Clonal Hematopoiesis and Premalignant Diseases

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Clonal hematopoiesis (CH) arises when mutations in the hematopoietic system confer a fitness advantage to specific clones, thereby favoring their disproportionate growth. The presence of CH increases with age and environmental exposures such as cytotoxic chemotherapy or radiotherapy. The most frequent mutations occur in epigenetic regulators, such as *DNMT3A*, *TET2*, and *ASXL1*, leading to dysregulation of tumor suppressor function, pathogen response, and inflammation. These dysregulated processes elevate risk of overall mortality, cardiovascular disease, and eventual hematologic malignancy (HM). CH is likely acting as an initiating event leading to HM when followed by cooperating mutations. However, further evidence suggests that CH exerts a bystander influence through its pro-inflammatory properties. Delineating the mechanisms that lead to the onset and expansion of CH as well as its contribution to risk of HM is crucial to defining a management and intervention strategy. In this review, we discuss the potential causes, consequences, technical considerations, and possible management strategies for CH in the context of HMs and pre-HMs.

The accumulation of mutations in overtly healthy tissues occurs during normal aging with somatic mosaicism being present from birth (Vilkkil et al. 2001; De 2011; Blokzijl et al. 2016; Vattathil and Scheet 2016). Although the majority of these mutations are neutral and do not favor clonal expansion (Martincorena et al. 2017), mutation-driven dominant clones have been observed in nearly all tissues, especially in the hematopoietic system (Bowman et al. 2018). Hematopoietic stem cells (HSCs) initiate hematopoietic development and only seldomly

enter into the cell cycle for self-renewal and replication (Catlin et al. 2011), with each HSC acquiring approximately one exonic somatic mutation per decade in a largely stochastic manner (Welch et al. 2012). A subset of these acquired mutations confers a growth advantage (Welch et al. 2012), producing a clonal expansion that has been termed “clonal hematopoiesis” (CH).

Although the genes most commonly mutated in CH are the same genes that drive malignant conditions like myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML),

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most persons with CH are overtly healthy and never progress to MDS/AML. Nonetheless, CH is not an inert condition. CH clones have been known to show elevated inflammation, impaired tumor suppressor function, risk of cardiovascular disease (CVD), and risk of eventual hematologic malignancy (HM) (Jaiswal et al. 2014; Xie et al. 2014; Genovese et al. 2015; Rasmussen et al. 2015; Wu et al. 2015). Indeed, CH confers a rate of progression to any HM of 0.5%–1% per year (Jaiswal et al. 2014). This rate of progression is similar to monoclonal gammopathy of undetermined significance (MGUS) and monoclonal B-cell lymphocytosis (MBL) with the notable exception that CH can potentially produce any HM as it can arise in less committed hematopoietic precursors. Recent case-control studies have sought to fine-tune this risk especially in AML (Abelson et al. 2018; Desai et al. 2018). Nonetheless, the certainty of progression to any one condition in any single person is still not absolute given the presence of CH, mutation pattern, and/or any given clinical parameter. Data in model systems have shown that the growth and oncogenic progression of CH mutations may be influenced by extrinsic factors, varying from microbial and inflammatory signals to vitamin C (Cimmino et al. 2017; Cai et al. 2018; Meisel et al. 2018). Thus, the contribution of CH to disease is likely to involve a complex interplay between stochastic molecular events and their interaction with an individual's “exposome” (Wild 2005). Understanding CH mutations in the context of these extrinsic influences will enable development of better predictive models to improve decision-making when CH is detected. Although the cardiovascular risks associated with CH are important and considerable, the present review will focus on presenting what is known about the genetics, risks, and effects of CH in regard to premalignant conditions and hematologic malignancies.

CLONAL HEMATOPOIESIS

CH is functionally defined by the disproportionate growth of hematopoietic clones harboring a common somatic alteration. The first description of CH arose from excess skewing of the

1:1 ratio of maternal to paternal X-inactivation normally observed in women by Gale in 1991 (Lyon 1961; Gale et al. 1991). Subsequent advances in molecular techniques enabled Busque and colleagues, in 1996, to make quantitative assessments showing that neonates and young women had significantly less X-inactivation skewing than women >60 yr old, suggesting that CH is an age-related phenomenon (Busque et al. 1996). Follow-up studies revealed that somatic mutations are the probable underlying drivers of CH with the finding that elderly women with nonrandom X-inactivation were significantly more likely to harbor *TET2* mutations (Busque et al. 2012), which until then had been principally described as a driver event in AML causing epigenetic dysregulation (Delhommeau et al. 2009; Chou et al. 2011; Moran-Crusio et al. 2011). Individuals with CH typically have hematologic parameters that are indistinguishable from normal individuals inclusive of white blood cell count, hemoglobin, red cell distribution width (RDW), and platelets. Notably, the presence of both CH and high RDW synergizes so as to worsen outcomes (Jaiswal et al. 2014). Many groups have shown RDW to be a poor prognostic factor in a variety of settings, suggesting it to be a marker of chronic disease (Baba et al. 2018; Zurauskaite et al. 2018).

MUTATION LANDSCAPE OF CLONAL HEMATOPOIESIS

The most common mutations driving CH occur in the epigenetic modifier genes *DNMT3A*, *TET2*, and *ASXL1* (Jaiswal et al. 2014; Xie et al. 2014; Genovese et al. 2015; Steensma et al. 2015). The prevalence of *DNMT3A* mutations is highest, representing ~45% of all CH mutations. Mutations in *TET2* and *ASXL1* also occur commonly but each represent only 10% or less of all CH mutations (Heuser et al. 2016), although variable coverage of these large tumor suppressor genes on exome/panel-based sequencing suggests the actual mutation frequency may vary in different cohorts. Other less frequent somatic mutations have been shown to occur in *TP53*, *PPM1D*, *JAK2*, *CBL*, and spliceosome genes (Jaiswal et al. 2014; Xie et al. 2014; Geno-

vese et al. 2015; Steensma et al. 2015; Coombs et al. 2017). Although the genes most commonly mutated in CH are the same genes that drive malignant conditions like MDS and AML, most persons with CH never progress to MDS/AML and otherwise show normal hematologic parameters. These same genes commonly mutated in CH occur as a function of age both in CH (Jaiswal et al. 2014; Genovese et al. 2015) and in overt myeloid neoplasms such as MDS, myeloproliferative neoplasms (MPNs), and AML. Nonetheless, CH is not an inert condition. CH clones have been known to show elevated inflammation, impaired tumor suppressor function, risk of CVD, and risk of eventual HM (Jaiswal et al. 2014; Genovese et al. 2015). Indeed, CH confers a rate of progression to any HM of 0.5%–1% per year (Jaiswal et al. 2014). This rate of progression is similar to MGUS and MBL, with the notable exception that CH can potentially increase risk of any HM as it arises in less-committed hematopoietic precursors. Recent case-control studies have sought to define this risk especially in AML (Abelson et al. 2018; Desai et al. 2018). Nonetheless, the certainty of progression to any one condition in any single person is still not absolute. Data in model systems have shown that the growth and oncogenic penetrance of CH mutations may be influenced by extrinsic factors, such as microbial and inflammatory signals, and nutritional factors like vitamin C (Cimmino et al. 2017; Cai et al. 2018; Meisel et al. 2018). Thus, the contribution of CH to disease is likely to involve a complex interplay between stochastic molecular events and their interaction with an individual's "exposome" (Wild 2005). Understanding CH mutations in the context of these extrinsic influences will enable development of better predictive models to improve decision-making when CH is detected. Although the cardiovascular risks associated with CH are important and considerable and reviewed elsewhere (Sano et al. 2018; Calvillo-Argüelles et al. 2019), the present review will focus on presenting what is known about the genetics, risks, and possible management strategies for CH in the context of premalignant conditions and hematologic malignancies (Fig. 1).

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CLONAL HEMATOPOIESIS ELEVATES RISK OF PROGRESSION TO HEMATOLOGIC MALIGNANCIES

Premalignant states are well-established in hematologic malignancies, including MBL as a precursor to chronic lymphocytic leukemia (CLL) and MGUS as a precursor to multiple myeloma (MM), progressing to full disease at a rate of 1% to 2% per year (Kyle et al. 2002; Rawstron et al. 2008). Certain higher-risk presentations of CH can confer similar rates of disease progression. Indeed, Jaiswal and colleagues tested for CH across genes recurrently mutated in myeloid and lymphoid malignancies in the peripheral blood of 17,182 individuals via whole-exome sequencing (WES) at 70× median coverage depth (Jaiswal et al. 2014). Using variant allele fraction (VAF) cutoffs of 3.5% of single-nucleotide variants (SNVs) and 7% for indels, the prevalence of CH increased in an age-dependent manner from 2.5% in the 50–59 age group to 9.5% in the 70–79 age group. The presence of CH in any driver gene increased the risk of HM by >11-fold (hazard ratio [HR]: 11.1, 95% confidence interval [CI] 3.9–33). A large clone size (VAF >10%) further elevated this risk to 49-fold (HR: 49, 95% CI: 21–120). Although the fold change in HM risk is high based on these data, the absolute risk is relatively low with only 4% of individuals with a CH mutation progressing to develop a HM during the study period of 95 mo corresponding to 0.5% per year overall in the presence of any CH. Large clone size (VAF >10%) elevated the progression rate to 1.0% per year, approaching progression rates similar to MBL or MGUS. All-cause mortality associated with RDW from 1.6 (95% CI: 1.2–2.1) in the absence of CH to 3.7 (95% CI: 2.2–6.5) in the presence of CH when RDW $\geq$ 14.5%. Similar contemporaneous studies arrived at similar conclusions with regard to risk of progression to HR and rates of elevated CH (Xie et al. 2014; Genovese et al. 2015).

TECHNICAL DEFINITION OF CLONAL HEMATOPOIESIS

Uniform standards for the detection and reporting of CH are absent and urgently needed. Tech-

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Figure 1. Progression from somatic mosaicism through clonal hematopoiesis (CH) into hematologic malignancy. A combination of intrinsic and/or extrinsic events favors expansion of hematopoietic cells harboring mutations that confer a fitness advantage (e.g., mutations *DNMT3A*, *TET2*, and *ASXL1*) leading to CH. CH is pro-inflammatory and produces a positive-feedback loop that produces conditions favorable for the CH clone's expansion (i.e., its own expansion). Acquisition of subsequent mutations leads to clonal cytopenia of uncertain significance (CCUS), aplastic anemia (AA), or myelodysplastic syndrome (MDS), and other overt hematologic malignancies (e.g., acute myeloid leukemia). Aberrant pro-inflammatory signals also potentially exert a bystander influence on neighboring non-CH cells, thereby favoring adverse outcomes in co-occurring cancers and cardiovascular disease. It is speculated here that the pro-inflammatory environment generated by CH may promote protective survival signaling through pathways such as nuclear factor kappa B (NF- κ B). The colored squares under each cell depict indicate somatic mutations. CH mutations are early events and followed by additional genetic lesions, including cooperating cancer mutations. Age increases risk of CH and hematologic malignancies.

nical variations such as read depth, VAF cutoffs, germline variant exclusion criteria, and the breadth of genes interrogated have complicated the comparison of CH rates across cohorts. For example, deep targeted error-corrected sequencing revealed that mutations can be identified in the blood of nearly all persons aged 50–70 at a sensitivity of 0.03% VAF (Young et al. 2016). Although somatic mosaicism in hematopoietic cells is common, the mutations that confer a selective advantage are likely to be most important to the relevance of CH as a clinical condition. To this end, Steensma and colleagues proposed a VAF of >2% in defining CH (Steensma et al. 2015). This cutoff, however, is driven by available technological capacity and limited

clinical data. Recent studies have shown elevated risk even at >1% VAF for certain mutation classes (Abelson et al. 2018; Desai et al. 2018). Studies that are scientifically, statistically, and clinically principled are required to establish more formal thresholds.

Overall, the true prevalence of CH is likely underestimated in most studies. For example, whole-exome studies performed at 70×–80× median depth of coverage will only achieve <5% sensitivity for mutations occurring at 2% VAF based on next-generation sequencing (NGS) sensitivity estimates modeled by Shin et al. (2017), thereby missing the vast majority of CH mutations at lower VAF. Earlier whole-exome studies (Jaiswal et al. 2014; Genovese

et al. 2015) were also lacking coverage for most coding exons for *TET2* (Genovese et al. 2014; Jaiswal et al. 2014). There is, moreover, an ascertainment bias in the selection of genetic events driving CH in which studies have generally examined small variants related to progression to HM. In an analysis of 11,000 adults of all ages, whole-genome sequencing (WGS) studies by Zink et al. (2017), searching for mutations in candidate and noncandidate driver genes, yielded a significantly higher prevalence of CH than what had previously been reported (Jaiswal et al. 2014; Genovese et al. 2015; Zink et al. 2017). With the inclusion of both leukemia driver mutations and nondriver mutations, ~50% of those 85 and older had evidence of CH in this cohort, compared with ~20% in data from other studies (Jaiswal et al. 2014; Genovese et al. 2015; Zink et al. 2017). More recently, Loh and colleagues showed clonal expansions in which CH is defined by larger-scale mosaic chromosomal alterations (Loh et al. 2018). As consensus forms and depth, uniformity, and breadth of coverage are standardized, the actual prevalence is likely >20%–30% in persons 50 years of age and above based on two recent high-depth studies (Abelson et al. 2018; Desai et al. 2018) as well as data from blood of solid tumor patients derived from MSK-IMPACT (Coombs et al. 2017).

Finally, different terminologies are currently being used to describe similar states of clonal expansion in the absence of cytopenia or dysplastic hematopoiesis. CH and “clonal hematopoiesis of indeterminate potential” (CHIP) roughly refer to the same hematologic process with differing levels of specificity (Bowman et al. 2018). CH has a broader definition as it is inclusive of any disruptive somatic event that attains a fitness advantage exceeding the background level of somatic mosaicism—not necessarily occurring in a HM-associated driver such as DNA structural abnormalities or skewed X-inactivation. The term age-related clonal hematopoiesis (ARCH) emphasizes the age association with the acquisition of such mutations but would seem to exclude CH mutations driven by therapy. In reality, although age is a principle driver, the events driving the expansion of CH clones are unknown in many cases and may arise from

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prior therapy, chemical, immunological, infectious, and age-related events that initiate and/or select particular clones.

CCUS AND ICUS

Idiopathic cytopenia of undetermined significance (ICUS) was proposed as a classification in 2005 and was defined as persistent cytopenias without evidence of dysplasia in the bone marrow and with normal cytogenetics, in the absence of any secondary cause (Wimazal et al. 2007). The largest study to date on patients determined to meet strict criteria for ICUS showed this entity to be quite rare (10 of 2899 patients over 12 yr) but evolution to MDS was common (6/10) and thus supports the hypothesis that this might actually represent a pre-MDS clinical phase (Steensma 2012). With better diagnostic tools incorporating standard karyotypic and targeted sequencing analyses, diagnosing MDS has become significantly more streamlined (Steensma et al. 2015). There remain, however, large portions of individuals with persistent cytopenias and somatic mutations in the absence of overt dysplasia. These instances of normal marrow morphology, but persistent cytopenias in the presence of somatic mutations, underscore our need for a more inclusive classification system (Jaiswal et al. 2014; Genovese et al. 2015; Steensma et al. 2015). When the term ICUS was derived, the ability to universally sequence patient samples was lacking, and thus it was unclear if this was a clonal process. Although many of the patients in the limited analysis above did eventually develop MDS, it remains unclear whether any of these patients had or developed somatic mutations. In an analysis of a clinical cohort of patients who met criteria for ICUS, between 21% and 33% had CH-associated mutations, and this prevalence was higher than the background rate of CH in an age-matched population (Hall et al. 2014; Kwok et al. 2014). In addition, these clonal cytopenias have a similar prevalence as MDS (Steensma et al. 2015). Yet, it should also be noted that progression of these clonal cytopenias to MDS is not universal, and so they remain distinct entities. Given our better understanding of ICUS and CH, it was proposed

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that we classify patients meeting criteria for ICUS and harboring CH-associated somatic mutation as CCUS. These entities exist on a spectrum with nonclonal ICUS having a very low progression risk and CCUS having a still low but increased risk of progression to MDS (Steensma et al. 2015).

CLONAL HEMATOPOIESIS IN APLASTIC ANEMIA

CH is a common finding in individuals with aplastic anemia (AA). In a study of 439 patients with AA undergoing immunosuppressive therapy (IST), 47% of patients showed evidence of CH (Yoshizato et al. 2015). Unlike CH in the general population, the predominant mutations were *BCOR/BCORL1*, *PIGA*, *DNMT3A*, and *ASXL1*, accounting for >75% of CH. Mutations in *TET2* were notably underrepresented. Selection pressure in the context of bone marrow failure thus favor patterns of clonal dominance that differ from age-related CH. The prognostic impact in AA varied dependent on the mutation with *PIGA*, *BCOR*, and *BCORL1* mutations predicting better improved overall survival (OS) and progression-free survival (PFS) with immunosuppressive therapy being compared than *TP53*, *DNMT3A*, *JAK2*, and *ASXL1*, showing improved OS (HR: 0.27, 0.09–0.78; *P* = 0.016). *PIGA* and *BCOR/BCORL1* mutations showed clonal attrition over time while *DNMT3A* and *ASXL1* mutations expanded. Despite mutational commonalities with MDS (e.g., *DNMT3A*, *ASXL1*, *RUNX1*, splicing), the average clone size of CH in the AA cohort was lower (<10% VAF) and the clone size substantially rapidly expanded 6 mo post-IST.

Overall, the heightened prevalence of CH mutations that are found in the general population among patients in premalignant states or smoldering disease suggests their causal influence in the stepwise progression to overt leukemias. These findings thus suggest the usage of mutation screening in identifying at-risk persons and, given the high prevalence of CH in the general population, the importance of identifying better methods of predicting who will progress to overt leukemia.

FOUNDER CLONAL HEMATOPOIESIS: KNUDSONIAN PROGRESSION TO LEUKEMIA

Genetics of cancer development are largely modeled around the Knudson “two-hit hypothesis” (Knudson 1971), and the available data suggest that this principle can govern progression from CH to overt leukemia. Accordingly, mutation of epigenetic regulators, such as *DNMT3A*, *TET2*, or *ASXL1*, commonly seen in CH, represent an initial “hit.” The ensuing epigenetic remodeling leads to premalignant changes—for example, reduced *GATA2* function leading to impaired differentiation (Madzo et al. 2014; Shih et al. 2015). A subsequent “second hit” promotes progression to overt malignancy. For example, a second-hit mutation in *NPM1* produces AML whereas a second-hit mutation in *RHOA* produces angioimmunoblastic T-cell lymphoma (AITL) (Tiacci et al. 2018). Thus, founder CH mutations show multilineage oncogenic potential that is determined, at least partly, by subsequent oncogenic mutations.

Recent studies from Jan and colleagues and Shlush and colleagues have delineated the clonal trajectory from the early CH-like mutations to overt AML through sequencing of preleukemic stem and progenitor cells (pre-LSCs) (Jan et al. 2012; Shlush et al. 2014). Through single-cell sequencing, Jan and colleagues described a stepwise accumulation of mutations arising initially in pre-LSCs but eventually developing into LSCs and overt AML on acquisition of secondary mutations (*FLT3*-ITD). *FLT3*-ITD was not present in any of the pre-LSCs with several mutations, including *SMC1A*, and *TET2* only present in the pre-LSCs. Similarly, Shlush and colleagues analyzed highly purified HSCs, with progenitor and mature cell fractions from the blood of AML patient fractions and using deep targeted sequencing with a read depth of ~250×, found recurrent *DNMT3A* mutations at high allele frequency but without the co-occurring *NPM1* mutations found in the AML blasts. Consistent with CH and previous mouse model data (Jan et al. 2012; Kim et al. 2013), these *DNMT3A* mutated pre-LSCs showed a self-renewal advantage over nonmutated HSCs in xenografts.

These data together provided support for the presence of pre-LSCs. Further analysis revealed these pre-LSCs exist in remission samples, suggestive of both chemotherapy resistance and cell population poised for relapse (Jan et al. 2012; Shlush et al. 2014).

CH only rarely progresses to AML. Case-control studies from multiple independent teams have sought to better define factors that distinguish CH from pre-AML (Abelson et al. 2018; Desai et al. 2018). Desai and colleagues deeply sequenced banked peripheral blood specimens from the Women's Health Initiative (WHI) study at a median of 9.6 yr before AML diagnosis alongside matched control participants who did not develop AML during an equivalent follow-up period. Overall, mutations were more common in pre-AML cases versus AML-free controls when present at >1% VAF (OR: 4.86, 3.07–7.77; $P = 3.8 \times 10^{-13}$). Particular mutation patterns conferred varying levels of risk when adjusted for age and co-mutations. Multiple mutations in *DNMT3A* or *TET2* were more adverse than single mutations: single *DNMT3A* (OR: 2.11, 1.19–3.75; $P = 0.011$); 2+ *DNMT3A* mutations (OR: 12.6, 3.02–52.92; $P < 0.001$); single *TET2* (OR: 3.29, 1.38–7.83; $P = 0.005$); and 2+ *TET2* (OR: 69.3, 3.76–1280.7; $P < 0.001$). The risk of AML progression for *DNMT3A* and *TET2* mutations was also dependent on allelic fraction with >10% VAF conferring more risk. The converse was true for mutations at any VAF > 1% in *TP53* (OR: 50.9, 2.76–943.01; $P < 0.001$), *IDH1* or *IDH2* (OR: 29.6, 1.51–582.25; $P < 0.001$), *JAK2* (OR: 5.83, 0.79–43; $P = 0.042$), and spliceosome genes (OR: 7.72, 1.75–34.02, $P = 0.002$). The concurrent study by Abelson and colleagues, using an independent cohort, revealed similar findings with regard to mutation-specific risks. Additionally, subsequent work by Young and colleagues, using a cohort comprised of the Nurses' Health Study and Health Professionals Follow-up Study found a similar elevated risk of AML with mutations at $\geq 1\%$ VAF (OR: 5.4, 1.8–16.6; $P = 0.003$) as well as elevated risk with *DNMT3A* R882H or R882C variants (OR: 7.3, 1.5–34.7; $P = 0.01$) (Calvillo-Argüelles et al. 2019). All studies found that pre-AML cases were devoid of *FLT3* or *NPM1*

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mutations with the exception of one case in the study by Desai and colleagues in which longitudinal follow-up at 1-yr postbaseline acquired an *NPM1* mutation followed by AML diagnosis nearly a month later. Abelson and colleagues further developed a predictive model heavily dependent on high RDW as an informative predictor of AML as much as 1 yr before diagnosis from readily available electronic health record data. However, improved sensitivity and specificity are required for a rare disease such as AML. Overall, the data from both studies suggest that, with further study and improved models, a monitoring strategy combining readily available EHR parameters, sensitive mutation testing, and perhaps other molecular parameters, can identify persons at higher risk of progression from CH to HM.

BYSTANDER CLONAL HEMATOPOIESIS: ACTION AT A DISTANCE

The effects of CH on cancer development likely extend beyond the initial genetic hit in the two-hit model of oncogenesis. Preclinical and epidemiological evidence suggests that CH mutations can produce a chronic inflammatory state (Cook et al. 2017; Cull et al. 2017; Fuster et al. 2017; Sano et al. 2018), which is known to be oncogenic and exert prosurvival effects outside the context of CH via multiple mechanisms, including NF- κ B activation (Philip et al. 2004). *TET2*-deficient macrophages produce elevated and prolonged expression of pro-inflammatory factors (e.g., IL-1, IL-6, TNF α , CCL-5, CXCL9) (Zhang et al. 2015; Fuster et al. 2017; Cai et al. 2018; Sano et al. 2018). Moreover, hematopoietic cells showing *TET2*-driven CH gain a growth advantage in the presence of an inflammatory environment containing TNF (Abegunde et al. 2018). Thus, CH simultaneously appears to provide an advantage to affected cells under inflammatory conditions while also propagating the inflammation. Cook and colleagues evaluated human serum and found associations between CH mutations and inflammation, revealing elevated IL-6 with *TET2*-driven CH and elevated CCL11 with *DNMT3A*-driven CH. CH of large clone size of >10% presented with elevated TNF α (Cook et al. 2017).

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Consistent with this concept, studies of solid tumor and multiple myeloma patients with incidental findings of CH show that CH-positive patients experience worse outcomes (Coombs et al. 2017; Mouhieddine et al. 2018). However, further studies are needed to characterize the inflammatory factors in the serum of these patients and determine if CH-driven signaling is enabling tumor survival or whether tumor-driven inflammation is causing expansion of CH clones or, as is likely, an interplay of both.

CLONAL HEMATOPOIESIS IS ASSOCIATED WITH ADVERSE SOLID TUMOR OUTCOMES

Coombs and colleagues systematically evaluated the impact of CH in solid tumor patients using the MSK-IMPACT panel (Coombs et al. 2017). In a large analysis of ~9000 individuals, ~25% of solid tumor patients had incidental CH and 4.5% harbored mutations in genes typically associated with leukemia and with VAF $\geq 10\%$ (presumptive drivers, CH-PD). Importantly, CH-PD was associated with shorter 24-mo survival: OS: 0.48 (0.40–0.57, 95% CI) with CH-PD versus 0.54 (0.52–0.56, 95% CI) without CH-PD. Cumulative incidence of secondary HM was significantly higher with CH-PD with 3.2% (1.4%–6.2%) of patients with CH-PD progressing to HM within 18 mo versus 0.3% (0.2%–0.6%) of patients without CH-PD ($P < 0.001$). Although the prevalence of CH was higher than previously reported in the general population by previous studies (Jaiswal et al. 2014; Genovese et al. 2015), normalization of sequencing and analytical methodologies is needed to make an accurate comparison given the differences in depth of coverage, limits of detection, and analytics. The study also found that *PPM1D* and *TP53* were associated with prior exposure to chemotherapy and radiotherapy, a finding similar to cohorts of patients with lymphoma and myeloma before ASCT (Abdel-Wahab et al. 2012; Husby et al. 2018; Mouhieddine et al. 2018).

Clonal Hematopoiesis and Risk of Therapy-Related Myeloid Neoplasms

The co-occurrence of CH with other cancers is known to result in worse outcomes in these in-

dividuals and an increased risk for treatment related myeloid neoplasms (t-MNs). In a small case-control study, Takahashi and colleagues showed the prognostic impact of CH mutations before receiving chemotherapy or radiation treatment. The HR for development of t-MNs in those with CH as compared with those without CH was 13.7, $P = 0.013$. Additionally, cumulative incidence of t-MNs at 5 yr was significantly higher in patients with CH (30% [95% CI: 16%–51%] vs. 7% [95% CI: 2%–21%], $P = 0.016$) (Takahashi et al. 2017). Given the universally poor outcomes that exist in those that develop t-MNs, close monitoring of blood counts and clone size in individuals with malignancies and co-existing CH could prove to be beneficial, although how to intervene should it become evident that a t-MN is likely requires further investigation and should be performed as part of a clinical trial.

ASSOCIATIONS BETWEEN CH AND MULTIPLE MYELOMA OUTCOMES

Mouhieddine and colleagues evaluated the impact of CH mutations on multiple myeloma (MM) outcomes post-ASCT in the presence and absence of the immunomodulatory (IMiD) agents lenalidomide and pomalidomide (Mouhieddine et al. 2018). HSCs of 629 MM patients were deeply sequenced to nearly 1000 $\times$ depth of coverage. Overall, having a CH mutation was associated with inferior OS and PFS in MM.

Importantly, the investigators found that the adverse impact of CH seen in MM patients was obviated by IMiD maintenance (lenalidomide, pomalidomide, etc.) therapy after ASCT such that OS and PFS differences were no longer significant. Although a mechanism remains to be described, one possibility is that IMiD therapy is acting via suppression of inflammatory factors normally up-regulated by CH such as TNF α , IL-6, IL-1, and IL-12 (Stahl and Zeidan 2017), thus preventing prosurvival signaling such as NF- κ B in the myeloma cells. Given the pleiotropic effects of IMiDs, further study is required to delineate the precise mechanism.

INHERITED PREDISPOSITION TO CH

Data suggest an inherited risk of CH in some cases, although this does not account for the majority of patients with CH. Zink and colleagues showed that an SNP (rs34002450) in the telomerase reverse transcriptase (*TERT*) was associated with increased CH (OR: 1.37; $P = 7.4 \times 10^{-12}$). In a study of 151,202 individuals with banked blood samples, Loh and colleagues showed several rare mosaic chromosomal alterations (mCA) that were associated with inherited risk for developing CH mutations (Loh et al. 2018).

Other groups have also shown a heritable aspect of CH. In an analysis of 2530 women, *TET2*, but not *DNMT3A*, was shown to have a fairly high risk of heritability as a function of age, between 2.24 and 2.65 times the normal risk in women >55 yr and 65 yr, respectively (Buscarlet et al. 2017). Interestingly, in the allogeneic transplantation setting, matched sibling donors who were donating to a sibling with myeloid disease were more likely to have CH compared with those donating to a sibling with a lymphoid malignancy (19.2% vs. 6.3%).

In addition, an analysis of several young AML patients with high mutational burden and genomic evidence of methylation damage, revealed germline mutations in *MBD4*. It appears that loss-of-function mutations in *MBD4* led to inactivating mutations in *DNMT3A* and acquisition of other high-risk mutations such as *IDH1* and *IDH2*, akin to the process by which CH leads to AML, albeit at an accelerated rate (Sanders et al. 2018). Methyl-binding domain 4 (*MBD4*) is a DNA glycosylase responsible for the removal of mispaired thymine as a function of base excision repair due to aberrant 5mC deamination.

PRECLINICAL DATA ON MODIFICATION OF CH

Intervention in CH first requires the development of evidence-based guidelines for determining at-risk people who are destined to develop CH-related diseases as well as data to prove that intervention/modification of CH is

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feasible and will ultimately lead to prevention of disease. Although data thus far suggest an elevated risk of AML mutations in certain genes, high allelic fraction, and multiple mutations, the risk of progression is relatively low (Genovese et al. 2014; Abelson et al. 2018; Desai et al. 2018). There have been several preclinical investigations at possible modes of intervention.

TARGETING HIGH-RISK MUTATIONS IN CH

Inhibitors of *JAK2*, *IDH1*, and *IDH2* are Food and Drug Administration (FDA)-approved targeted therapies with favorable safety profiles. The *JAK2* inhibitor ruxolitinib is used in *JAK2*+ myelofibrosis and has been shown to reduce symptom burden while slightly reducing the mutant allele burden (Verstovsek et al. 2012). *IDH1* and *IDH2* inhibitors ivosidenib and enasidenib respectively, are both approved for use in relapsed/refractory AML (Stein et al. 2017; DiNardo et al. 2018) and are currently being investigated in clinical trials for use in other *IDH1*+ and *IDH2*+ MPNs as well as upfront in AML in combination with induction chemotherapy and in MDS (NCT03839771, NCT03515512, NCT03471260). There are, currently, no clinical trials investigating the safety or efficacy of targeting either *JAK2*, *IDH1*, or *IDH2* in the context of CH. Given the greater tendency of *IDH1/2* and *JAK2* mutations in CH to progress to AML (Desai et al. 2018), targeting these mutations with already approved drugs needs requires investigation, most likely in the context of patients showing abnormal hematological parameters.

Vitamin C to Compensate for *TET2* Haploinsufficiency

TET2 mutations exert their effects through altered methylation patterns in affected cells, ultimately leading to CH and possibly MDS or AML. Restoration of normal *TET2* enzymatic function was hypothesized to halt or reverse this process. This hypothesis was tested in a *TET2* knockdown mouse model (Cimmino et al. 2017). Reversible *TET2* knockdown recapitulated the effects of *TET2* deletion, leading to

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aberrant HSC self-renewal and disease development. In this same mouse model, exogenous vitamin C was shown to promote restoration of normal *TET2* enzymatic function by increasing DNA demethylation in embryonic stem cells and induced pluripotent stem cells leading to enhanced 5-hydroxymethylcytosine (5-hmC) levels (Cimmino et al. 2017). Studies suggesting that this may be true in humans are lacking.

Low Glucose and Metformin

Tet2 protein is destabilized by high glucose levels, a process mediated by the inhibition of AMP-activated protein kinase (AMPK)-dependent phosphorylation of TET2 at serine-99, leading to marked reduction of 5-hydroxymethylcytosine levels (Wu et al. 2018). This effect was rescued by metformin in mouse models. Thus, loss of *TET2* enzymatic function via protein destabilization has the potential exacerbate the loss of *TET2* function observed in *TET2*-driven CH, thereby potentiating the effects of CH. Interestingly, large epidemiological analyses have suggested that diabetes is linked to an increased risk for cancer, with type 2 diabetes conferring a 20% increase in HM risk (Gallagher and LeRoith 2015; Lauby-Secretan et al. 2016). Thus, the complex interplay of diabetes and cancer requires further study in the context hematopoiesis.

Inhibition of Inflammation

CH is itself pro-inflammatory and simultaneously confers a fitness advantage under pro-inflammatory conditions (Cook et al. 2017; Cull et al. 2017; Abegunde et al. 2018). Thus, intervening in this pattern of runaway inflammation may mitigate the effects of CH. In CVD, it has already been shown that IL-1 blockade with canakinumab shows improved responsiveness in patients with *TET2*-driven CH (Svensson et al. 2018). In an unrelated preclinical study, Cai and colleagues exposed *TET2* knockout (*TET2*-KO) mice to inflammatory stress, leading to consistently higher levels of IL-6 being seen in these mice. IL-6 was found to result in hyperactivation of the *SHP2*-*STAT3* signaling axis and increased

expression of anti-apoptotic long noncoding DNA, *Morrbid* (Cai et al. 2018). Inhibition of IL-6 signaling via knockout of *Morrbid* or via small-molecule inhibition on the *SHP2*-*STAT3* pathway with SHP099 or E330 impaired clonal expansion of *TET2* KO HSPCs. Thus, inhibition of IL-6, IL-1, or other pro-inflammatory cytokines using either directed monoclonal antibody or anti-inflammatory small molecules may have the potential for modification of CH and/or mitigating the bystander effects of CH mediated through inflammatory signaling.

CLINICAL FOLLOW-UP OF PATIENTS WITH CLONAL HEMATOPOIESIS

CH is being increasingly detected in patients during workup of cytopenias and as part of tumor genomic reports for both solid and hematologic malignancies. As data on the effect of CH accumulate, there is obvious clinical interest in managing the risks associated with CH. Because CH is associated with increased cardiovascular mortality, the presentation of CH—particularly mutations in *DNMT3A* and *TET2* at high VAFs and/or multiple mutations—should prompt a referral to cardiology for cardiovascular risk assessment and management. The presence of high-risk CH in patients with malignancy (*TP53*, *PPM1D*, clonal complexity, high VAF CH defined as >10% VAF, *DNMT3A*-R882) may influence decisions regarding the use of radiation or chemotherapy in some cases. For example, the presence of these high-risk mutations could prompt a discussion in the setting of autologous transplant if there are alternative therapies available. Regardless, the risks and benefits of either approach needs to be discussed with patients. In the absence of data to support repeat mutation testing, patients with CH should be followed with blood counts only as standard of care and repeat testing of mutations should only be considered as part of a clinical trial or in the context new or worsening cytopenias.

CONCLUDING REMARKS

The prevalence of CH is more common than originally put forward given recent findings

from high-depth, high-sensitivity sequencing studies (Young et al. 2016; Coombs et al. 2017; Abelson et al. 2018; Desai et al. 2018; Calvillo-Argüelles et al. 2019; Young et al. 2019). Current evidence suggests mutations of >1% VAF can be of clinical relevance for progression to malignancy (Abelson et al. 2018; Desai et al. 2018; Young et al. 2019). Standardization of methodologies and development of evidence-based guidelines are required to determine detection limits and parameters for identification of clinically significant CH mutations. In addition to providing founding genetic lesions for hematologic cancers, CH is a predictor of adverse risk in existing cancers ranging from solid tumors to multiple myeloma (Coombs et al. 2017; Mouhieddine et al. 2018), suggestive of a CH-driven bystander influence. Further studies are required to ascertain whether this possible bystander influence is mediated through inflammation or by other mechanisms. CH further confers elevated susceptibility to secondary therapy-related HMs (Coombs et al. 2017; Takahashi et al. 2017) and thus potentially impact guidelines for use of radiotherapy and/or chemotherapy in cancer patients who present with CH as a comorbidity.

Preclinical data suggest that CH may be intervened on, although any intervention would require considerable risk–benefit analysis. Preliminary evidence suggests that vitamin C, metformin, and anti-inflammatory agents may represent possible modifying interventions in CH. On further preclinical evaluation and confirmation in CH-focused studies, observational trials may be warranted in humans to test the impact of potential interventions on CH clones — preferably in patients already exposed to these drugs. Regardless, screening of CH in individuals at high risk of myeloid neoplasms could prove to be beneficial right now in cancer patients undergoing genotoxic therapy. Overall, management guidelines for CH are urgently needed for both HM and CVD risk. Thus, formation of an international advisory committee of clinical experts, precision medicine specialists, and epidemiological and laboratory medicine experts is required.

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