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Concise Review: Age-Related Clonal Hematopoiesis: Stem Cells Tempting the Devil

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Key Words. Hematopoiesis • Hematologic malignancies • Aging • Stem cells

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Received January 16, 2018; accepted for publication April 19, 2018; first published online in STEM CELLS *EXPRESS* April 30, 2018

http://dx.doi.org/10.1002/stem.2845

Available online without subscription through the open access option

ABSTRACT

The recent characterization of clonal hematopoiesis in a large segment of the aging population has raised tremendous interest and concern alike. Mutations have been documented in genes associated with hematological cancers and in non-driver candidates. These mutations are present at low frequency in the majority of individuals after middle-age, and principally affect the epigenetic modifiers *DNMT3A* and *TET2*. In 10%–40% of cases, the clone will progress to meet the diagnostic criteria for Clonal Hematopoiesis of Indeterminate Potential, which is associated with an increased risk of hematological cancer and cardiovascular mortality. Blood cell parameters appear unmodified in these individuals, but a minority of them will develop a hematologic malignancy. At this time, the factors put forward as potentially influencing the risk of cancer development are clone size, specific gene, specific mutation, and the number of mutations. Specific stress on hematopoiesis also gives rise to clonal expansion. Genotoxic exposure (such as chemotherapy), or immune attack (as in aplastic anemia) selects/provides a fitness advantage to clones with a context-specific signature. Clonal hematopoiesis offers a new opportunity to understand the biology and adaptation mechanisms of aging hematopoiesis and provides insight into the mechanisms underlying malignant transformation. Furthermore, it might shed light on common denominators of age-associated medical conditions and help devise global strategies that will impact the prevention of hematologic cancers and promote healthy aging. STEM CELLS *2018;36:1287–1294*

SIGNIFICANCE STATEMENT

Hematologists have been fascinated by the concept of clonality for decades; it is the basis for cellular expansion and malignancy. Recent characterization of clonal hematopoiesis in a large segment of the aging population has raised tremendous interest and concern alike. Mutations have been documented in genes associated with hematological cancers. Although blood cell production is maintained in these individuals, a minority of them will progress to hematologic malignancies. The risk factors for progression need to be precisely identified. Clonal hematopoiesis offers a new opportunity to understand the biology and adaptation mechanisms of aging and mechanism of malignant transformation.

INTRODUCTION AND PROBLEMATIC

Human hematopoietic stem cells (HSC) have the enormous task of providing 10^{10} to 10^{12} blood cells daily for an entire lifespan. Remarkably, aging minimally affects output, despite being associated with a myeloid proliferation bias [1], decreased bone marrow cellularity [2], reduced lymphopoiesis [3], and less erythropoiesis (increased anemia) [4]. Furthermore, there is a strong association between age and myeloid-derived hematological cancers such as acute myeloid leukemias (AML), myelodysplastic syndromes (MDS), and myeloproliferative neoplasms (MPN). The incidence of AML increases more than 20-fold between the ages of 25 and 75, and that of MDS increases more than 300-fold (http://www.cancer.gov/statistics) [5–7].

Recently, acquired mutations conferring clonal growth advantage have been documented in aging healthy individuals with a normal hematopoietic phenotype, challenging the putative relationship between clonality and malignancy. In this article, we provide an historical context to these discoveries, discuss the repercussions on normal and neoplastic hematopoiesis as well as on non-hematological conditions, and highlight some key issues warranting further clarification. For the purpose of this review, age-related clonal hematopoiesis (ARCH) refers to clonal hematopoiesis caused by any type of acquired clonal event, whereas clonal hematopoiesis of indeterminate potential (CHIP) refers to that caused by a mutation in a candidate driver (CD) gene (e.g., *DNMT3A* or

Table 1. Etiologies of XCI skewing (modified from [9])

Primary
Non-random XCI (embryonic)
Selection (constitutional)
Positive
X-linked adrenoleukodystrophy [10]
Age-associated hemizygous cell selection [11]
Negative (X-linked disease allele)
X: autosome translocation [9]
Hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency [12]
X-linked dyskeratosis congenital [13]
Blood cell lineage specific
Agammaglobulinemia [14]
X-linked severe combined immunodeficiency (SCID) [15]
Wiskott-Aldrich syndrome [16]
Acquired
HSC drift
Clonal hematopoiesis
Hematologic malignancies (reviewed in [17])
Age-related clonal hematopoiesis (ARCH) [18]
With mutation in Candidate Driver (CD) genes (CHIP) [19]
Other clonal markers in Non Driver candidates

Abbreviations: CHIP, clonal hematopoiesis of indeterminate potential; HSC, hematopoietic stem cell; XCI, X-chromosome inactivation.

other) with a variant allele frequency (VAF) greater than 2%.

Clonal Dominance Demonstrated by X-Chromosome Inactivation Skewing in Aging Females

The first evidence suggesting that aging hematopoiesis might be subjected to clonal evasion and dominance came from analysis of X-chromosome inactivation (XCI) ratios in normal aging females. The analysis of XCI is a clever application of Mary Lyon's fundamental observation that each X-chromosome in excess of one is *randomly* inactivated in cells of the developing female embryo [8]. Therefore, following XCI, females present a mosaic pattern of two cell populations, one expressing maternal (Xm), the other paternal (Xp) X-linked genes. Deviation from the theoretical 1:1 ratio between each population is called skewing and likely has multifold etiologies (Table 1). The first XCI-based clonality studies were published five decades ago [20–22], and set the stage for the subsequent unravelment of some of the basic tenets of modern oncology (reviewed in [17]). Twenty years ago, following a lead by Martin Fey [23], we studied normal aging females and showed a significant increase in the prevalence of skewing in their blood cells after the age of sixty [18]. This has been confirmed by others [24–26]. A number of etiologic mechanisms have been put forward, including acquired clonal hematopoiesis, stochastic clonal dominance caused by HSC depletion, and genetic predisposition [26–29]. The later has been supported by three twin studies [30–32], and by Abkowitz et al., [11] who provided evidence for an X-linked genetic component causing hemizygous cell selection in Safari cats.

Clonal Acquired Events in Normal Aging Individuals

We sought to further investigate the cause of acquired XCI skewing by performing exome sequencing on DNA from polymorphonuclear cells (PMN) and buccal epithelial cells. We first analyzed cells from three elderly women with known skewing in their PMN, and identified somatic mutations in *TET2, DNMT3A,* and *SLC39A12* in one them [19]. Extension of the analysis to various age groups led to the identification of missense, nonsense, and frameshift somatic *TET2* mutations in the DNA from PMN, but not that from lymphocyte or epithelial cells, in 10/179 (5.6%) elderly subjects with XCI skewing, in 0/105 elderly subjects without XCI skewing, and in 0/96 younger subjects with XCI skewing. The hematologic parameters of *TET2* mutant individuals did not differ from those of age-matched counterparts. This study was the first to demonstrate that acquired mutation in a myeloid cancer associated gene (*TET2*) is age-dependent and compatible with normal hematopoiesis. It also suggests that acquired mutation in a CD gene is a small contributor to acquired XCI skewing in the general population. Simultaneously, Laurie et al. showed an age-dependant clonal mosaicism for large chromosomal anomalies using single nucleotide polymorphism (SNP) microarray data from over 50,000 individuals recruited for genome-wide association studies [33]. Clonal mosaicism was detected in 2%–3% of elderly individuals in contrast to 0.5% of individuals younger than 50 years. Furthermore, the presence of clonal mosaicism was associated with a 10-fold increased risk of developing a hematological cancer. Jacobs et al. [34] and Forsberg et al. [35] reported similar findings.

In 2014, three groups reported analysis of DNA exome datasets from large cohorts of subjects and documented age-dependent mutations in CD genes [36–38]. These three cohorts had different inclusion criteria (non-hematological cancer, psychiatric disorder, or cardiovascular disease), yet they identified a similar set of genes, suggesting a universal age-associated phenomenon. Although more than 70 different genes were identified, the most frequently mutated ones were the epigenetic modifiers *DNMT3A, TET2,* and *ASXL1*. Among the other significant culprits were *TP53 JAK2, SF3B1, CBL, SRSF2, PPM1D, and BCOR*. Importantly, the prospective data available for two of these studies revealed that subjects with clonal hematopoiesis had a roughly 10-fold heightened risk of developing an hematological cancer [37, 38]. That said, the relative risk of mortality was only 1.4, and clonal hematopoiesis was also associated with an increased risk of cardiovascular events. Given the uncertainty about their clinical impact, mutations in CD genes have been coined "CHIP" [39].

Several ensuing studies have aided characterizing and quantitating ARCH and CHIP. Using a gene-targeted approach, we recently analyzed a large cohort of 2,530 normal individuals, aged 55 to 100 years-old, in search of mutations in CD genes [40]. We documented a 2- to 3-fold higher prevalence of mutations, and a different proportion between affected genes. The majority of mutations (92.8%) involved *DNMT3A* or *TET2* - genes central to DNA methylation and demethylation. Clone size (VAF) was significant in a large proportion of affected individuals the average VAF was 14.3%, which corresponds to 28.6% of cells originating from mutated stem cells. Almost half of the mutated cohort (48.7%) had a VAF $\geq$10%. No single mutation hotspot was identified in either *DNMT3A* or *TET2*. There were no significant differences in blood cell parameters (cell numbers and indices) between mutant and aged-matched controls, except for a tendency toward reduced PMN in *TET2* mutants. The striking preponderance of mutations in *DNMT3A* and *TET2* is intriguing, and hardly

reconcilable with random, genome-wide acquisition of mutation. It is unclear if these genes are inherently hypermutable or if there are specific factors (yet to be identified) affecting the HSC or its microenvironment that instigate a hypermutable state. Adding to the complexity of the origin of these mutations, we recently discovered that mutations in *DNMT3A* and *TET2* occur at different levels of the hematopoietic hierarchy. *DNMT3A* lineage restriction patterns are compatible with a pluripotent stem cell origin, whereas *TET2* mutations occur mainly in myeloid cells and sometimes in B-cells [41].

Using an ultra-sensitive error-corrected sequencing method, Young et al. demonstrated mutations in *TET2* or *DNMT3A* at very low frequency in 95% of individuals aged 50 to 60 years-old, suggesting that these mutations are almost ubiquitous after the age of fifty [42]. Using a whole-genome sequencing (WGS) approach on a large cohort from Iceland, Zink et al. confirmed the high prevalence of mutations in CD genes, but showed an even higher prevalence of mutation in non-driver candidates, thus suggesting that ARCH is inevitable [43].

Etiologies of ARCH: Survival of the Fittest

Aging

Aging HSC face the potential of exhaustion. Several parameters differentiate old versus young HSC (reviewed in [44]), such as cumulating random DNA damage [45], reduced telomere length [46, 47], increased polarity [48], reduced autophagy [49], and epigenetic reprogramming [50]. Some of these changes are presumably signs of imminent demise whilst others are possibly adaptation mechanisms for sustainability. One thing is certain: throughout the aging process, less and less HSC contribute to hematopoietic output, but this does not impact overall production. Prospective accumulation of random DNA damage is in line with the WGS data published by Zink et al. showing a higher prevalence of mutations in non-driver candidates than in CD genes [43]. Mutations in non-driver candidates may be hallmarks of a drift associated with reduced HSC pool and stochastic dominance. Alternatively, some of these mutations may be linked to unidentified CD genes. As stated above, the main recognized CD genes are *DNMT3A* and *TET2* [40]. Studies in mice have demonstrated that loss of function of either of these genes leads to increased HSC self-renewal capacity. Loss of *Dnmt3a* leads to expansion of HSC number but at the expense of loss of differentiation [51]. It is also associated with an almost infinite serial transplantation capacity and with an immortalization of HSC in vivo [52]. Similarly, conditional *Tet2* loss in the hematopoietic compartment induces $Lineage^{-}Sca1^{+}cKit^{+}$ (LSK) cell expansion along with decreased 5-hydroxymethyl-cytosine (5hmC) levels and increased self-renewal capacity of HSC [53–55]. The specific *TET2* or *DNMT3A* mutations that we and others have documented in the blood cells of aging individuals are mainly loss-of-function mutations, and they include indels, frameshift, nonsense, splice site, and missense mutations which abrogate catalytic function. It is therefore reasonable to speculate that HSC harboring a mutation in either of these genes have an increased self-renewal capacity, allowing them to outcompete non-mutated HSC. Simply put, mutation in *TET2* or *DNMT3A* might provide a sort of "fountain of youth" to aging HSC. Of note, despite the almost ubiquitous presence of these mutations over the age of fifty [42], only a subset of individuals will develop a clone with a VAF greater than 2% over time, suggesting inter-individual variation in the competitiveness of mutated versus non-mutated HSC. The relative competitiveness between HSCs may related to intrinsic or extrinsic factors.

Table 2. Factors influencing acquisition/progression of clonal hematopoiesis of indeterminate potential

Age (HSC/microenvironement)
Genetic predisposition [40, 43, 56]
Inflammation [57]
Genotoxic exposure [58]
Immune attack [59]
Smoking [60]
Others to be defined

Abbreviation: HSC, hematopoietic stem cell

Knowledge about the factors (other than aging) that predispose to acquisition/progression of these mutations is so far incomplete (Table 2). Genetic predisposition may play a role. Zink et al. have shown an association between a small deletion in *TERT* and clonal hematopoiesis [43], and we have documented familial aggregation for *TET2* mutation [40]. The MPN literature already acknowledges that genetic predisposition to acquire *JAK2 V617F* mutation [61] and certain germline variations at the *TERT* locus are linked to familial clustering of these diseases (reviewed in [62]). Clonal hematopoiesis may also be influenced in part by the microenvironment. Vas et al. have shown that aging microenvironment influences clonality of malignant cells in a HSC-independent manner, and may therefore contribute to ARCH [63]. The association of CHIP with cardiovascular [37, 38, 64] and chronic pulmonary [40, 43] diseases raises the possibility that age-associated chronic inflammation, often called «*inflammaging*» [65], may be a key common denominator between these medical conditions and the emergence of clonal hematopoiesis. There are evidences suggesting that a pro-inflammatory milieu favors mutated stem cells. For example, chronic myelogenous leukemia (CML) stem cells have increased expression of IL-1 receptor and sensitivity to IL-1 in comparison to normal HSC, and are therefore favored in an inflammatory environment [66]. Abegunde et al. demonstrated that a pro-inflammatory environment supported by TNF-α promotes the expansion of *Tet2* mutant clones in mice [57]. It is currently unclear if *inflammaging* promotes clonal hematopoiesis independently from other age-associated medical conditions or if they are somehow linked. Interestingly, *Tet2* mutant myeloid cells have an impaired capacity to resolve inflammation caused by increased production of IL-6 [67], and *Tet2* deficient mice have a predisposition to artherosclerosis [68]. It is tempting to hypothesise that low grade inflammation promotes initial clonal expansion of mutated HSC which, as the clone size increases, further impede the control of inflammation, ultimately affecting organs such as the heart, lung, pancreas, etc. (Fig. 1 A).

Genotoxic Exposure

Clonal analyses of blood from patients who have developed treatment-related AML (t-AML) have been instrumental in

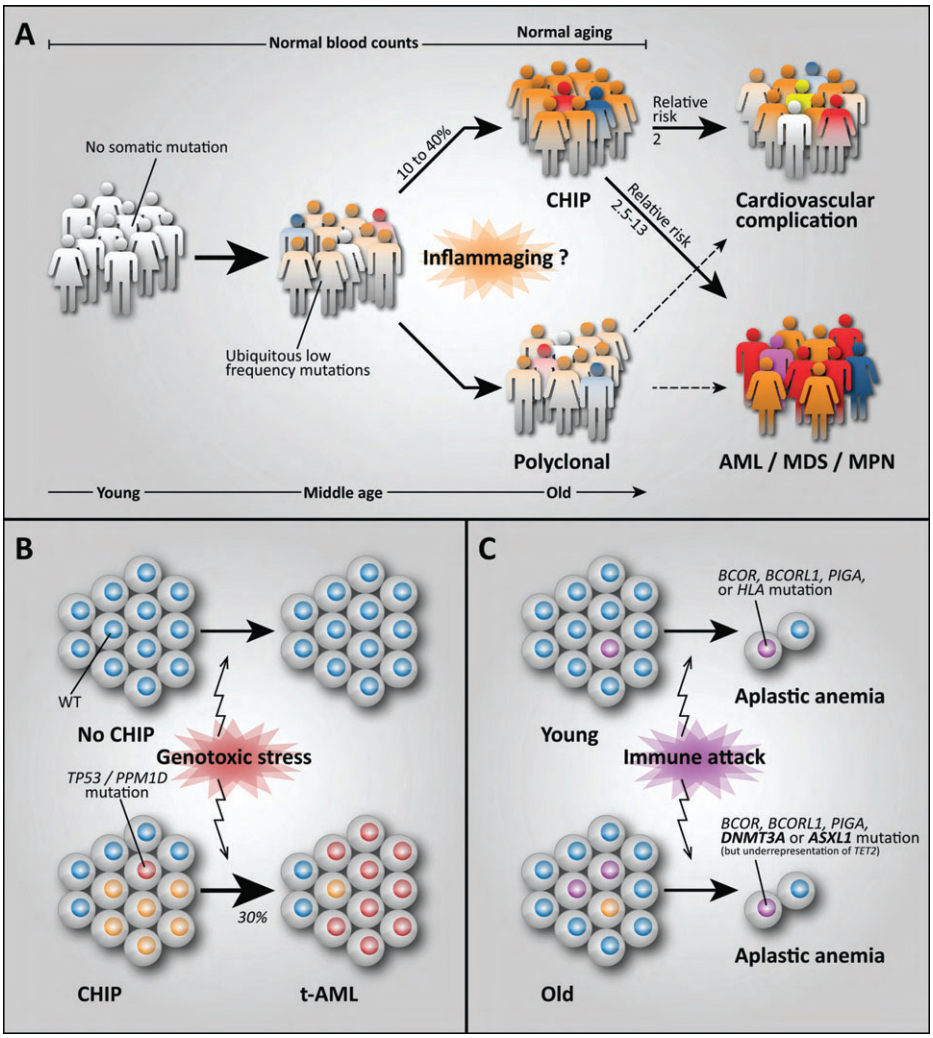

Figure 1. **(A):** Evolution of CHIP in the aging population. Mutations begin to appear in middle-aged subjects at a low frequency. In a subset of subjects (10%–40%), the clone will expand to meet diagnostic criteria of CHIP (VAF > 2%). Factors influencing clonal expansion are enumerated in Table 2 and include inflammation. Fitness of wild-type (WT) HSC may be key in maintaining polyclonal hematopoiesis. Blood counts remain normal in polyclonal and CHIP individuals. A small proportion of subjects with CHIP will progress to hematological cancer under the influences enumerated in Table 3. A proportion of subjects with CHIP will develop cardiovascular disease. Subjects without CHIP may also develop hematological cancers or cardiovascular disease but have a lower relative risk. **(B):** Specific mutations such as *TP53* or *PPM1D* may provide HSC with resistance to genotoxic stress such as chemotherapy or radiotherapy and promote clonal expansion. These mutations may lead to development of t-AML. **(C):** Immune attack of HSC promotes selection of cells with a mutational profile different than that of aging or genotoxic stress. In younger patients this includes mutations in PIGA, BCOR/BCORL1, and HLA deletions. In older subjects, mutations in DNMT3A and ASXL1 are also documented, but TET2 mutations are underrepresented. Abbreviations: CHIP, Clonal Hematopoiesis of Indeterminate Potential; HSC, hematopoietic stem cells; MDS, myelodysplastic syndromes; MPN, myeloproliferative neoplasms; t-AML, treatment-related acute myeloid leukemias; WT, wild-type.

elucidating the physiopathology of these complications. Wong et al. documented that 4 out of 22 patients with t-AML had, prior to exposure to chemotherapy, the exact *TP53* mutation documented in their AML specimen, but at a very low VAF, supporting expansion of this clone under the influence of cytotoxic agents [58]. Gillis et al. also identified pre-existing *TP53* mutation as a risk factor for t-AML [69]. Interestingly, they showed that *TET2* mutations were prevalent pre-chemotherapy but not associated with treatment-related hematological cancer. Recently a genomic study on a large cohort of patients found *TP53* and *PPM1D* mutations to be enriched in therapy-induced MDS compared with primary MDS [70], offering supporting evidence that clones harboring these mutations thrive under therapy-induced selective pressure. Likewise, mutations in the *TP53* modulator *PPM1D* have been detected in the blood of patients with ovarian [71] and lung [72] cancer following therapy [73–75]. Studying a large cohort of patients treated for non-hematological cancers, we recently documented that CHIP was prevalent in 25% of them, associated with age, prior radiation, and tobacco smoking [60]. The strongest association was found between *TP53* and *PPM1D* mutations and prior treatment with chemotherapy and radiation. Taken together, these data suggest that exogenous genotoxic stress selects naturally occurring clones that harbor mutations conferring resistance to these toxic agents. It may be possible to identify patients at risk of

Table 3. Factors influencing progression to hematologic cancers

Variant allele frequency [86]
Specific gene [86]
Specific mutation (hot spot) [86]
Number of mutations [87]
Presence of cytopenias [88]
Others to be defined

developing treatment-related cancer prior to treatment. However, the predictive value of mutations for this rare complication may be specific for certain genes (such as *TP53* and *PPM1D*) and not be the same for other genes (such as *TET2* for example). Screening for mutations in individuals submitted to intensive chemotherapy/radiotherapy may allow a better evaluation of the risk-benefit ratio of therapy (Fig. 1 B). *TP53* mutations may also be found in the context of germline predisposition syndromes (reviewed in [76]), and must be differentiated from acquired mutations as management may be different [77]. Interestingly, the acquisition of *TP53* mutation has been documented in close to half of children with Shwachman-Diamond syndrome, and may be important in their progression to leukemia [56].

Immune Attack

Hematopoiesis in condition such as aplastic anemia or other bone marrow failure syndromes are subjected to intense stress. This offers an opportunity to contextualize HSC mutated clone selection and expansion (reviewed in [78]). The pattern of mutations identified in aplastic anemia bares certain differences with that found in the general aging population. Aplastic anemia patients have a high prevalence of mutations (45%), which can be subcategorized as mutations more specific to aplastic anemia (*PIGA*, *BCOR, and BCORL1*) and other mutations classically documented in ARCH (*DNMT3A* and *ASXL1)* [79]. The incidence of *DNMT3A* and *ASXL1* mutations increases with age, while those in *PIGA* and *BCOR/BCORL1* are equally represented across all age groups [79]. *PIGA* and *BCOR/BCORL1* mutations have been associated with increased responsiveness to immunotherapeutic intervention, while classical ARCH mutations have been associated with less sensitivity. Aplastic anemia is also subjected to loss of HLA class I allele, which may help escape immune attack [80, 81]. Therefore, it seems as if, depending on the specific mutation, some may favor evasion of immune attack while others may favor HSC self-renewal (Fig. 1 C). Interestingly, *TET2* mutation was underrepresented in a cohort of aplastic anemia patients including older subjects, suggesting that *TET2* mutation is less common in these patients compared with the general aging population [40]. This may be hypothetically related to our recent documentation that in contrast to *DNMT3A*, *TET2* mutations do not occur at the multipotent stem cell level [41]

ARCH and CHIP Relationship to Hematological Cancer Development

ARCH has been described as an inevitable consequence of aging [43]. The conditions associated with its progression support an increased fitness of affected HSC compared with wild-type ones, suggesting a potential utility for this phenomenon in maintaining normal blood counts. However, there are incriminating evidences that these clones may progress to hematologic cancers. The first evidence comes from analysis of the clonal architecture of patients with AML or MDS. Several investigators performing genomic analyses on clonal colonies or using single cell analysis of primary samples of AML patients have documented a chronology of mutation acquisition where the initial clone had mutation in CD genes found in CHIP such as *DNMT3A*, *TET2, or ASXL1* [82–84]. Further, these clones are present in a subset of remission samples, and can serve as a reservoir for clonal evolution and relapse [82]. The second evidence comes directly from the analysis of outcome of subjects with clonal hematopoiesis. Jaiswal [38] and Genovese [37] documented an approximately 10-fold increased risk of developing a hematological cancer, a risk comparable to the monoclonal gammopathy of undetermined significance (MGUS) transformation rate to multiple myeloma of 1% per year. However, there is a controversy on the exact risk facing individuals with CHIP [78]. Novel data support a lower RR than first reported in these seminal studies [37, 38]. First, the increased prevalence of CHIP documented using more precise technology [40] would per se change the RR calculation. Second, the definition of hematological cancer were broad and included both myeloid and lymphoid (including MGUS) pathologies. In fact, a total of 20 patients had hematological cancers (12 myeloid, 8 lymphoid), only one subject had a *TET2* mutation (85 years-old with a diffuse large cell lymphoma) and 4 had *DNMT3A* mutations. Five had passenger mutations and 3 had a *JAK2* mutation later associated with a MPN. Further, the clonal patients in Jaiswal's study were significantly older than those wild-type who developed cancer (73 vs. 57 years-old). At a minimum, this would mitigate the RR for *TET2,* and to a lesser extent *DNMT3A,* which are the most prevalent in the population. In further support of a low risk, a recent study demonstrated uncompromised 10-year survival in elderly subjects (over 85) carrying such mutations [85]. Moreover, Zink et al. documented a RR of hematological cancer of 2.43 for subjects with at least one mutation in whole genome data.

The uncertainty on the RR of hematological cancer progression in individuals with clonal hematopoiesis and the need for clinically applicable screening strategies call for the identification of risk factors modulating the oncogenic penetrance of CHIP. This has been recently done by Abelson et al. [86] who looked at samples obtained an average of 6 years prior to AML and compared their clonal architecture to that of healthy aged-matched controls. They showed that certain variables were strongly associated with AML progression. These included the identity of the mutated gene (splicing factors favor AML), the number of mutations (any type), the number of AML associated mutation, and the VAF of clone. They devised AML prediction tool that could calculate the risk of AML progression 7 years before diagnosis with a RR of 20. However, this tool is aimed at predicting AML and not all hematological cancers as described above. The hematological context in which clonal hematopoiesis is documented is also important. Malcovati et al. showed that when clonal hematopoiesis is documented in individuals with unexplained cytopenias (therefore not meeting CHIP criteria), the positive predictive value of progression to a

myeloid cancer is very high (0.81). Increased VAF (>10%), carrying 2 or more mutations, and spliceosome mutations in combination with epigenetic regulators were all associated with a greater predictive value of myeloid cancer. Interestingly, mutation in *TET2* alone had the lowest predictive value for myeloid cancer.

Taken together these studies indicate that there is a clear relationship between ARCH, but more specifically CHIP, to the development of blood cancers. This risk is dependent on several variables (Table 3). Prediction models should be developed to help clinicians appropriately define an individual's risk for development of hematological cancer or a cardiovascular event in order to provide counseling and intervention to patients at greater risk. This is the first opportunity for hematologists to develop preventive strategies for these deadly disorders.

Conclusion

Hematologists and oncologists have been fascinated by the concept of clonality for decades; it is the basis for cellular expansion and development, but also of malignancy. Recent technological advances in next generation sequencing (NGS) provided invaluable tools for the characterization of clonal hematopoiesis, and have led to unprecedented observations. They have already furthered our understanding of normal hematopoiesis, and provided insight into the mechanisms associated with aging and cancer development. Systematic and prospective studies of large cohorts are now required to decipher the mechanisms of clonal dominance and, more importantly, those whose deregulation lead to malignant transformation. As the segment of the population reaching older age is relentlessly increasing, it is a medical priority to identify the basis of hematological cancer progression and to devise prevention strategies to prevent transformation in subjects with clonal hematopoiesis.

Acknowledgments

Part of the original work that supported this review was funded for L.B. by the Canadian Institutes of Health Research (CIHR) and Leukemia Lymphoma Society of Canada (LLSC), and for R.L.L. by the NIH National Cancer Institute (NCI) grant P30 CA008748 (Cancer Center Support Grant). L.B. wrote the manuscript; M.B. did all figures; M.L., L.M. and R.L.L. critically revised the manuscript.

Disclosure of Potential Conflicts of Interest

R.L. declares employment/leadership relationship with Qiagen. The other authors indicated no potential conflicts of interest.

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