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Clonal Hematopoiesis and risk of Acute Myeloid Leukemia

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ABSTRACT

Acute Myeloid Leukemia, the most common form of acute leukemia in adults, is an aggressive hematopoietic stem cell malignancy that is associated with significant morbidity and mortality. Though AML generally presents *de novo*, risk factors include exposure to chemotherapy and/or radiation, as well as both familial and acquired bone marrow failure syndromes. Clonal Hematopoiesis (CH) refers to an expansion of blood or marrow cells resulting from somatic mutations in leukemia-associated genes detected in individuals without cytopenias or hematological malignancies. While CH is considered part of normal ageing, CH is also significantly associated with cardiovascular disease, solid tumors, and hematological malignancies. In this review, we will discuss evidence linking CH with the development of AML, as well as describe challenges in and strategies for monitoring patients with high risk CH mutations.

Introduction

Acute Myeloid Leukemia is the most common acute leukemia in adults with a median age at diagnosis of 66 years according to the SEER database [1]. In the United States, the age-adjusted incidence of AML in 2007–2011 was 13.0 per 100,000 persons per year with a mortality of 7.1 per 100,000 persons per year [1]. For older patients, long-term outcomes are dismal, with cure rates of less than 10% and median survival of less than a year [2,3]. The pathogenesis of AML is characterized by serial acquisition of somatic mutations, but how and when these events occur is unknown. Several large-scale sequencing studies have shown that acquired mutations in leukemia-associated genes, are common in older people and associated with an increased risk of hematological malignancies [4–6]. Clonal Hematopoiesis (CH) refers to an expansion of blood or marrow cells resulting from somatic mutations in leukemia-associated genes detected in individuals without cytopenias or hematological malignancies [7]. CH is believed to occur as a result of age-related hematopoietic stem cell attrition, combined with acquired clonal populations of cells with enhanced fitness and survival [8]. With novel deep-sequencing technologies, CH mutations have found to be nearly ubiquitous in older patients [9]. Thus, clearly, the frequency of detectable mutations is much higher than the rate of hematological malignancies in the general population. Identification of cell-intrinsic and extrinsic factors related to the malignant evolution of CH is an area of intensive research and will hopefully lead to strategies for early detection, monitoring, and, eventually, disease interception.

CH and AML risk in normal populations

The association between CH and the development of AML was established by two seminal studies that compared peripheral blood samples from healthy individuals banked several years before the diagnosis of AML to matched controls who never developed AML

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Table 1
Clinical data from studies evaluating risk of AML with the presence of CH mutations in the general population C.I.: Confidence Interval.

	Study design	Prevalence of CH in cases and controls	VAF cut off	Association with CH with AML risk
Desai et al. [10]	Case control: 212 AML cases and 212 age matched controls	Cases: 68.62% Controls: 30.94%	1%	● Odds of developing AML: OR 4.86 (95% C.I. 3.07–7.77, P < 0.001) ● High risk mutations: *TP53, IDH1/2,* Spliceosome, *TET2, DNMT3A* ● Increased risk with clonal complexity ● Increased risk with more than one variants in *DNMT3A* and *TET2* ● Increased risk with high VAF (> 10%) ● Shorter time to AML with *TP53*, clonal complexity, *PHF6* and *RUNX1*
Abelson et al. [11]	Case Control: 95 AML cases and 414 age matched controls	Cases: 73.4% Controls: 36.7%	0.5%	● Risk of developing AML over 10 years per 5% increase in clone size: HR 3.0 (95% C.I. 1.5–5.0 appx.) ● High risk muations: TP53, IDH, Spliceosome, TET2, DNMT3A, ASXL1, JAK2 ● Increased risk with clonal complexity ● Increased risk with high VAF
Young et al. [12]	Case Control: 35 AML cases and 70 controls	Cases: 73.4% Controls: 36.7%	1%	● Odds of developing AML: OR 5.4 (95% C.I. 1.8–16.6, p = 0.003) ● DNMT3A R882 H/C associated with increased odds of AML (OR 14.0, 95% C.I. 1.7–113.8, p = 0.01) ● No association with time to AML ● No association with clonal size

[10,11](Table 1). Desai et al. evaluated 212 AML cases and 212 matched controls and found an association between CH mutations with variant allele frequency (VAF) $>$ 1% and increased odds of AML (OR 4.86, 95% C.I. 3.07–7.77) [10]. Abelson et al. (n = 95 AML cases and 414 controls) showed an increased risk of developing AML in individuals with CH mutations with a cut off over 0.5% VAF [11]. In yet another study by Young et al., of 35 AML patients and matched controls, the odds of developing AML were increased using a VAF cutoff of $\geq$1% (OR: 5.4, 95% CI: 1.8–16.6; p = 0.003) [12]. These three studies lowered the cutoff of detection of CH below the 2% VAF that had been previously utilized for defining Clonal Hematopoiesis of Indeterminate Potential (CHIP) [7] and found a positive association at the lower cutoff values.

The Desai and Abelson studies reached similar conclusions. Utilizing targeted deep sequencing, both studies found CH in approximately 70% of patients who eventually developed AML, versus approximately 30% in controls, with an average latency of 8.9 years and 6.8 years, respectively, for the two studies [10,11]. The most common mutations detected in patients who eventually developed AML included *DNMT3A*, *TET2*, spliceosome mutations, *TP53*, *IDH1*, *IDH2*, and *JAK2*. While other studies of CH have shown that these mutations can be readily identified in healthy older patients using deep sequencing [9], an increased risk of AML has been shown only with VAF $>$ 0.5–1%. Even using this cutoff in the high risk genes, individuals who harbor these mutations may not develop AML. Thus, increased understanding of the specific mutation pattern, clonal complexity, and behavior of AML-predisposing clones is required.

Clonal complexity in CH mutations increase risk of AML

Clonal complexity, defined as the presence of more one CH mutation, is a strong predictor of increased risk of AML, with AML risk increasing proportionately with clonal complexity. In the study by Desai et al., 46.8% of cases with AML had multiple CH mutations a median of 8.9 years before diagnosis of AML, while only 5.5% of controls had a co-mutation [10]. In both the Desai and Abelson studies, the number of mutations per individual increased with age [10,11]. Of note, the presence of more than 4 mutations at baseline was only found in individuals who eventually developed AML [10]. The presence of co-mutation in DNMT3A and spliceosome genes was also associated with an increased odds of developing AML within 5 years (OR 14.8, 95% C.I. 1.63–136, p = 0.01) [10]. It remains unknown whether multiple mutations arise from different clones, or from serial acquisition of new mutations in a single clone. It is possible that the risk of AML may be different based on the origin of these mutations. Future studies using single cell sequencing may prove helpful in answering this question.

The risk of AML is mutation-specific

DNMT3A (DNA methyltransferase 3 alpha) and TET2 (Ten-Eleven Translocation-2) are the most prevalent mutations in individuals with CH accounting for 45–55% and 10–30% of all CH mutations respectively [13,14] and obviously the most common mutations in individuals who eventually develop AML. *DNMT3A* and *TET2* are important genes involved in regulation of DNA methylation and are recurrently found mutated in 23–25% and 8–10% respectively of patients with AML [15,16]. Although the presence of *DNMT3A* and *TET2* CH mutations is associated with an increased risk of AML (Desai et al. *DNMT3A*: OR 2.6, *TET2*: OR 5.79, p $<$ 0.001) [10], the vast majority of individuals with CH mutations in *DNMT3A* and *TET2* will never develop AML. Therefore, it is essential to identify distinguishing features between individuals with *DNMT3A* and *TET2* who will and will not eventually progress to AML. The presence of *DNMT3A* and *TET2* mutations with high allele frequencies ($>$ 10% VAF) is associated with a higher risk of AML, as is clonal complexity (presence of a co mutation in a different gene). In the study by Desai et al., the presence of more than one variants in the *DNMT3A* and *TET2* genes was also more commonly present in individuals who eventually developed AML while the control population mostly had single variant mutations in *DNMT3A* or *TET2* (*DNMT3A* 2 + vs. 1 variants OR 12.6 vs. 2.1, *TET2* 2 + vs. 1 variants OR 69.3 vs. 3.29) [10]. Interestingly, while *DNMT3A* R882 mutations are the most common subtype of DNMT3A mutations (65%) in AML [16], their prevalence is lower in CH comprising of 18% of all *DNMT3A* variants [10,12]. While Desai et al. and Abelson et al. did not find a difference in the risk of AML by DNMT3A subtype [10,11], Young et al. demonstrated a 14-fold increase in risk of AML with DNMT3A R882 H/C mutations (OR 14, 95% C.I. 1.7–113.8, p = 0.01) [12].

IDH1/2 (*isocitrate dehydrogenase 1/2*) mutations have been associated with significant risk of AML progression in population studies [10,11]and generally mutated in 15–20% of newly diagnosed AML [15,16]. In Desai et al. and Young et al., all individuals with *IDH1/2* mutations at the time of baseline sampling ultimately progressed to AML [10,12]. The *IDH* mutations seen in these studies were in the known pathogenic hotspot regions that are frequently seen in AML. Importantly, IDH mutations are associated with a long latency of up to 20 years prior to the development of AML, although rising VAF was associated with faster time to overt disease [10]. In Abelson et al., although *IDH1/2* mutations were associated with an increased risk of AML, 20% of individuals with an *IDH2* mutation did not develop AML during the study follow-up period [11]. It is possible that these individuals continue to be at risk for AML development. Unlike with *DNMT3A* and *TET2*, the risk of AML with *IDH1/2* CH mutations was independent of clone size (VAF) [10].

TP53 (tumor protein p53) mutations comprise another CH mutation subgroup with a high risk for developing AML. *TP53* mutations comprise 4% of CH mutations in the population [14]and have been reported to be present in 7% of newly diagnosed AML [15,16]and 23% of therapy related AML [17–21]. In Desai et al., *TP53* CH mutations were present only in pre-AML cases, not in controls (OR 47.2, p $<$ 0.001) [10]. Abelson et al., also reported an increased risk of AML (HR 12 per 5% increase in clone size, 95% C.I. 5.0–50.0), but *TP53* mutations were also identified in controls who did not develop AML [11]. While *TP53* mutations are known to occur in patients with therapy-related myeloid neoplasms and evidence points toward clonal selection post chemotherapy [22,23], the *TP53* mutations detected in the Desai et al. study were reported in patients who did not have a history of cancer or chemotherapy exposure [10]. The behavior of clones arising competitively at clinically significant VAFs (> 1%) in the presence of exposure-naive hematopoietic stem cells may be different with respect to risk of AML compared to expansion of clones post-chemotherapy. The high risk of AML with *TP53* was seen regardless of low (< 10%) versus high (≥10%) VAF or the specific location of the *TP53* mutation. *TP53* mutations were also associated with increased odds of developing AML within 5 years (OR 5.24, 95% C.I. 1.9–14.49, p = 0.001) [10].

Spliceosome mutations: SRSF2 *(serine and arginine rich splicing factor 2), U2AF1 (U2 small nuclear RNA auxiliary factor 1), SF3B1(splicing factor 3b subunit 1), ZRSR2 (zinc finger CCCH-type, RNA binding motif and serine/arginine rich 2)* comprise 5% of CH mutations [14]but are very common in MDS (50%) and AML (denovo AML: 10%, secondary AML 10–55%) [24]. The presence of spliceosome mutations was associated with increased odds of developing AML (OR 7.43, 95% C.I. 1.71–32.22, p = 0.002) [10]. When assessed individually, *U2AF1* seems to be associated with a higher risk of AML compared to other spliceosome mutations [11]. As with mutations in *IDH* and *TP53*, the risk of AML with spliceosome mutations was independent of clone size [10,11]. Though *SRSF2* and *IDH2* mutations are frequently co-mutated [10], spliceosome mutations retained an independently increased risk of AML, even with adjustment for concomitant *IDH* co-mutations.

JAK2-V617F (Janus Associated kinase 2) mutations are commonly present in myeloproliferative disorders (MPD) [25]and have been associated with increased risk of thrombosis even in patients who do not have a diagnosis of MPD. The presence of *JAK2-V617F* mutations as part of CH was associated with increased risk of AML in the Abelson and Desai studies (OR 6.1, 95% C.I. 1.2–61.1, P = 0.03) [10,11]. Interestingly, in patients with MPD, the presence of a *TET2*-mutated clone prior to acquisition of *JAK2* mutation had a negative impact on the ability of *JAK2* to up regulate genes associated with myeloproliferation and a decreased clinical response to ruxolitinib [26]. The impact of concomitant TET2 mutations on the risk of AML in patients with CH who have *JAK2-V617F* is unknown.

ASXL1 (ASXL transcriptional regulator 1) mutations are the most common mutations after *DNMT3A* and *TET2* and make up 9% of CH mutations [14]but are relatively uncommon in AML (1.5%) [16]. Although common in CH, *ASXL1* mutations have an unclear impact on risk of AML. While Abelson et al. found an increased risk of AML with the presence of *ASXL1* [11], no association was found in the study by Desai et al. [10]. Thus, the specific risk for AML conferred by *ASXL1* mutations remains controversial.

PHF6 (PHD finger protein 6) and RUNX1 (runt related transcription factor 1) mutations are not commonly found as part of CH mutations (n = 3/424 in Desai et al., n = 8/105 in Young et al.) but warrant discussion as all individuals with *PHF6* mutations in the above mentioned studies [10–12], developed AML and in one study was associated with rapid development of AML within 5 years [10]. In the study by Desai et al., *PHF6* (n = 3) was always co-mutated with *RUNX1* (n = 3) thus making it difficult to ascertain whether the rapid development of AML and even the absolute risk seen in the study was attributable to the presence of *PHF6* or *RUNX1* [10]. However, in the study by Abelson et al. and Young et al., *PHF6* mutations always progressed to AML while *RUNX1* mutations did not carry this absolute risk, although 80% of those patients eventually developed AML [11]. Interestingly, efforts are underway to prospectively monitor patients with known RUNX1 familial predisposition syndromes as these individuals carry a high risk of AML (median incidence 35%) and are known to have a high prevalence of CH mutations (67%) [27]. The relative contribution of CH in these patients to the risk of AML is unknown.

Size of the CH clone may impact risk of AML

For mutations in *IDH* and *TP53*, the risk of AML is independent of baseline clone size [10]. Baseline VAF ≥10% was associated with higher odds of developing AML than VAF < 10% for mutations in *DNMT3A* (OR 4.8 vs. 2.5, p = 0.002), *TET2* (OR 20.4 vs. 3.6, p = 0.004) and spliceosome genes (OR 14.1 vs. 3.4, p = 0.019) [10]. It should be noted that, with serial follow-up, most of the clones associated with development of AML increased in VAF up to the time of disease diagnosis. In Abelson et al., the risk of AML increased two-fold with each 5% increase in VAF, except for *DNMT3A* and *TET2* mutations, where the proportionate increase in risk was lower [11]. Clone sizes over 30% for *DNMT3A* and 10% for *TET2* were only found in 1% of controls in the Desai et al. study [10]. In contrast, Young et al. did not identify differences in VAF between individuals who progressed to AML vs. those who did not [12]. Potential explanations for this finding include the smaller size of the study and differences in blood collection time points prior to AML diagnosis.

CH and risk of therapy-related myelodysplastic syndrome (tMDS) and AML

A unique aspect and application of CH and its impact on risk of AML is in the area of therapy related MDS/AML. CH mutations are being increasingly and sometimes erroneously reported as part of tumor genomic reports [28–31] and are associated with poor outcomes in solid malignancies [32]. The risk of tMDS/AML with CH mutations has been reviewed in detail by the authors [33] and is discussed briefly below.

CH increases risk of t-MDS/AML

Several studies have linked the presence of CH with risk of tMDS/AML [32,34–36]. Two case-control studies of patients who received chemotherapy for solid or hematological malignancies showed significantly increased prevalence of CH among cases versus controls [34,35]. In studies by Takahashi et al. and Gillis et al., CH mutations were present in 70% and 62% of cases who developed tAML, versus 30% and 27% of controls, respectively. Takahashi et al. reported that chemotherapy-treated patients with CH mutations had a 30% 10-year cumulative incidence of tAML, versus 7% in patients without CH [35]. Similarly, Gillis et al. found that CH mutations led to increased odds of AML (OR 5.75, C.I. 1.5–25.09), with positive predictive and negative predictive values of 34.8% and 89.1%, respectively [34]. Most of the CH mutations were present in blood samples prior to chemotherapy administration, leading to the hypothesis that they may expand under selective pressure from chemotherapy.

Patients undergoing autologous stem cell transplantation are also at increased risk of t-MDS/AML due to the combination of chemotherapy-induced cytotoxic damage and selection pressure/cellular stress from rapid hematopoietic stem cell proliferation. In Gibson et al., the 10-year cumulative incidence of tMDS/AML was 14.1% and 4.3% in patients with and without CH, respectively [36]. *TP53* mutations and clonal complexity, defined as more than one mutation, at the time of autologous transplant were also associated with increased risk of tMDS/AML, with cumulative incidence of t MDS/AML at 10 years for > 1 mutation 25% vs. 9.9% for a single mutation. As with the studies of chemotherapy-treated patients [34,35], most of the CH mutations identified in patients undergoing autologous transplantation were found in baseline blood samples drawn prior to the transplant. Some of the CH clones were shown in serial samples to expand in size after transplant, while others remained stable until the time of progression to tMDS/AML.

Role of TP53 and PPM1D CH mutations in t-MDS/AML

TP53 and *PPM1D* mutations as part of CH clones are enriched in patients who eventually develop tMDS/AML. *TP53* mutations are present in 23% of tMDS/AML *TP53* [18,21]and have been reported to be enriched as part of CH in patients exposed to radiation and/or chemotherapy [22,23,32]. As CH mutations in *TP53* are also known to occur in patients without exposure to chemotherapy [33] and still lead to increased risk of *de novo* AML, it is possible that *TP53* mutated clones would be especially prone to expansion after chemotherapy, as they are inherently chemotherapy-resistant [23]. In other scenarios where *TP53* clones were shown to appear only after chemotherapy, it is possible that they are either newly emerging somatic mutations or under the baseline detection threshold.

PPM1D mutations are rarely seen in *de novo* AML, but are detected in 20% of patients with tMDS/AML [37]and 2–18% of patients treated with chemotherapy [38]. These mutations lead to inhibition of p53-mediated apoptosis [39,40]and have been linked to specific chemotherapy exposures, including etoposide, platinum, cytarabine and doxorubicin [37]. Unlike *TP53* mutations, *PPM1D* mutations are generally not associated with radiation [32]. Interestingly, while CH mutations in *TP53* usually expand over time leading up to and forming the bulk population of mutant clones in t-MDS/AML, *PPM1D* mutations usually comprise of only a small fraction of tMDS/AML myeloid cells. Mutations other than TP53 and *PPM1D* have also been identified in tMDS/AML [22]and little is understood, to date, about their relative contributions to the disease biology. Importantly, there is no mutation or pattern of mutations that is known to result in definitive progression to tMDS/AML.

Risk of MDS/AML with CH in acquired aplastic anemia

In a study of 438 patients with aplastic anemia (AA) undergoing immunosuppressive therapy (IST), 47% of patients were reported to harbor CH mutations [41]. Yoshizato et al. demonstrated that these mutations were generally present at diagnosis at low allele frequencies (< 10%) and over time increased in size. The patterns of *DNMT3A*, *RUNX1* and *ASXL1* mutations in AA closely resemble age-related CH in the general population in terms of C-T transitions as well as clonal expansion over time. *BCOR* and *PIGa* mutations, which are uniquely seen with high frequencies in AA, remain relatively stable over time and tend to be associated with better response to IST and longer overall survival (HR 0.27, 0.09–0.78; $P = 0.016$) [41]. As patients with AA have a 10–15% 10-year risk of MDS/AML [42], distinguishing CH mutations and patterns that would identify individuals at high risk for transformation is of clinical interest. As expected, increased clonal complexity and acquisition of new clones as well as telomere attrition preceded the development of AML of AML [41]. Yet another study of 150 AA patients demonstrated that CH mutations excluding *PIGa* were detected in 19% of patients with 38% of those patients progressing to MDS/AML compared to 6% in patients who did not harbor a CH mutation [43]. Still, not all expansions of CH mutations lead to MDS/AML and complex interactions between clonal dominance, stem cell attrition, and immune factors are almost certainly involved in malignant transformation. In the subset of patients were serial blood samples were available before and after IST, Yoshizato et al. demonstrated that CH clones rapidly expanded after IST use ($p < 0.001$) [41]. It is not clear whether IST itself contributes to expansion of CH clones. While the rate of progression to MDS/AML in AA patients who underwent allogeneic transplant is reportedly lower than in those treated with IST, there are multiple confounders in this observation (age, prior therapies, time to treatment, etc.) and the potential role of CH is unknown.

Risk of MDS/AML with CH in inherited bone marrow failure syndromes

Interestingly, CH clones have been associated with improved marrow function and blood counts in patients with inherited bone marrow failure syndromes, possible due to positive selection of mutant clones that enhance fitness and are able to provide transient hematopoietic support [44]. For example, Schwachman-Diamond syndrome (SDS) and Diamond-Blackfan Anemia (DBA) are associated with increased ribosomal stress and increased TP53 driven apoptosis leading to ineffective hematopoiesis [44,45]. Clones harboring CH mutations in *TP53* would have selective advantage in these individuals. Although the selective advantage and clonal fitness of CH clones in the setting of inherited bone marrow failure syndromes, may lead to functional complementation and rescue of hematopoiesis, some of these clonal selections set the stage for a higher risk of MDS/AML [44].

TP53 mutated clones have selective advantage in bone marrow failure syndromes like Schwachman-Diamond syndrome (SDS) and Diamond-Blackfan Anemia (DBA), leading to *TP3* activation, ribosomal stress, and growth arrest [44]. In SDS, recurrent chromosomal abnormalities in isochromosome 7, del (20q) and monosomy 7 have been reported. While isochromosome 7 and del (20q) have generally been linked to a benign clinical course, monosomy 7 was associated with progression to AML [46,47]. *TP53* mutations were noted in 48% of patients with SDS [48] and in another study *TP53* mutations were present in all AML patients with biallelic *SBDS* mutations [49]. Since 36% of patients with SDS syndrome are expected to develop AML by age 30 years [50]and the general prevalence of *TP53* CH mutations is approximately 48% [48], detection and monitoring of *TP53* CH, as well as potential clinical trials of disease interception strategies may be appropriate for these patients. *TP53* CH mutations are also known to occur in DBA, although with lower rates of progression to MDS/AML with a cumulative incidence of 5% by 46 years of age [51].

In patients with Fanconi Anemia (FA), development of CH has been associated with improved in blood counts [52]. Of note, patients with FA who progress to MDS/AML, often have a complex karyotype at diagnosis of MDS/AML without concomitant *TP53* mutation. The occurrence of CH mutations in *RUNX1*, as well as the chromosomal abnormalities 3q+ and monosomy 7/del (7q), have been strongly correlated with malignant transformation in FA patients, suggesting alterative pathways of progression [53–55]. Patients with Severe Congenital Neutropenia (SCN) have an increased risk of AML with a cumulative incidence of 22% after 15 years of G-CSF use [56]. While acquired mutations in *CSF3R* are common in patients with SCN, additional mutations in *RUNX1* were present only in patients who eventually developed AML, suggesting that late acquisition of *RUNX1* may play an important role in the evolution of AML in SCN patients [57].

The mechanisms that govern the development of CH in patients with inherited bone marrow failure syndromes and their ultimate progression to AML are varied depending on the underlying germ line defect. The detection of specific CH mutations in the right context should trigger a closer follow up and bone marrow evaluation.

Correlation of CH with clinical and laboratory values

CH mutations have been associated with smoking and increased red cell distribution width (RDW). In an attempt to identify a population at risk for AML and CH, Abelson et al. demonstrated that the presence of increased RDW (> 14) was associated with an increased the risk of AML in a large electronic health database ($p = 0.0016$) [11]. Similar increased risk with regards to RDW was seen in the case control cohort of 95 cases and 412 controls (with CH mutation testing) and this effect was significant even when individuals without CH mutations were excluded from analysis. Using a machine learning algorithm model on electronic health records that incorporated RDW and other blood count measurements, AML could be predicted 6–12 months prior to diagnosis with a sensitivity of 25% and specificity of 98.2%. However, the correlation of this model prediction to actual detection of CH in these patients remains unknown.

Conclusions, challenges, and future directions

While the association between CH and increased risk of AML has been well-established in the literature, significant work remains to be done before this information will become clinically actionable. While CH mutations have been associated with an increased risk of AML in various clinical scenarios (Fig. 1), clearly it would be both unfeasible and massively anxiety-inducing to propose screening of general populations for CH with the objective of identifying individuals at risk for AML. However, as a result of expanded applications of sequencing panels, CH is already being identified in a variety of clinical scenarios, including in tumor genomics reports from patients with both hematologic and solid malignancies, in liquid biopsies performed for solid tumor patients, and in increasingly popular and readily available personal genomics platforms [29–31,58]. CH mutations are also frequently discovered during the evaluation of unexplained cytopenias. Doctors and many patients are well-aware that CH has been associated with increased cardiovascular mortality, as well as with potentially inferior cancer outcomes, and it is completely understandable that the identification of CH, whether expected or incidental, generates both concern and a desire to intervene.

As of this publication, the CH genes associated with risk of AML transformation include *DNMT3A, TET2, IDH1, IDH2, TP53, SRSF2, U2AF1, SF3B1, ZRSR2,* and *PPM1D* [11,12,32–36]. If an individual is identified to have one or more of these high-risk genes, serial follow-up with monitoring of the complete blood count (CBC) is reasonable. It is difficult to make definitive recommendations regarding the VAF, but the following parameters can be considered in selecting individuals for monitoring:

- CH mutations in *IDH*, *TP53*, spliceosome genes at any VAF
- CH mutations in *DNMT3A* and *TET2* with high VAF (> 10%)
- CH mutations with clonal complexity, including multiple mutations per gene

The optimal timing for clinical follow-up is also unknown, but we currently suggest at least twice yearly CBC monitoring, with close examination of a manual differential. We consider more frequent monitoring in individuals with *DNMT3A* and *TET2* mutations over 30% VAF and/or in those with clonal complexity with concomitant detection of more than 4 CH mutations. If abnormalities and/or changes in the CBC are detected, we consider bone marrow biopsy and more frequent monitoring, but it must be stated that there are no data yet confirming that this type of monitoring will either save lives or reduce disease morbidity. Serial assessments of CH in individuals with or without evidence of an underlying disease are highly controversial and should ideally be performed as part of prospective clinical studies. Disease interception strategies in high risk individuals are currently lacking. If such strategies become available in the future, there are several questions with regards to ethics, efficacy, timing of intervention and a consensus on clinical endpoints need to be answered before such trials could be envisioned.

We believe that the most immediate application of the discovery that specific CH mutations are associated with increased risk of AML is to start prospective monitoring and laboratory investigation trials of individuals at highest risk for this scenario, including

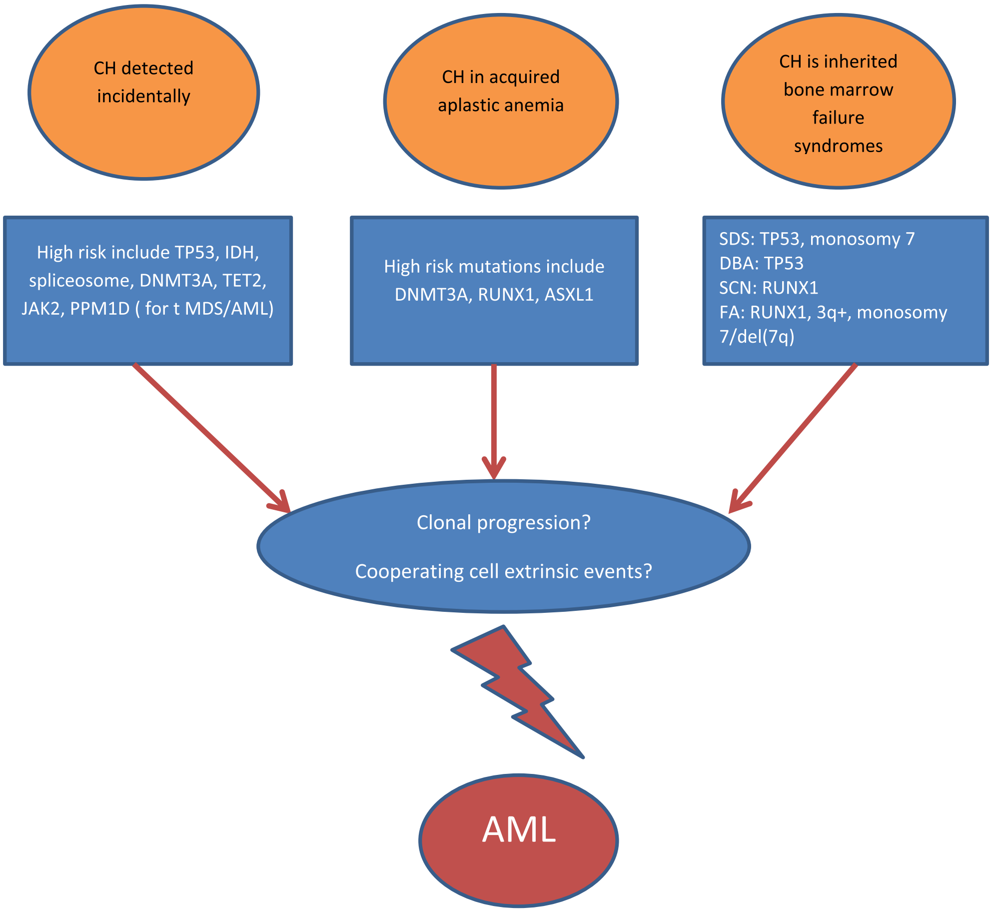

Fig. 1. Association of high risk CH mutations and risk of AML in various populations. CH mutations can be detected incidentally in patients during workup of cytopenias, as part of tumor genomic profile in solid and non-myeloid hematological malignancies and available commercial whole genome testing. CH testing can also be done during work up of congenital and acquired bone marrow failure syndromes. SDS: Schwachman-Diamond syndrome; DBA: Diamond-Blackfan Anemia (DBA); SCN: Severe Congenital Neutropenia; FA: Fanconi Anemia.

chemotherapy-treated patients with persistent, post-treatment CBC abnormalities. Other populations that have high risk of MDS/AML include inherited and acquired bone marrow failure syndromes as well as individuals with known familial predisposition syndromes (e.g. RUNX1, Li-Fraumeni, etc). We envision prospective monitoring of CBC, bone marrow biopsies, and clonal complexity and VAF, with the ultimate goal of disease interception strategies for individuals with evidence of progression.

Summary: The presence of Clonal Hematopoiesis (CH) is associated with increased risk of both *de novo* and therapy-related AML. CH mutations in *TP53, IDH1/2, DNMT3A, TET2* and spliceosome mutations have been associated with newly diagnosed AML, while *PPM1D* and *TP53* CH mutations confer a higher risk of tMDS/AML. Strategies to identify and monitor high-risk CH mutations in appropriate patient populations are needed in conjunction with ongoing translational efforts to better define the disease pathogenesis.

Practice points

- CH may be identified in a variety of scenarios, including as part of molecular genetics reports associated with solid and hematological malignancies, liquid biopsies obtained for solid tumors, and commercially available personal genomics platforms.
- The presence of CH in high risk mutations is associated with increased risk of AML and should prompt referral to a specialist in this area.
- Widespread screening for CH in general populations is not recommended at this time.

Research agenda

- Factors that govern the unequivocal progression of CH mutations towards AML need to be determined.
- Prospective monitoring of CH mutations as part of clinical trials is warranted to determine optimal monitoring strategies
- Clinical milestones prompting early intervention strategies in patients with high-risk CH mutations need to be tested in clinical

trials

Conflicts statement

Pinkal Desai, Duane Hassane and Gail J. Roboz: No conflicts to report with regard to the content of this article.

References

[1] The incidence, prevalence and mortality data in facts 2016-2017 reflect the statistics from the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) program.
[2] Buchner T, Berdel WE, Haferlach C, Haferlach T, Schnittger S, Muller-Tidow C, et al. Age-related risk profile and chemotherapy dose response in acute myeloid leukemia: a study by the German Acute Myeloid Leukemia Cooperative Group. J Clin Oncol: Off J Am Soc Clin Oncol 2009;27(1):61–9.
[3] Burnett AK, Hills RK, Milligan DW, Goldstone AH, Prentice AG, McMullin MF, et al. Attempts to optimize induction and consolidation treatment in acute myeloid leukemia: results of the MRC AML12 trial. J Clin Oncol: Off J Am Soc Clin Oncol 2010;28(4):586–95.
[4] Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med 2014;371(26):2488–98.
[5] Xie M, Lu C, Wang J, McLellan MD, Johnson KJ, Wendl MC, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat Med 2014;20(12):1472–8.
[6] Genovese G, Kahler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med 2014;371(26):2477–87.
[7] Steensma DP, Bejar R, Jaiswal S, Lindsley RC, Sekeres MA, Hasserjian RP, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood 2015;126(1):9–16.
[8] Bowman RL, Busque L, Levine RL. Clonal hematopoiesis and evolution to hematopoietic malignancies. Cell stem cell 2018;22(2):157–70.
[9] Young AL, Challen GA, Birmann BM, Druley TE. Clonal haematopoiesis harbouring AML-associated mutations is ubiquitous in healthy adults. Nat Commun 2016;7:12484.
[10] Desai P, Mencia-Trinchant N, Savenkov O, Simon MS, Cheang G, Lee S, et al. Somatic mutations precede acute myeloid leukemia years before diagnosis. Nat Med 2018;24(7):1015–23.
[11] Abelson S, Collord G, Ng SWK, Weissbrod O, Mendelson Cohen N, Niemeyer E, et al. Prediction of acute myeloid leukaemia risk in healthy individuals. Nature 2018;559(7714):400–4.
[12] Young AL, Tong RS, Birmann BM, Druley TE. Clonal haematopoiesis and risk of acute myeloid leukemia. Haematologica 2019.
[13] Buscarlet M, Provost S, Zada YF, Barhdadi A, Bourgoin V, Lepine G, et al. DNMT3A and TET2 dominate clonal hematopoiesis and demonstrate benign phenotypes and different genetic predispositions. Blood 2017;130(6):753–62.
[14] Heuser M, Thol F, Ganser A. Clonal hematopoiesis of indeterminate potential. Deutsches Arzteblatt international 2016;113(18):317–22.
[15] Patel JP, Gonen M, Figueroa ME, Fernandez H, Sun Z, Racevskis J, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. N Engl J Med 2012;366(12):1079–89.
[16] Cancer Genome Atlas Research N, Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med 2013;368(22):2059–74.
[17] Papaemmanuil E, Dohner H, Campbell PJ. Genomic classification in acute myeloid leukemia. N Engl J Med 2016;375(9):900–1.
[18] Metzeler KH, Herold T, Rothenberg-Thurley M, Amler S, Sauerland MC, Gorlich D, et al. Spectrum and prognostic relevance of driver gene mutations in acute myeloid leukemia. Blood 2016;128(5):686–98.
[19] Volkert S, Kohlmann A, Schnittger S, Kern W, Haferlach T, Haferlach C. Association of the type of 5q loss with complex karyotype, clonal evolution, TP53 mutation status, and prognosis in acute myeloid leukemia and myelodysplastic syndrome. Genes Chromosomes Cancer 2014;53(5):402–10.
[20] Sebaa A, Ades L, Baran-Marzack F, Mozziconacci MJ, Penther D, Dobbelstein S, et al. Incidence of 17p deletions and TP53 mutation in myelodysplastic syndrome and acute myeloid leukemia with 5q deletion. Genes Chromosomes Cancer 2012;51(12):1086–92.
[21] Kadia TM, Jain P, Ravandi F, Garcia-Manero G, Andreef M, Takahashi K, et al. TP53 mutations in newly diagnosed acute myeloid leukemia: clinicomolecular characteristics, response to therapy, and outcomes. Cancer 2016;122(22):3484–91.
[22] Wong TN, Miller CA, Jotte MRM, Bagegni N, Baty JD, Schmidt AP, et al. Cellular stressors contribute to the expansion of hematopoietic clones of varying leukemic potential. Nat Commun 2018;9(1):455.
[23] Wong TN, Ramsingh G, Young AL, Miller CA, Touma W, Welch JS, et al. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. Nature 2015;518(7540):552–5.
[24] Sperling AS, Gibson CJ, Ebert BL. The genetics of myelodysplastic syndrome: from clonal haematopoiesis to secondary leukaemia. Nat Rev Canc 2017;17(1):5–19.
[25] Jones AV, Kreil S, Zoi K, Waghorn K, Curtis C, Zhang L, et al. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood 2005;106(6):2162–8.
[26] Ortmann CA, Kent DG, Nangalia J, Silber Y, Wedge DC, Grinfeld J, et al. Effect of mutation order on myeloproliferative neoplasms. N Engl J Med 2015;372(7):601–12.
[27] Churpek JE, Pyrtel K, Kanchi KL, Shao J, Koboldt D, Miller CA, et al. Genomic analysis of germ line and somatic variants in familial myelodysplasia/acute myeloid leukemia. Blood 2015;126(22):2484–90.
[28] Bolton KL, Gillis NK, Coombs CC, Takahashi K, Zehir A, Bejar R, et al. Managing clonal hematopoiesis in patients with solid tumors. J Clin Oncol : Off J Am Soc Clin Oncol 2019;37(1):7–11.
[29] Severson EA, Riedlinger GM, Connelly CF, Vergilio JA, Goldfinger M, Ramkissoon S, et al. Detection of clonal hematopoiesis of indeterminate potential in clinical sequencing of solid tumor specimens. Blood 2018;131(22):2501–5.
[30] Ptashkin RN, Mandelker DL, Coombs CC, Bolton K, Yelskaya Z, Hyman DM, et al. Prevalence of clonal hematopoiesis mutations in tumor-only clinical genomic profiling of solid tumors. JAMA oncology 2018;4(11):1589–93.
[31] Coombs CC, Gillis NK, Tan X, Berg JS, Ball M, Balasis ME, et al. Identification of clonal hematopoiesis mutations in solid tumor patients undergoing unpaired next-generation sequencing assays. Clin Cancer Res : Off J Am Assoc Cancer Res 2018;24(23):5918–24.
[32] Coombs CC, Zehir A, Devlin SM, Kishtagari A, Syed A, Jonsson P, et al. Therapy-related clonal hematopoiesis in patients with non-hematologic cancers is common and associated with adverse clinical outcomes. Cell stem cell 2017;21(3):374–382 e4.
[33] Desai P, Roboz GJ. Clonal Hematopoiesis and therapy related MDS/AML. Best Pract Res Clin Haematol 2019;32(1):13–23.
[34] Gillis NK, Ball M, Zhang Q, Ma Z, Zhao Y, Yoder SJ, et al. Clonal haemopoiesis and therapy-related myeloid malignancies in elderly patients: a proof-of-concept, case-control study. Lancet Oncol 2017;18(1):112–21.
[35] Takahashi K, Wang F, Kantarjian H, Doss D, Khanna K, Thompson E, et al. Preleukaemic clonal haemopoiesis and risk of therapy-related myeloid neoplasms: a case-control study. Lancet Oncol 2017;18(1):100–11.
[36] Gibson CJ, Lindsley RC, Tchekmedyian V, Mar BG, Shi J, Jaiswal S, et al. Clonal hematopoiesis associated with adverse outcomes after autologous stem-cell transplantation for Lymphoma. J Clin Oncol : Off J Am Soc Clin Oncol 2017;35(14):1598–605.
[37] Hsu JI, Dayaram T, Tovy A, De Braekeleer E, Jeong M, Wang F, et al. PPM1D mutations drive clonal hematopoiesis in response to cytotoxic chemotherapy. Cell stem cell 2018;23(5):700–713 e6.

[38] Kahn JD, Miller PG, Silver AJ, Sellar RS, Bhatt S, Gibson C, et al. PPM1D-truncating mutations confer resistance to chemotherapy and sensitivity to PPM1D inhibition in hematopoietic cells. Blood 2018;132(11):1095–105.

[39] Dudgeon C, Shreeram S, Tanoue K, Mazur SJ, Sayadi A, Robinson RC, et al. Genetic variants and mutations of PPM1D control the response to DNA damage. Cell Cycle 2013;12(16):2656–64.

[40] Lu X, Nguyen TA, Zhang X, Donehower LA. The Wip1 phosphatase and Mdm2: cracking the "Wip" on p53 stability. Cell Cycle 2008;7(2):164–8.

[41] Yoshizato T, Dumitriu B, Hosokawa K, Makishima H, Yoshida K, Townsley D, et al. Somatic mutations and clonal hematopoiesis in aplastic anemia. N Engl J Med 2015;373(1):35–47.

[42] Li Y, Li X, Ge M, Shi J, Qian L, Zheng Y, et al. Long-term follow-up of clonal evolutions in 802 aplastic anemia patients: a single-center experience. Ann Hematol 2011;90(5):529–37.

[43] Kulasekararaj AG, Jiang J, Smith AE, Mohamedali AM, Mian S, Gandhi S, et al. Somatic mutations identify a subgroup of aplastic anemia patients who progress to myelodysplastic syndrome. Blood 2014;124(17):2698–704.

[44] Schaefer EJ, Lindsley RC. Significance of clonal mutations in bone marrow failure and inherited myelodysplastic syndrome/acute myeloid leukemia predisposition syndromes. Hematol Oncol Clin N Am 2018;32(4):643–55.

[45] Elghetany MT, Alter BP. p53 protein overexpression in bone marrow biopsies of patients with Shwachman-Diamond syndrome has a prevalence similar to that of patients with refractory anemia. Arch Pathol Lab Med 2002;126(4):452–5.

[46] Pressato B, Valli R, Marletta C, Mare L, Montalbano G, Lo Curto F, et al. Deletion of chromosome 20 in bone marrow of patients with Shwachman-Diamond syndrome, loss of the EIF6 gene and benign prognosis. Br J Haematol 2012;157(4):503–5.

[47] Minelli A, Maserati E, Nicolis E, Zecca M, Sainati L, Longoni D, et al. The isochromosome i(7)(q10) carrying c.258 + 2t > c mutation of the SBDS gene does not promote development of myeloid malignancies in patients with Shwachman syndrome. Leukemia 2009;23(4):708–11.

[48] Xia J, Miller CA, Baty J, Ramesh A, Jotte MRM, Fulton RS, et al. Somatic mutations and clonal hematopoiesis in congenital neutropenia. Blood 2018;131(4):408–16.

[49] Lindsley RC, Saber W, Mar BG, Redd R, Wang T, Haagenson MD, et al. Prognostic mutations in myelodysplastic syndrome after stem-cell transplantation. N Engl J Med 2017;376(6):536–47.

[50] Donadieu J, Delhommeau F. TP53 mutations: the dawn of Shwachman clones. Blood 2018;131(4):376–7.

[51] Vlachos A, Rosenberg PS, Atsidaftos E, Alter BP, Lipton JM. Incidence of neoplasia in Diamond Blackfan anemia: a report from the Diamond Blackfan anemia registry. Blood 2012;119(16):3815–9.

[52] Soulier J, Leblanc T, Larghero J, Dastot H, Shimamura A, Guardiola P, et al. Detection of somatic mosaicism and classification of Fanconi anemia patients by analysis of the FA/BRCA pathway. Blood 2005;105(3):1329–36.

[53] Quentin S, Cuccuini W, Ceccaldi R, Nibourel O, Pondarre C, Pages MP, et al. Myelodysplasia and leukemia of Fanconi anemia are associated with a specific pattern of genomic abnormalities that includes cryptic RUNX1/AML1 lesions. Blood 2011;117(15):e161–70.

[54] Cioc AM, Wagner JE, MacMillan ML, DeFor T, Hirsch B. Diagnosis of myelodysplastic syndrome among a cohort of 119 patients with fanconi anemia: morphologic and cytogenetic characteristics. Am J Clin Pathol 2010;133(1):92–100.

[55] Tonnies H, Huber S, Kuhl JS, Gerlach A, Ebell W, Neitzel H. Clonal chromosomal aberrations in bone marrow cells of Fanconi anemia patients: gains of the chromosomal segment 3q26q29 as an adverse risk factor. Blood 2003;101(10):3872–4.

[56] Alter BP, Giri N, Savage SA, Peters JA, Loud JT, Leathwood L, et al. Malignancies and survival patterns in the National Cancer Institute inherited bone marrow failure syndromes cohort study. Br J Haematol 2010;150(2):179–88.

[57] Skokowa J, Steinemann D, Katsman-Kuipers JE, Zeidler C, Klimenkova O, Klimiankou M, et al. Cooperativity of RUNX1 and CSF3R mutations in severe congenital neutropenia: a unique pathway in myeloid leukemogenesis. Blood 2014;123(14):2229–37.

[58] Phallen J, Sausen M, Adleff V, Leal A, Hruban C, White J, et al. Direct detection of early-stage cancers using circulating tumor DNA. Sci Transl Med 2017;9(403).