In dyskeratosis congenita (DC), which is caused by loss-of-function mutations in genes that regulate telomere maintenance (e.g. *TERT*), mechanisms of somatic normalization include direct reversion, mutation of the unaffected wild-type *TERT* promoter (thereby leading to increased telomerase activity), and *POT1* loss-of-function mutations (facilitates telomere elongation).[60] *TP53* inactivation and chromosome 7 loss are the major causes of somatic transformation in DC.[61]

Similar to IBMFSs, the unique selection pressures introduced by familial AML/MDS disorders results in the development of specific profiles of CH mutations. Individuals with germline pathogenic variants (without a hematological malignancy) in *RUNX1* and *GATA2* had high rates of CH (35 and 22% respectively) at all ages, whereas individuals with pathogenic *DDX41* germline variants had a low CH prevalence (3%).[62] In addition, *RUNX1* germline variant carriers had a unique CH mutational spectrum with a high frequency (42%) of *BCOR* variants. These

findings collectively underscore the importance of integrating germline genetics into our understanding of CH biology. Such integration may improve risk stratification, inform surveillance strategies for individuals with hereditary predisposition to hematologic malignancies, and provide opportunities for mechanistic studies that explore how inherited variation shapes the evolutionary landscape of somatic hematopoietic clones.

Genome-wide association studies using large biobanks with linked phenotypic data have identified several common germline loci that increase susceptibility to CH, both overall and in specific genes. Most of the genes associated with CH globally have known roles in telomere biology, DDR, hematological malignances (e.g. *TET2*, *SETB1*, *RUNX1*, *ETV6*, *GATA*, *PTPN11A*, *MPL*), or HSPC biology (e.g. *CD164*, *LY75*, *SMC4*).[19,21,63,64] Among these, variants at the *TERT* locus associated with increased telomerase activity and telomere length consistently show the strongest association with overall CH risk.[27,64] Mendelian randomization analysis supports a causal role of increased telomere length CH risk.[41] In line with this, a small family-based study found that germline heterozygous loss-of-function mutations in *POT1*, a telomere maintenance gene, confer a high risk of increased telomere length and CH risk, as well as a diverse spectrum of benign and malignant neoplasms.[65] Rare variants in *CTC1*, another important regulator of telomeres which has been implicated in DC, have been associated with global CH risk.[63] These findings collectively demonstrate a critical role for telomere biology in CH pathogenesis, suggesting that reduction of normal telomere shortening with aging promotes the development of CH.

Common germline variants in several DDR genes also predispose individuals to CH overall, including genes associated with well-established cancer predisposition syndromes (e.g. *CHEK2*, *ATM*, *PARP1*, *TP53*).[41,63] In addition, our group recently identified 22 new CH predisposition genes (20 CH gene-specific),[21] 14 of which have recognized roles in the DDR (*ATR*, *RAD51D*, *FANCI*, *NBN*, *RTEL1*, *ERCC1*, *ERCC2*, *ERCC3*, *ERCC4*, *MUTYH*, *NTHL1*, *LIG4*, *ERCC6L2*, *PRDM9*).

Previous studies have also identified common germline variants that drive CH in specific genes.[19,63,64,66] Most notably, a promoter variant at *TCL1A* associated with decreased *TCL1A* expression predisposes to *DNMTA* CH, but is protective from CH driven by other genes including *TET2*, *ASXL1*, *SF3B1*, and *SRSF2*.[41] This difference was driven by the differential impact of the allele on CH expansion rate, resulting in slower growth of non-*DNTM3A* CH.[67] In addition, functional studies showed that *TCL1A* drives HSPC clonal expansion and that its expression is induced by introduction of mutations in *TET2* or *ASXL1*, but not *DNMT3A*. The results implicate *TCL1A* as a key mediator of the fitness advantage of many commonly mutated CH genes. Similarly, a variant in the *CD164* locus, which is involved in HSPC migration, is associated with *DNMT3A* and *ASXL1* CH, but not *TET2* CH for reasons that remain unclear.[41,63]

Overall, the link between germline variants and gene-specific CH predisposition appears to be driven primarily by the influence of germline variants on clonal fitness rather than direct mutagenesis, as mutational signatures were largely comparable between carriers and noncarriers, and dominated by the age-related SBS1 signature.[21] However, longitudinal studies

comparing CH evolution in germline carriers and noncarriers would be needed to clarify the relative contribution of mutational acquisition versus expansion.

Acquired Disorders

Aplastic anemia is a bone marrow failure disorder characterized by pancytopenia and bone marrow aplasia. Acquired forms of the disorder are usually caused by autoimmune destruction of HSPCs and associated with CH in approximately 50% of patients.[68] The genes most frequently involved with somatic mutations in acquired aplastic anemia include *DNMT3A*, *PIGA*, ASXL1, *BCOR*, and *BCORL1*. The unique enrichment of *PIGA*, *BCOR*, and *BCORL1* somatic mutations in acquired aplastic anemia may support their role in escape from immune-mediated destruction, although the mechanisms are not well characterized.

Drivers of CH Progression to MN

Clonal Evolution

Over the past five years our understanding of the determinants of clonal behavior and their influence on progression to MN has advanced substantially. Studies have employed diverse approaches to characterize clonal dynamics including serial measurement of CH through longitudinal aging studies,[69] phylogenetic analysis through sequencing of individual HSPC clones,[11,69] and clonal growth estimation from single time point data using passenger mutation analysis.[67] These complementary methodologies have yielded several broad insights across CH driver mutations. CH clones generally expand at an accelerated rate earlier in life, followed by a slower steady exponential rate during older age (at least age 55 years old).[69] Growth rates vary considerably between different CH mutations, with some mutations exhibiting age-dependent variability in growth.[69] Larger CH clone size, higher clonal growth rates, and a greater number of CH mutations are all associated with an increased risk of progression to MN.[7,69] Finally, CH mutations are typically acquired decades before manifesting clinically.[11]

Specific CH driver mutation genes play a central role in dictating clonal behavior, including both the risk and type of progression to MN. *DNMT3A* CH generally has a low clonal growth rate (~5% per year), though there is marked growth variation by age—faster at younger ages and slower at older ones.[69] The risk of progression to MN with *DNMT3A* mutations is relatively low and is driven primarily by an increased risk of AML.[41] In contrast, *TET2* mutations exhibit an intermediate clonal growth rate (~10% per year) which is stable across all ages, with one study suggesting *TET2* overtakes *DNMT3A* as the most common CH mutation in individuals older than 75 years old.[69] *TET2* CH confers an intermediate risk of progression to MN, particularly MDS and CMML.[41] Despite having opposite effects on DNA methylation, loss-of-function mutations in *TET2* and *DNMT3A* both drive CH clonal expansion. *ASXL1* mutations also demonstrate intermediate growth rates and are strongly associated with progression to AML and MDS risk.[41,69] Mutations in *IDH1* and *IDH2* are less common, have an intermediate-to-high growth rate (which is stable over time), and strongly associated with risk of progression to AML.[22,69] In addition, *IDH1/2* mutations are largely mutually exclusive with each other and *TET2*

mutations in MN (reflecting a shared pathogenic mechanism discussed later in this review),[70] which has been validated using single-cell DNA-sequencing approaches to reconstruct phylogenetic trees of the major driver clones in AML cases.[71,72]

Mutations in splicing factors (including *SRSF2*, *SF3B1*, and *U2AF1*) are rare before age 50 years old, but when present at older ages are associated with rapid clonal growth rates (15-20% per year).[69] The reasons for its late onset are incompletely understood, but likely reflect age-related changes in selection pressures, rather than a unique restriction of these mutations to older individuals. Recent work has shown that splicing factor mutations promote CH expansion in part by mitigating telomere shortening in HSPCs with aging.[73] Investigating the molecular basis of this phenomenon has been difficult because current *in vitro* and *in vivo* models of splicing factor mutations often show reduced clonal fitness, the opposite of which is observed in humans.[74] Splicing factor mutations are typically mutually exclusive because their co-expression results in synthetic lethality.[75] Splicing factor-driven CH has a high risk of progression to all MN subtypes.[41] Interestingly, *SF3B1* CH is associated with a favorable prognosis upon progression to MDS,[76] but an adverse prognosis upon progression to AML,[77] highlighting the context-dependent effects of specific splicing factor mutations.

While CH clones harboring the *JAK2* V617F hotspot mutation generally have intermediate growth rates, they are unique in demonstrating unpredictable growth dynamics at older ages.[69] In contrast, mutations in other MPN drivers (*CALR* and *MPL*) are less frequently observed in CH, consistent with their lower prevalence in MPN.[41] The canonical MPN driver mutations (*JAK2*, *CALR*, *MPL*) are mutually exclusive and exert strong influence on the risk of developing specific MPN subtypes. *JAK2* V617F mutations are associated with all MPN types including polycythemia vera, primary myelofibrosis (PMF), and essential thrombocythemia (ET); whereas *CALR* mutations are restricted to ET and PMF, and *MPL* mutations to ET. JAK2 V617F VAF significantly influences MPN subtype distribution: higher VAFs (>50%), often reflecting homozygous mutations due to copy-neutral loss of heterozygosity, are strongly associated with polycythemia vera and myelofibrosis, whereas lower VAFs, consistent with heterozygous mutations, are more commonly observed in essential thrombocythemia.[78] The factors that influence the progression of MPN driver mutant CH to specific MPN subtypes remains poorly understood.

The growth of DDR-mutated CH is generally modest in the absence of genotoxic therapy-induced selective pressure. *TP53* mutations are typically associated with a low clonal growth rate, which further decelerates with aging, whereas *PPM1D*-mutant CH demonstrates an intermediate growth rate.[69] Both *TP53*- and *PPM1D*-mutant clones are linked with a relatively average risk of progression to MN. This may help explain why MN uncommonly occur in patients with Li Fraumeni syndrome (pathogenic germline *TP53* variants) in the absence of prior genotoxic therapy.[22,23,79,80] In contrast, exposure to cytotoxic chemotherapy or radiation therapy drives expansion of *TP53*- and *PPM1D*-mutant CH clones and increases the risk of MN.[49,54,81] The selection advantage provided by somatic *TP53* mutations is not restricted to genotoxic therapies, but is also observed with lenalidomide[82] and MDM2 inhibitors.[83]

Although *NPM1* and *FLT3* are common driver mutations in *de novo* AML, they are rarely detected in CH. In the UK Biobank cohort, only 2 out of ~200,000 individuals were found to have a *NPM1* driver mutation with normal blood counts.[84] Both individuals developed AML within 6 months of the sample collection. The near lack of *FLT3* and *NPM1* mutations in CH studies may reflect a rapid progression to *de novo* AML.

Factors other than CH mutations play an important role in governing clone behavior and include both cell-intrinsic and extrinsic factors. The relevance of non-mutational factors is highlighted by several observations. Low frequency CH mutations (micro-CH) are ubiquitous with aging (>95% after 50 years old), however only a small minority of these clones expand to meet criteria for CHIP (VAF of 2%).[13] Parallel observations have been made in other somatic tissues including the skin and esophagus, which have frequent oncogenic mutations, but low overall rates of progression to carcinoma.[2,3] The same driver mutations have variable growth rates between different clones.[67,69] Expanded clones lacking known drivers are not uncommon, particularly at advanced ages.[27] While acquisition of somatic alterations is typically involved in driving progression to MN, a large proportion of *JAK2* CH that progresses to MPN[85] and a subset of *SF3B1* CH to MDS[86] without the acquisition of new somatic alterations.

Cell-intrinsic factors that govern clonal behavior beyond individual CH mutations are diverse, and several examples are highlighted below. Clinical markers of ineffective erythropoiesis, such as increased mean corpuscular volume (MCV) and red cell distribution width (RDW), as well as the presence of cytopenias, are associated with an elevated risk of MN even after accounting for specific CH mutations.[41] Germline genetic variation also plays a substantial role. In the UK Biobank cohort, 14 out of 98 CH predisposition genes were associated with increased risk of hematological malignancy, eight of which were linked with CH overall.[21] Moreover, CH carriers with germline variants in these 14 CH predisposition had a higher risk of developing a hematological malignancy compared with CH carriers lacking the variants.[21] The previously discussed *TCL1A* promoter variant, which confers gene-specific effects on CH predisposition, is also associated with slower expansion of non-*DNTM3A* CH.[67] In addition, germline pathogenic variants in *DDX41* exhibit a markedly higher penetrance of MN in men compared to women for unknown reasons.[87] Down syndrome (trisomy 21) is frequently associated with hematologic abnormalities, including neonatal transient abnormal myelopoiesis (TAM). TAM is a preleukemic syndrome unique to Down syndrome, which typically presents with circulating blasts that originate from the fetal liver and harbor somatic *GATA1* mutations, and resolves spontaneously within a few months.[88] Notably, approximately 20% of neonates with Down syndrome have detectable somatic *GATA1* mutations in the absence of hematologic features of TAM.[89]

Beyond selection of DDR mutations with genotoxic therapy, the extent to which cell-extrinsic factors influence CH progression to MN is less well established. Multiple preclinical models have demonstrated that systemic inflammation can promote the expansion of specific CH clones, particularly those with *TET2*- and *DNMT3A*-mutations.[90] However, there is limited data supporting this in humans. We recently identified a number of plasma proteins associated with CH (N = 34) and subsequent risk of progression to MN (N = 115) in the UK Biobank cohort.[91] The proteins associated with MN risk were enriched for involvement in regulation of the innate

and adaptive immune system, and improved risk prediction beyond clinical and CH-related features.[91] Longitudinal studies in humans are needed to further characterize the extent to which inflammatory stress might influence CH evolution.

CH Risk Stratification

CH is ubiquitous with aging, but progression to MN only occurs in a small subset of patients (<1% per year among those with CHIP). As knowledge of the factors driving progression of CH to MN increases, there has been growing interesting developing models to predict risk of progression. Such models are essential for consideration of interventional trials to prevent malignant transformation, given that the overall risk of MN in unselected CH populations is low, and therefore the potential harms of preventative approaches could outweigh their benefits. Recently, two groups developed models for predicting MN risk that have gained prominence in the CH field.[22,23] Both efforts used the UK Biobank, which is uniquely suited for this purpose due to its in-depth genetic (including CH mutation profiling) and longitudinal (>10 years) health information on over 450,000 individuals.

Weeks et al[23] employed a decision tree-based machine learning model to identify demographic factors, CH clone characteristics, and laboratory values predictive of incident MN. From this, they created the Clonal Hematopoiesis Risk Score (CHRS), which stratifies individuals with CHIP and CCUS into three risk groups. The estimated 10-year probability of incident MN was 52% in the high-risk group (1% of individuals), 8% in intermediate-risk group (10%), and <1% in low-risk group (89%). The CHRS model incorporates the following binary features, listed in order of decreasing risk contribution: presence of high-risk mutation, MCV ≥100, RDW ≥15, VAF ≥0.2, ≥2 CH mutations, presence of cytopenia, age ≥65 years, and presence of single *DNMT3A* mutation. Gu et al[22] used Cox regression to develop MN-predict, a set of models that estimate the risk of progression to MPN, MDS, or AML separately. These models rely on features similar to those in CHRS, but also allow for utilization of optional parameters such as BMI and clinical laboratory value, which can improve prediction accuracy.

However, it is important to note that both CHRS and MN-predict were developed using data from the UK Biobank, a cohort enriched for healthy volunteers predominantly of European ancestry, which limits their generalizability to other patient populations.[33] This cohort also lacks longitudinal blood count data to assess cytopenia persistence and bone marrow biopsies were not performed to exclude MN including MDS—both of which are required to meet diagnostic criteria for CCUS. To address these limitations, Xie et al[92] developed the clonal cytopenia risk score (CCRS) using real-world data from patients evaluated at tertiary referral centers who met diagnostic criteria for CCUS (and underwent bone marrow biopsy). The CCRS model stratifies individuals with CCUS into three risk groups based on three binary variables, listed in order of decreasing risk contribution: presence ≥2 mutations, platelet count <100 x 10^9/L, and presence of splicing factor mutation. The estimated two-year probability of incident MN was 37% in the high-risk group (10% of individuals), 14% in intermediate-risk group (39%), and 6% in low-risk group (51%). Notably, the incidence of MN observed in this real-world cohort was substantially higher than that observed among healthy participants in the UK Biobank cohort (~1%).[23] Further

refinement of CH/CCUS risk prediction models in real-world patient populations will be critical to guide future therapeutic intervention trials.

Interventional Studies in High-Risk CH

Advances in CH risk stratification have identified populations at significant risk of progression to MN based on clinical and molecular features,[22,23] thereby creating opportunities for therapeutic intervention trials. The increasing use of next-generation sequencing in oncology—both for detecting circulating tumor DNA and assessing for hereditary cancer syndromes—has also led to more frequent detection of CH as part of routine clinical care. Together, these developments have provided strong impetus to investigate targeted approaches to mitigate malignant transformation, particularly in individuals with high-risk clonal profiles. Several such approaches are now being investigated in clinical trials, which are summarized in Figure 2 and Table 1.

Ascorbic Acid (Vitamin C)

TET family methylcytosine dioxygenases, including TET2, catalyze the oxidation of methylated DNA, thereby promoting DNA demethylation, which has key roles in regulation of gene expression.[93] Loss-of-function mutations in *TET2* are very common in CH (second after only *DNMT3A* CH) and confer a fitness advantage to HSPCs by impairing DNA demethylation, ultimately resulting in enhanced self-renewal and impaired differentiation. Preclinical studies demonstrated that treatment with ascorbate (vitamin C), which is a cofactor for TET2, can restore TET2 activity in the setting of *TET2* haploinsufficiency, resulting in increased DNA demethylation, normalization of HSPC function, and impaired leukemogenesis.[94,95] This compelling preclinical data had led to the initiation of two clinical trials studying vitamin C in CCUS. The phase 2 trial of single-agent high-dose IV ascorbic acid in *TET2*-mutant CCUS (NCT03418038) has readout and did not identify any clinical responses at 20 weeks by International Working Group MDS criteria (out of 8 patients eligible for response assessment) or significant changes in *TET2* VAF.[96] Results from a phase 2 study of oral vitamin C in low-risk MN including CCUS (NCT03682029) are pending.

IDH1/2 Inhibition

Mutations in *IDH1* (R132) and *IDH2* (R140 or R172) are associated with high-risk CH and represent common early clonal leukemogenic events in MN. Isocitrate dehydrogenase (IDH) enzymes normally catalyze the conversion of isocitrate to alpha-ketoglutarate (αKG). These missense IDH mutations result in production of the oncometabolite 2-hydroxyglutarate (2-HG), which competitively inhibits αKG-dependent enzymes (including *TET2*), resulting in broad effects including altered metabolism, aberrant DNA and histone methylation, and differentiation blocks.[70] Mutant specific small molecule inhibitors of these enzymes are already approved for IDH-mutated AML, including ivosidenib and olutasidenib (*IDH1*), as well as enasidenib (*IDH2*). Given that IDH mutations are early clonal events in the natural history of MN and that IDH inhibitors have a reasonably good safety profile, IDH inhibition is an ideal target for initial CH intervention studies. Our group is leading two early phase clinical trials studying enasidenib in

IDH2-mutant (NCT06240754) and ivosidenib in *IDH1*-mutant CCUS (NCT05030441). There is also an active trial (NCT06566742) assessing olutasidenib in *IDH1*-mutant CCUS and low-risk MN. Initial results from our IDH1-mutant CCUS trial (median follow-up of 16 months) demonstrated that ivosidenib was well tolerated and induced high rates of hematologic response (>80%) and *IDH1* mutation clearance (>40%).[97]

Interluekin-1β (IL-1β) Inhibition

The relationship between CH, aging, and inflammation is complex and likely involves bidirectional causality between the three. This has been thoroughly reviewed in detail elsewhere.[98,99] In brief, both CH (particularly *TET2* mutant) and aging are associated with elevated levels of proinflammatory cytokines, which can provide a selection pressure for expansion of specific CH clones with a relative fitness advantage in the context of systemic inflammation (*TET2* and *DNMT3A* CH).[90] Preclinical studies in mice have shown that *TET2* loss in hematopoietic cells results in accelerated atherosclerosis and increased proinflammatory cytokines (including IL-1β and IL-6) secretion by macrophages.[100,101] Furthermore, IL-1 (which increased with aging) induces expansion of $Tet2^{+/-}$ HSPCs in mouse models of $Tet2^{+/-}$ driven CH, whereas genetic and pharmacological inhibition of IL-1 signaling impairs expansion of $Tet2^{+/-}$ clonal expansion.[102] These findings collectively provide a strong biological rationale for the therapeutic targeting of IL-1β in CH and CCUS, which has fueled subsequent clinical investigation in this space.

Canakinumab is an anti-IL-1β monoclonal antibody that inhibits IL-1β from binding to and activating the IL-1 receptor. The large phase 3 CANTOS trial assessed if treatment with canakinumab reduces major adverse cardiovascular events (MACE) in patients with prior myocardial infarction and an elevated baseline C-reactive protein (CRP) level.[103] The trial found that treatment with canakinumab (compared to placebo) resulted in ~15% relative risk reduction in MACE, increased incidence of fatal infections, and no difference in overall survival. Subsequent exploratory analyses of CANTOS showed that: CH mutations in *TET2* were more common than in *DNMT3A* (which could be related to the inclusion criteria requirement of an elevated CRP level); patients with *TET2* CH who were treated with canakinumab had a decreased risk of MACE (compared to placebo); and canakinumab treatment was associated with decreased risk of incident anemia and improved hemoglobin response (particularly among patients with concurrent CH mutations and anemia).[104,105] A randomized placebo controlled clinical trial (NCT05641831) of canakinumab in patients with CCUS is currently underway and aims to determine if canakinumab IL-1β inhibition can prevent or delay MN development (primary endpoint of time to MN). A recent small single arm trial of canakinumab in lower-risk MDS demonstrated a modest 17% overall response rate, with all responders (N = 4) having an IPSS-M score <0.[106]

The NLRP3 inflammasome pathway is aberrantly activated in many MN, leading to the caspase-1-dependent secretion of the pro-inflammatory cytokines IL-1β and IL-18, which drive inflammation and ineffective hematopoiesis.[107] The small molecule NLRP3 inhibitor DFV890 is

currently under investigation in low-risk MN including high-risk CCUS (NCT05552469) as well as *TET2*- and *DNMT3A*-mutant CHIP with coronary heart disease (NCT06097663).

TGF-β/SMAD Inhibition

Luspatercept is a recombinant fusion protein composed of the extracellular domain of the activin receptor type IIB and the Fc domain of immunoglobulin G1 that is FDA approved for treating anemia in patients with low-risk MDS and beta-thalassemia. It binds to multiple transforming growth factor-beta (TGF-β) superfamily ligands resulting in inhibition of SMAD2 and SMAD3 signaling, which promotes effective maturation of erythroid progenitor cells.[108] Luspatercept is particularly efficacious for treating anemia due to MDS with *SF3B1* mutations and/or ring sideroblasts,[109] which is characterized by pronounced ineffective erythropoiesis and an indolent clinical course.[110] Interestingly, no change in the VAF of *SF3B1* or other somatic mutations has been observed in luspatercept responders compared with nonresponders.[111] Additional clinical trials will be required to determine if luspatercept has disease-modifying activity in MDS or CCUS. A clinical trial (NCT06788691) of luspatercept in patients with CCUS is ongoing.

CDK4/6 Inhibition

Cyclin-dependent kinase 4 and 6 (CDK4/6) play a key role in cell cycle regulation, promoting progression through the G1/S checkpoint by phosphorylating the retinoblastoma protein (Rb). CDK4/6 inhibitors are used widely in combination with endocrine therapy to treat hormone receptor positive breast cancer, which are sensitive to the inhibitors due to intact Rb function and reliance on CDK4/6 signaling for cell cycle progression.[112] HSPCs are also reliant on CDK4/6 activity for cell cycle progression and undergo transient G1 arrest after CDK4/6 inhibition.[113] Trilaciclib is a CDK4/6 inhibitor that was developed to protect HSPCs from DNA damaging agent-induced myelosuppression by inducing G1 arrest prior to DNA damaging agent administration.[114] It is now FDA-approved for the prevention of chemotherapy-induced myelosuppression in patients with extensive-stage small cell lung cancer, a malignancy intrinsically resistant to CDK4/6 inhibition due to loss of Rb function, thereby explaining why trilaciclib does not compromise the antitumor efficacy of chemotherapy. Given the myeloprotective effects of trilaciclib, our group recently investigated the effect of trilaciclib on chemotherapy-related expansion of CH clones with DDR mutations (e.g. *TP53*, *PPM1D*, *CHEK2*). We found that trilaciclib reduced expansion of DDR CH by 32% when given prior to cytotoxic chemotherapy in four randomized clinical trials and in a *TP53* mutant CH mouse model.[115]

Given the poor outcomes associated with therapy-related myeloid neoplasms (tMN), strategies to mitigate cytotoxic chemotherapy-driven expansion of DDR CH, particularly *TP53*-mutant clones, are warranted and may reduce progression to tMN. A pragmatic approach is to quantify MN risk using validated clinical prediction models that integrate CH mutational data with routine laboratory parameters,[22,23,92] and to incorporate these individualized estimates when weighing the absolute benefit of adjuvant chemotherapy—whose survival advantage in some early-stage settings is modest—against long-term tMN risk. CH status could also be obtained from cell-free

DNA assays already obtained as part of standard oncologic care. Modeling analyses suggest that a subset of patients with early-stage breast cancer have a predicted MN risk that exceeds the expected absolute survival benefit from adjuvant chemotherapy (which frequently includes doxorubicin, a therapy associated with a high risk of MN), highlighting the potential utility of CH-based risk stratification to inform adjuvant treatment decisions.[49]

Hypomethylating Agents

Hypomethylating agents such as decitabine have demonstrated efficacy in most MN, while maintaining a favorable safety profile. A clinical trial (NCT06802146) evaluating decitabine/cedazuridine (an oral formulation of decitabine) in patients with high-risk CCUS is ongoing.

Metformin

Metformin is widely used due to being the first-line treatment for type 2 diabetes mellitus. It has a complex and incompletely understood mechanism of action that involves inhibition of hepatic gluconeogenesis and mitochondrial respiration.[89] A pair of preclinical studies from earlier this year demonstrated that the competitive advantage of murine *Dnmt3a* R878H HSPCs is dependent on increased mitochondrial respiration and can be reduced by treatment with metformin.[116,117] Functional studies revealed that metformin treatment normalized the aberrant DNA methylation and gene expression profiles driven by *Dnmt3a* R878H. In addition, analysis of the UK Biobank showed that metformin use was associated with a lower prevalence of *DNMT3A* R882 CH. A small phase 2 clinical trial (NCT04741945) is investigating metformin in patients with CCUS and low-risk MDS is ongoing.

Miscellaneous

Preclinical studies have demonstrated that statins can exert anti-cancer effects *in vitro*.[118] Statins also possess anti-inflammatory effects in addition to their lipid-lowering properties.[119] Observational data in MDS indicate that statin use is associated with improved overall survival and a reduced risk of progression to acute leukemia, as shown in a large retrospective database analysis employing propensity score matching.[120] These findings provided the impetus for a prospective clinical trial (NCT05483010), which is currently evaluating high-intensity statin therapy in patients with CCUS and low-risk MDS.

Curcumin, the active ingredient in turmeric, is a polyphenol with antioxidant and anti-inflammatory properties. It is currently being evaluated in a placebo controlled clinical trial (NCT06063486) of patients with low-risk MN including CCUS. The primary end point for the study is the change in inflammatory cytokine levels and inflammation-related symptomatology.

Clinical Trial Design Considerations

Designing interventional trials for CH presents significant challenges. Because CH is a premalignant condition, potential interventions must be risk-informed and have a favorable toxicity profile. Trials should have clinically meaningful end points and avoid the overmedicalization of otherwise healthy individuals with low-risk CH features. To date, CH trials have largely focused on patients with CCUS; accordingly, the following discussion of clinical trial design considerations centers on this population. CCUS is particularly appropriate for interventional trials, as the risk of progression to MN among individuals with CH appears to reside almost entirely in those who develop a preceding cytopenia.[121]

Most CH trials have modeled their key end points from those used in low-risk MDS, such as hematological improvement (Table 1). However, reduction in transfusion burden or infections are the only cytopenia-related outcomes recognized as clinically meaningful by the FDA in hematological malignancies. Thus, studies that show improvement in neutropenia, for example, may not be considered "clinically meaningful". The clinical relevance of currently accepted endpoints in studies of low-risk MDS remains under debate. For example, while there are multiple FDA-approved therapies for low-risk MDS that result in reduced transfusion burden, none have been prospectively shown to alter the natural history of the disease, such as improvement of overall survival.[122] Thus, it would be prudent for the CH field to learn from the persistent challenges faced in drug development for low-risk MDS[123] and design feasible studies that can demonstrate improvement in quality of life and the potential for disease modification.

Randomized, placebo-controlled trials with disease-free survival as the primary endpoint are the gold-standard for chemoprevention trials. However, this end point is limited by the natural history of CH, where progression typically occurs over many years. Among healthy individuals in the UK Biobank the 10-year risk of MN is approximately 52% for those with high-risk CH, a group that represents only 1% of individuals with CHIP/CCUS.[23] Intermediate-risk CH is more common (~10% of CHIP/CCUS), but only 8% of carriers progress to MN over a 10-year period.[23] Progression rates are higher among patients with CCUS evaluated at tertiary referral centers, but vary widely between studies ranging from ~20% at 4 years[92] to 95% at 10 years[26]. In addition, the absence of standard-of-care interventions may make participation in long-term placebo-controlled studies undesirable for some patients.[124]

Surrogate endpoints may enable more feasible trial designs but must be chosen carefully to ensure clinical relevance. Given that CH expansion is well-recognized to be part of the natural history of CH progression to MN, interventions that reduce the burden of high-risk CH clones (as reflected by VAF) should reduce MN development. However, the direct impact of a reduction in CH VAF on MN risk has not been measured in prospective trials. Furthermore, reductions in CH mutant VAF can be confounded by the variable distribution of CH-mutant cells across hematopoietic lineages, which in turn can fluctuate with clinical context (e.g. active infection).[125] However, the achievement of measurable residual disease (MRD) negativity in hematological malignancies is clearly associated with improved clinical outcomes across a variety of malignancies including chronic phase chronic myeloid leukemia, AML, acute lymphocytic

leukemia, and multiple myeloma.[126] Thus, we believe that achievement of MRD negativity for high-risk CH clones represents a valid potential surrogate end point for MN prevention. This outcome is feasible for potent targeted therapies directed against high-risk CH mutations.

Finally, the inherent rarity of high-risk CHIP/CCUS, especially specific biological/molecular subtypes, poses significant challenges in recruiting study participants. Currently, screening for CH is not part of standard clinical practice. While targeted sequencing to identify somatic mutations in myeloid driver genes is increasingly common in the work-up of unexplained cytopenias, it is not uniformly applied. Thus, identification of individuals with high-risk forms of CH is challenging. Disease rarity combined with inefficiencies within the current clinical trial infrastructure and difficulties in securing interest from pharmaceutical industry partners for rare diseases make high-risk, gene-specific CH trials challenging to conduct. Decentralized clinical trials (DCT) may represent an attractive approach for uncommon diseases. DCT are clinical studies in which some or all trial-related activities are conducted remotely using digital health technologies (e.g. telemedicine visit), instead of at a traditional centralized clinical research site (e.g. office visit at an academic medical center).[127] This model can reduce barriers to clinical trial participation including geographic distance, time toxicity, and financial burden. Hybrid versions can also be used which incorporate traditional design features (e.g. sample collection at local laboratories). DCT are best suited for well-characterized therapies with good safety profiles used in relatively healthy patient populations with a low rate of serious adverse events.

In summary, to fully realize the potential of CH interventional studies to prevent MN, innovative and flexible clinical trial designs are needed. Rare diseases are subject to similar unique investigational and regulatory approaches.[128] High-risk CH should be included under this umbrella. Adoption of the rare disease regulatory paradigm including flexible evidence standards, and consideration of alternative data sources (including historic controls) will be needed to facilitate development and approval of therapies for individuals with high-risk CH. Encouragingly, the FDA has recognized the need to evolve its regulatory strategies for personalized therapies in rare diseases and recently announced plans for new drug development processes in this space, including the Plausible Mechanism Pathway and the Rare Disease Evidence Principles program.[129]

Conclusions

The CH field has made remarkable progress in the short time since the seminal studies by Jaiswal et al[7] and Genovese et al[6] were published in 2014. These investigations provided an unprecedented window into the earliest stages of carcinogenesis, elucidating how intrinsic, environmental, genetic, and acquired factors—often in a mutation-specific manner—govern CH initiation, clonal dynamics, and risk of progression to MN. Much of this progress has been driven by large-scale population (e.g. UK Biobank) and longitudinal sequencing studies, which are only feasible due to the unique ability to sample the hematopoietic system through peripheral blood draw. In turn, these discoveries have enabled the development of risk stratification models to identify individuals with CH at high risk for progression to MN, thereby opening the door for therapeutic intervention trials, which are now actively enrolling patients.

Despite these advances, significant unanswered questions remain. Key priorities for the field include defining the cell-intrinsic and -extrinsic mechanisms underlying the distinct clonal behaviors of specific driver mutations over time (particularly splicing factor mutations); elucidating the basis of hematopoietic oligoclonality that emerges with aging and its frequent association with CH lacking a known driver; and determining whether therapeutic intervention can alter the natural history of CH.

Moving forward, translating insights from CH biology into effective invention approaches will require careful balancing of the risk and benefits of intervention, as the absolute risk for progression to MN is modest even among individuals with high-risk CH. Ultimately, these efforts will be essential for determining optimal clinical management of CH, which is increasingly being identified in clinical practice, and will deepen our understanding of early carcinogenesis in humans, regardless of the trial outcomes.

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Tables

Table 1. Overview of the interventional clinical trials in clonal hematopoiesis and clonal cytopenia of undetermined significance.

Study ID	Phase	Intervention	Mechanism	Population	Target Enrollment	Primary End Point(s)
NCT03418038	2	Vitamin C (IV)	Enhance TET2 activity	*TET2* CCUS	10	Hematologic response rate
NCT03682029	2	Vitamin C (oral)	Enhance TET2 activity	CCUS & low risk MN	109	Δ VAF at 12 months
NCT05030441	2	Ivosidenib	IDH1 inhibitor	*IDH1* CCUS	20	Hematologic improvement rate
NCT06566742	2	Olutasidenib	IDH1 inhibitor	*IDH1* CCUS & low risk MN	15	Safety
NCT06240754	2	Enasidenib	IDH2 inhibitor	*IDH2* CCUS	15	Best hematologic response
NCT05641831	2	Canakinumab	Anti-IL-1β	CCUS	110	Time to MN
NCT05552469	1	DFV890	NLRP3 inhibitor	CCUS & low risk MN	105	Safety
NCT06097663	2	DFV890 or	NLRP3 inhibitor	*TET2* & *DNMT3A* CHIP with CHD	31	Δ IL-6 & IL-18
		MAS825	IL-1β x IL-18 BsAb			Δ IL-6
NCT06788691	2	Luspatercept	TGF-β superfamily ligand trap	CCUS	50	Hematologic improvement rate
NCT06802146	1	Decitabine/cedazuridine	DNA hypomethylating agent	CCUS	108	Feasibility failure rate
NCT04741945	2	Metformin	Mitochondrial modulation	CCUS & low risk MDS	40	Safety & feasibility
NCT05483010	2	High-intensity statin	Anti-inflammatory	CCUS & low risk MDS	16	Δ CRP
NCT06063486	2	Curcumin	Anti-inflammatory	CCUS & low risk MN	30	Δ cytokines & symptoms

IV: intravenous; *TET2*: tet methylcytosine dioxygenase 2; CCUS: clonal cytopenia of undetermined significance; MN: myeloid neoplasm; Δ: change in; *IDH1*: isocitrate dehydrogenase 1; *IDH2*: isocitrate dehydrogenase 2; IL-1β: interleukin-1β; NLRP3: NLR family pyrin domain containing 3; IL-18: interleukin-18; BsAb: bispecific antibody; *DNMT3A*: DNA methyltransferase 3 alpha; CHIP: clonal hematopoiesis of indeterminate potential; CDH: coronary heart disease (CHD); TGF-β: transforming growth factor beta; MDS: myelodysplastic syndrome; CRP: C-reactive protein.

Figure Legends

Figure 1. Evolution of clonal hematopoiesis to myeloid neoplasms. Hematopoietic stem cells acquire somatic mutations throughout life. Infrequently, these mutations confer a selective growth advantage, resulting in clonal expansion, which is termed clonal hematopoiesis (CH). Several risk factors for CH have been identified, many of which preferentially select for specific mutations. Common routes of progression from CH to specific myeloid neoplasms are shown and are driven by the acquisition of additional cooperating mutations. CCUS: clonal cytopenia of undetermined significance; MDS: myelodysplastic syndrome; AML: acute myeloid leukemia; CMUS: clonal monocytosis of undetermined significance; CMML: chronic myelomonocytic leukemia; MPN: myeloproliferative neoplasm.

Figure 2. Summary of the intervention approaches to mitigate malignant transformation in high-risk clonal hematopoiesis and clonal cytopenia of undetermined significance. CH: clonal hematopoiesis; CCUS: clonal cytopenia of undetermined significance; IDH1/2: isocitrate dehydrogenase 1 and 2; TET2: tet methylcytosine dioxygenase 2; IL-1β: interleukin-1β; NLRP3: NLR family pyrin domain containing 3; TGF-β: transforming growth factor beta; SMAD: SMAD family member 2; CDK4/6: cyclin-dependent kinase 4 and 6.
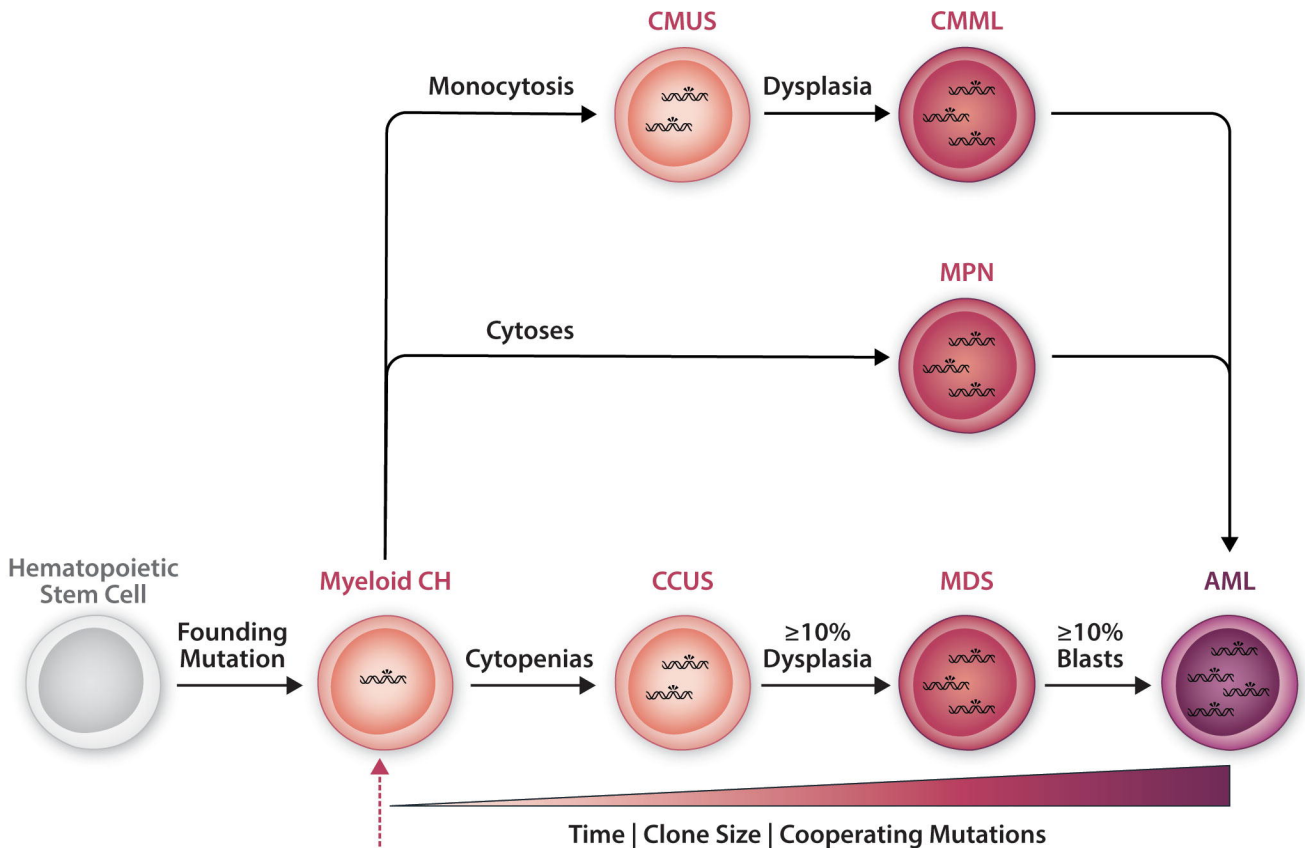
Approaches for High-Risk CH/CCUS Intervention

Treatment

IDH1/2 Inhibition
• Ivosidenib
• Olutasidenib
• Enasidenib

Krebs Cycle

TET2 Activation
• Vitamin C

Interleukin-1β Inhibition
• Canakinumab (anti-IL-1β)
• DFV890 (NLRP3 inhibitor)

Mitochondrial Modulation
• Metformin

Anti-Inflammatories
• Statins
• Curcumin

Hypomethylating Agents
• Decitabine

TGF-β/SMAD Inhibition
• Luspatercept

Prevention

Smoking Cessation
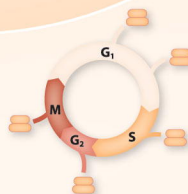
CDK4/6 Inhibition
• Trilaciclib