It was not long ago that we first started to appreciate the prevalence of clonal hematopoiesis, defined as the presence of somatic mutations in blood cells of individuals with no history of hematologic malignancy. This phenomenon was quickly associated with an increased risk of death, both from development of subsequent hematologic malignancy as well as cardiovascular disease. Since that time, significant advances have been made in understanding the relationship of clonal hematopoiesis to both of these phenomena.

With respect to evolution to hematologic malignancy, several studies have used population sampling (Women’s Health Initiative, Nurses’ Health Study, Health Professionals Follow-up Study, and others) to compare characteristics of clonal hematopoiesis between individuals who developed acute myeloid leukemia (AML) and age-matched controls. The risk for development of leukemia correlated with clonal hematopoiesis involving myeloid malignancy driver genes, with further risk stratification relating to specific genes as well as clonal hematopoiesis burden (assessed by variant allele frequency, VAF). For example, IDH1 and IDH2 mutations have been associated with a significantly increased risk of development of leukemia (1), whereas DNMT3A and TET2, the genes most frequently involved in clonal hematopoiesis, carry a lower risk of leukemic progression (1, 2). Despite reported differences in the magnitude of the increased risk, it is clear from the current evidence that mutations in myeloid driver genes increase risk for leukemia. This stands in comparison to “other” clonal hematopoiesis, where the particular mutated genes have not been specifically associated with hematologic malignancy and their role in clonal expansion and leukemic progression is less clear.

In this issue of Blood Cancer Discovery, Feusier and colleagues report a “pan-cancer” assessment of hotspot mutations across select hematologic malignancies, focusing on those that are likely to have higher rates of preceding clonal hematopoiesis, including acute and chronic leukemias, myelodysplastic syndrome, and myeloproliferative neoplasms (3). Through curation of almost 50 studies, all of which used next-generation sequencing (NGS) techniques and the majority of which performed whole-exome sequencing (WES), they were able to identify a large number of new hotspots in these hematologic malignancies. Nearly 20% (79/434) of these were not identifiable in any single study as hotspots, and more than 50% (222/434) had not been annotated as hotspots in hematologic malignancies in the Catalogue Of Somatic Mutations In Cancer (COSMIC), albeit many as general oncogenic hotspots.

This expansion of the range of hematologic malignancy hotspot mutations goes beyond an improved understanding of the molecular repertoire of hematologic malignancies. A majority of the population-based case-control clonal hematopoiesis studies noted above evaluated the risk for AML transformation using panel-based deep error-corrected sequencing to look at hotspot and potential driver mutations. Thus, more complete knowledge of relevant mutations may increase our detection of patients at highest risk for malignant transformation. In addition, although most studies focused on the risk for AML transformation specifically, these authors considered other hematologic malignancies that may arise from preexisting clonal hematopoiesis. This may also improve identification of molecular and clinical risk factors for progression to a broader suite of myeloid malignancies.

Second, the authors performed clonal hematopoiesis analysis on more than 4,500 individuals, including more than 400 children. They used publicly available databases from the 1000 Genome Project (adults only, across a variety of populations) and two population studies of WES that included...
children to evaluate patients with (i) autism and their unaffected siblings and parents, and (ii) type II diabetes including individuals ages 0 to 85. They assessed for clonal hematopoiesis in the genes on their expanded hotspot list as well as in an unscreened fashion across the exome. It should be noted that the strength of evaluation provided by WES comes at lower coverage in some hotspot regions, potentially leading to lower reported rates of clonal hematopoiesis than would be seen using targeted, deeper coverage sequencing.

The genes most frequently affected by clonal hematopoiesis in this study included DNMT3A, TET2, and TP53. Across the published literature, DNMT3A and TET2 are invariably the most frequently mutated genes in clonal hematopoiesis and are among the most commonly mutated genes in AML and other myeloid malignancies. However, the most commonly mutated genes in clonal hematopoiesis beyond DNMT3A and TET2 differ based on cohort or specific study. The authors speculate that the difference in prevalence may be due to differences in age distribution across studies. Indeed, while ASXL1 has been reported frequently mutated in patients with clonal hematopoiesis, it has been associated with older age (4). Correspondingly, in studies less skewed toward older individuals (2, 5), other genes are more commonly mutated than ASXL1. The authors also note that some AML-associated hotspots are less likely to be associated with clonal hematopoiesis. Notable examples, which are consistent with prior literature (1, 2), include mutations in NPM1 and FLT3, which likely have a role in clonal evolution and leukemic transformation, but not in the earliest stages of stem cell expansion. The relatively high frequency of TP53 mutations in this cohort is interesting given its high-risk nature in most case-control studies, and the role of TP53 in early clonal expansion versus leukemic progression may be allele or context specific (1, 2).

When evaluating the prevalence of clonal hematopoiesis in this study across hotspots, rates are roughly equivalent to previous reports using similar technology, and prevalence increases with age, as expected. In general, the frequency of clonal hematopoiesis depends significantly on the sensitivity of the assay used (whole-exome sequencing < targeted panels < error-corrected sequencing). Notably, however, VAFs below that detectable by standard WES or targeted panels may be less clinically relevant, as most studies evaluating clonal hematopoiesis with more sensitive assays have not been able to associate lower VAF clones (<0.01) with progression to leukemia or other adverse sequelae (6, 7). Correspondingly, many studies have associated clones with higher VAFs to have higher risk of leukemic transformation (1, 2). As such, it is important to consider clonal hematopoiesis a quantitative trait, such that low clonal burden clonal hematopoiesis is highly prevalent but likely of little clinical consequence, whereas clonal hematopoiesis detectable in standard NGS assays with a VAF of 0.02 to 0.05 or higher has greater relevance to leukemic progression and other nonmalignant diseases.

Another feature of high-risk clonal hematopoiesis frequently reported in population-based case-control studies is “complex” clonal hematopoiesis, or clonal hematopoiesis involving more than one mutated gene (1, 2). Here, the authors report that such complex events are rare in their cohort, with only six individuals in the broader clonal hematopoiesis assessment having more than one mutated gene, and none with more than two. This frequency is lower than that previously reported [Desai and colleagues report 5.5% in controls (1), and Acuna-Hidalgo and colleagues report approximately 1% with a skew toward older age (5)]. This may be attributable to the use of lower-depth WES instead of targeted panels, limiting coverage and sensitivity to identify second, likely lower-frequency, events. While the evaluation here may be missing some detection of complex clonal hematopoiesis, the addition of the newly identified hotspots to more sensitive assays may, in the future, provide the best assessment of risk associated with complex clonal hematopoiesis, especially if the presence of more than one clonal hematopoiesis mutation has independent prognostic relevance.

Although preleukemic rearrangements have been known to exist in cord blood (8) for almost two decades, there is currently no published work describing the prevalence and clinical relevance of clonal hematopoiesis in the general pediatric population. This work demonstrates the first evidence that clonal hematopoiesis is observed in children outside of those with advanced malignancies. Given the techniques utilized here and the rarity of such events, specific rates and patterns are merely speculative, and thus there is more work to be done to fully characterize this phenomenon. It should be noted that several studies to date using more sensitive techniques have demonstrated higher than expected rates in young adults ages 20 to 29 [Acuna-Hidalgo report 2.3% in this age range (5), and the Qatari cohort in this study reported associated ages, with a rate of 2.5% in relaxed clonal hematopoiesis in ages 20 to 29 (3)], supporting the notion that mutational clonal hematopoiesis likely does begin in childhood. We would expect that subsequent studies of general pediatric cohorts and pediatric patients treated with oncologic or biologic therapies will better delineate the frequency and clinical implications of clonal hematopoiesis in children and determine whether clonal hematopoiesis in children carries risks similar to those in adults, with an increased risk of subsequent leukemia and/or nonmalignant clonal hematopoiesis-driven complications.

The understanding of clonal hematopoiesis has evolved rapidly since the initial observations in the 1990s, the first NGS-based characterization of clonal hematopoiesis in 2012, and the first comprehensive population-based studies in 2014. It is now well understood that individuals with clonal hematopoiesis are at higher risk for cardiovascular events as well as hematologic malignancy, and further details both explaining those outcomes as well as better defining how specific risks and predictions should be applied are constantly being updated. With respect to defining risk of hematologic malignancy, previous work has consistently supported the notion that clonal hematopoiesis involving genes or loci affected in hematologic malignancy provide some of the highest risk of progression to leukemia. This work by Feusier and colleagues expands on the list of relevant loci to better define the spectrum of events that might contribute to this risk and provides a “road map” for hotspot-based analyses of clonal hematopoiesis in large patient cohorts investigating a diversity of disease risks and contexts.

Nearly every tissue in the body with malignant potential has recognized or postulated premalignant precursor states, and...
and with the recognition of the ubiquity of clonal hematopoiesis, the hematopoietic system appears to be no different. Some of these premalignant states are easier to screen for than others, such as colorectal adenomas or cervical intraepithelial neoplasia. Arguably, the blood is the most accessible tissue, and thus a more refined understanding of the risk factors for malignant transformation should allow for the development of a highly successful screening program. We have begun to characterize the elements that will play a role in the design of such a program, but the details are still emerging. As outlined above, this work helps clarify one piece of the puzzle, building on much previous work that established the importance of identifying mutationally driven clonal hematopoiesis. Additional inclusion of analysis for mosaic chromosomal alterations, which has also been demonstrated to predict for risk of leukemic progression, both independently and in combination with mutational clonal hematopoiesis (9, 10), will certainly strengthen this developing framework.

The last two complementary layers of a successful program will include the incorporation of germline predisposition and environmental factors. While some environmental factors have been identified, such as smoking or exposure to cytotoxic chemotherapy, the understanding of the interplay between germline predisposition to clonal hematopoiesis (4) and hematologic malignancy has just begun to unfold. More broadly, this work highlights the role of specific disease alleles in clonal expansion in the hematopoietic context, and we look forward to similar studies in different histologic contexts linking specific driver mutations with clonal expansion and progression to the broader spectrum of human cancers.

**Authors’ Disclosures**

R.L. Levine is on the supervisory board of Qiagen and is a scientific advisor to Loxo (until 2019), Imago, C4 Therapeutics, Mana, Auron, Ajax, Kurome, Mission Bio, Prelude, Scorpion, and Isoplexis, each of which includes an equity interest; receives research support from and has consulted for Celgene and Roche, has received research support from Constellation, Roche, and Prelude Therapeutics, and has consulted for Bridge Therapeutics, Bristol-Myers Squibb, Lilly, Incyte, Novartis, and Janssen; and has also received honoraria from AstraZeneca, Constellation, Lilly, and Amgen for invited lectures and Gilead for grant reviews. No disclosures were reported by the other author.

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