Clonal Hematopoiesis

A Recently Described Clinical Entity that is Very Common and Has Major Clinical Consequences

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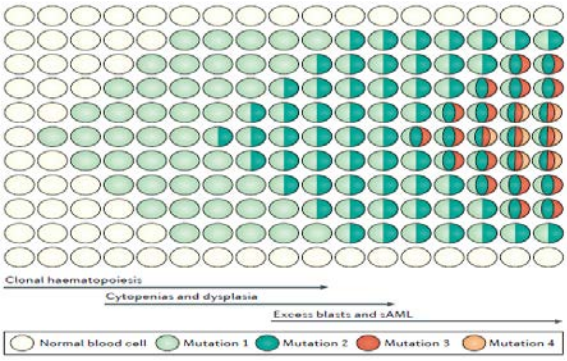

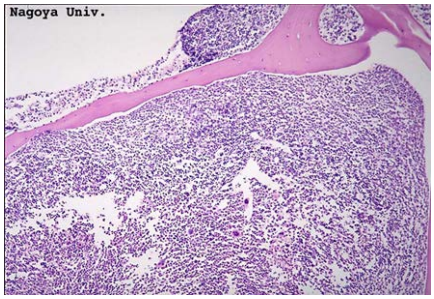

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The purpose of this lecture is to provide an overview of the recently described clinical entity, clonal hematopoiesis, in terms of its discovery, pathogenesis, clinical presentation, management, and future research directions.

Educational Objectives:

1. **Understand the concepts of normal and clonal hematopoiesis**
2. **Understand the frequency and degree of clonal hematopoiesis in the older population.**
3. **Understand the clinical consequences of clonal hematopoiesis in terms of increased risk of blood malignancy and atherosclerotic cardiovascular disease.**
4. **Understand the different ways that clonal hematopoiesis can present in the clinic.**
5. **Understand the pathogenesis of the disorder and potential future directions in management.**

Introduction

It has recently been discovered that a very large percentage of otherwise apparently healthy older individuals have a large fraction of their circulating blood cells derived from a single hematopoietic stem cell (HSC) clone, carrying mutations that are typically associated with myeloid neoplasms such as acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). (1-6) These persons usually have a normal CBC or only very mild abnormalities. However they have an increased risk of developing more serious medical problems, not surprisingly AML or MDS, but also atherosclerotic cardiovascular disease (ASCVD) such as myocardial infarction (MI) or stroke. The risk for cardiovascular disease is as significant as the risk conveyed by smoking or uncontrolled hyperlipidemia and is associated with a markedly increased risk of mortality.

This clinical entity, called clonal hematopoiesis (CH), is just in the process of being defined in terms of clinical manifestations and pathogenesis. Recent studies have shed light on the potential mechanisms of CH and its association with cardiovascular disease and investigators are beginning to propose ideas for potential management strategies.

Background

Normal non-clonal hematopoiesis

HSC give rise to hematopoiesis, producing all the mature blood cells which populate the body. The process proceeds through a cascade of highly regulated proliferation and differentiation. Each person has approximately 20,000 HSC and many of these are at any given time giving rise to hematopoiesis, so that there is no dominance of cells derived from one clone or another.

Acquisition of mutations leads to clonal dominance

However a single HSC can acquire mutations in a gene or genes which control proliferation and differentiation. These mutations can cause the stem cells to proliferate excessively and give rise to a higher percentage of blood cells than would normally be the case; the mutation-containing HSC clone and its progeny gain a “clonal dominance” over the remainder of the normal HSC and their progeny. With time, additional mutations can occur, causing the abnormal HSC clone to become more and more dominant, eventually leading to a blood cancer such as AML or MDS.

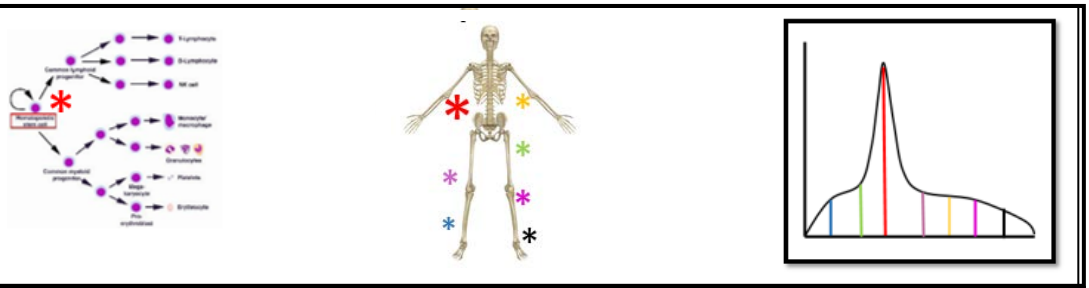

Figure 1. A single HSC acquires mutations in a gene or genes which give the cell a growth advantage, leading to clonal dominance over the rest of the normal HSC and their progeny

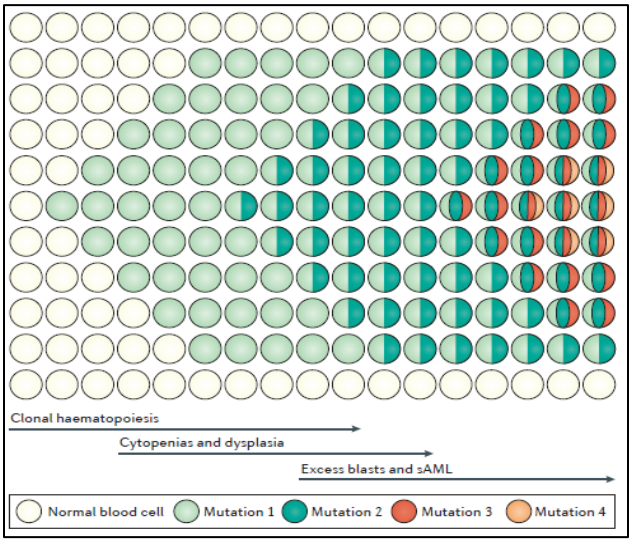

Figure 2. Clonal evolution. A single mutation initiates a process of clonal evolution in which subsequent mutations are associated with increasingly malignant behavior. (From Sperling et al. Nat Rev Cancer. 17:5-19, 2017)

Myeloid malignancy associated mutations

The first AML genome was sequenced in 2008. Since then, thousands of AML genomes (and genomes of other myeloid neoplasms such as MDS and myeloproliferative neoplasms) have been sequenced (7,8). We now know almost all of the genes which, when mutated, cause myeloid neoplasms. These mutated genes cause excessive proliferation and growth and impaired differentiation of hematologic cells and we now know, at least in broad terms and sometimes in exquisite detail, the actual mechanisms by which these mutated genes have their effects. The broad classes of gene mutation mechanisms include: transcription factor fusions, NPM1 mutation, tumor suppressors, DNA methylation, activated signaling, myeloid transcription factors, epigenetic modifiers, cohesion complex genes, and spliceosome complex genes. (7-9)

Most cases of AML or MDS, by the time they are diagnosed, have multiple mutations in genes from various classes; for example, a given AML case may have mutations in DNMT3A, a gene involved in DNA metalation, NPM1, a gene involved in regulation of several cellular processes, and FLT3, a gene involved in growth factor signaling. The typical AML case contains 3-5 DNA coding region mutations that are driving the disease.

It is possible to define the clonal hierarchy of a case of a given hematologic malignancy, most directly by single cell sequencing (10). We can now identify which mutation occurred first to give an HSC clone a growth advantage, and then which additional mutations were sequentially acquired to give the HSC additional growth advantage until it had frank malignant behavior. Full-blown cases of myeloid malignancy are comprised of multiple sub-clones with evidence of branching and convergent evolution, but always having begun with a single growth advantage-promoting mutation.

Although these myeloid malignancy genes have been increasingly appreciated in the world of malignant hematology, it has only recently become appreciated that a large percentage of otherwise apparently normal persons have a large fraction of cells circulating in their bloodstream with at least one, and sometimes more, of these mutations.

Discovery of CH in the general population

The first clue that CH may exist in a large percentage of the population was the observation of skewed lyonization in the blood of older women (11). Each female cell has two X-chromosomes and in each cell one X-chromosome is inactivated by a process called lyonization (named after its discoverer). This process is random and so any population of female cells should consist of cells in which one X-chromosome or the other has been inactivated in a 50:50 ratio. Detection of skewed lyonization suggests the dominance of a clone which contains a particular version of the X-chromosome. Studies in 1998 demonstrated the presence of skewed lionization, suggesting clonal dominance, in 38 % of women over age 60. DNA sequencing of a population of older women in 2012 made the striking observation of a mutated myeloid malignancy gene, TET2, in 5% of these patients (12).

In 2014, three groups reported large-scale analyses of several thousand persons to investigate the question of clonal hematopoiesis in the general population (1-3). Each study involved reanalyzing data from previous sequencing of large populations of persons that had been done to investigate risk for other diseases, such as diabetes and schizophrenia.

For example, one study reanalyzed 17,182 persons who had had blood cells sequenced (without regard to hematologic malignancies) as part of a type 2 diabetes association study or as part of the Jackson Heart study, a population-based cohort study (1). This group reanalyzed the prior sequencing for 160 genes commonly mutated in hematologic malignancies. Recurring mutations were discovered in several cancer genes at a variant allele frequency (VAF) of at least 2% (which translates roughly to about 4% of cells) in approximately 10% of individuals age 70 or older. Mutated genes included DNMT3A, TET2, ASXL1, TP53, JACK2, SF3B1, SRSF2, among others, genes which are highly associated in the hematologist's mind with myeloid malignancies. Most individuals with a mutation had one mutation; a small percentage had two. Mutations were present, on average, at a VAF of 10% (roughly 20% of cells) but ranging as high as 50% (roughly 100% of cells). The most common mutations were cytosine → thymidine mutations, a mutational signature of aging. Indeed, the prevalence of mutation increased steadily with age, being present in 0% of persons under age 40, 2-3% in persons age 40-59, 5% in persons age 60-69, 9.5-11.7% in persons age 70-89 and 18.4% in persons age 90 and older.

Similar findings were reported in two other large studies of 12,380 and 2,728 persons (2,3). These studies concurred in showing a high prevalence of older persons with somatic mutations in myeloid malignancy genes which were causing CH in a high fraction of circulating blood cells.

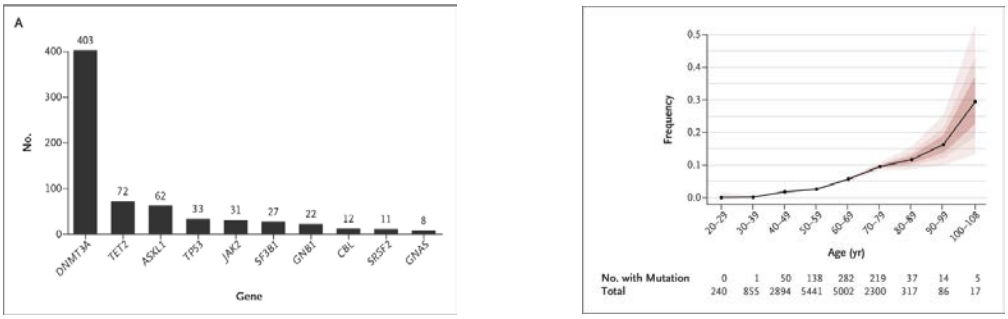

Figure 3. (A) Mutations in myeloid malignancy genes detected in blood cells of otherwise apparently normal persons. (B) The incidence of myeloid gene mutation increased with age. 10% of individuals age 70-79 have evidence of CH. (From reference 1)

Consequences of CH

Hematologic Malignancies

As might be expected, having a mutation in a myeloid malignancy gene was associated with an increased risk of developing a hematologic malignancy (1-3). The risk was 11-fold overall and 49-fold in persons who had a VAF of the mutant gene ≥ 10%, translating roughly to 20% of cells. But, because the risk of hematologic malignancy in the population is relatively low, even a substantially increased risk of developing hematologic malignancy does not translate to a high absolute risk; the risk of developing a hematologic malignancy in a patient with clonal hematopoiesis is 0.5-1% per year. As expected, in some persons with clonal hematopoiesis who do develop hematologic malignancies it has been possible to observe the clonal evolution of the disease from the initial CH-related mutation to full blown disease with multiple mutations.

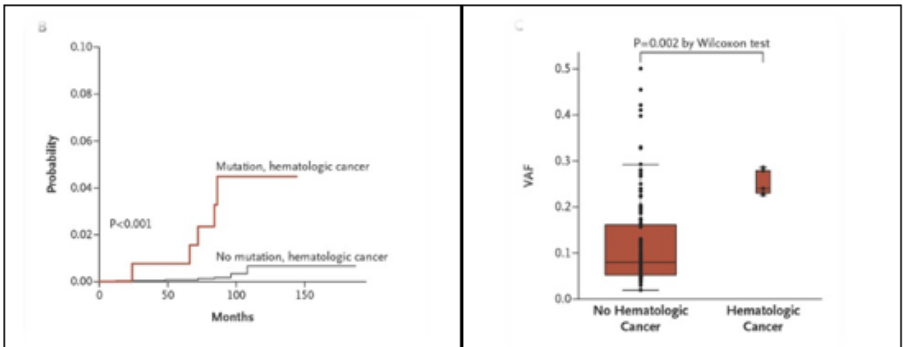

Figure 4A. Incidence of hematologic cancers are markedly increased in patients with mutations. (From reference 1)

Figure 4B. Higher VAFs were seen in patients who subsequently developed hematologic malignancies

Atherosclerotic Cardiovascular Disease and Mortality

A CH mutation is associated with a marked increase in mortality, with a hazard ratio of at least 1.4; the risk is particularly high in persons age ≥70 years. Because of its low absolute incidence, hematologic malignancy does not contribute to the increased overall risk of mortality. Rather, the increased risk was associated with *cardiovascular disease* (1-3). The hazard ratios for coronary heart disease and ischemic stroke were 2.0 and 2.6 respectively for individuals with mutations. The risk conferred by mutation remained significant after multivariable analysis for diabetes, hypertension, body mass index, smoking, and hypercholesterolemia, especially in persons with VAFs (≥10%). Additional studies have confirmed the markedly increased risk of cardiovascular disease in patients with clonal hematopoiesis mutations (13).

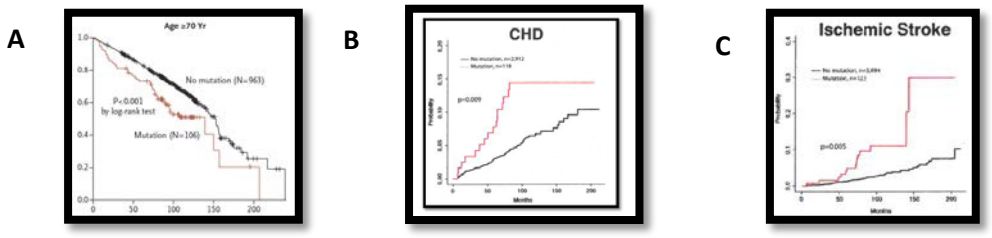

Figure 5. (A) Mortality is worse in patients with mutations. This is caused by a marked increase in (B) coronary heart disease and (C) ischemic stroke. (From reference 1)

Why is the Risk of Cardiovascular Disease Increased in CH?

Current Models for Atherosclerosis

Atherosclerosis begins with the subendothelial accumulation of apoB-containing lipoprotein (apoB-LP) that are made by the liver and small intestine and contain a lipid core of cholesterol fatty acyl esters and triglycerides (14). Activated endothelial cells then recruit blood monocytes which then differentiate into macrophages and take up apoB-LP; these lipid-laden cells, called foam cells, contribute to amplified apoB-LP retention, resulting in plaque growth, a lipid-filled necrotic core and thinning fibrous cap, and susceptibility to rupture. Thus, activated macrophages play a key role in early and advanced stages of atherosclerosis; pathways have been defined for macrophage priming and activation and mediation of inflammation through the NLRP3 inflammasome, which leads to production of mediators of inflammation, especially IL1β (14,15).

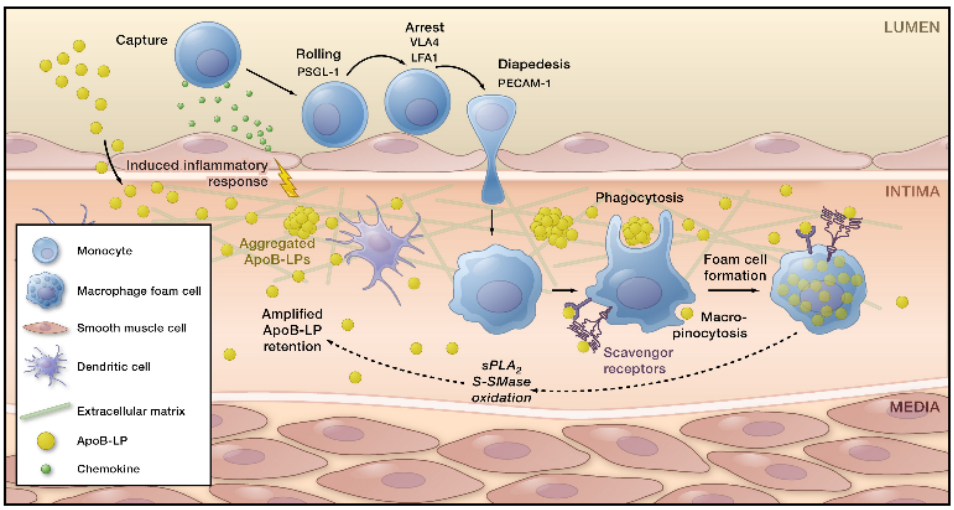

Figure 6. Model for Atherosclerosis. Atherosclerosis is initiated by deposition of cholesterol-containing apoB-LP in the subendothelium. Macrophages migrate to the site, take up apoB-LP particles, and become foam cells. See text above and references 14 for additional details. (From reference 14)

Investigating Mechanisms of CH-Mediated Atherosclerosis

At least two groups have developed animal models for clonal hematopoiesis and used them to investigate mechanisms of atherosclerosis in patients with CH (13,16). For example, Fuster et al. (16) started with a mouse that is atherosclerosis-prone because of lack of the LDL receptor (ldlr -/-), irradiated it, and replaced its bone marrow with a mixture of donor bone marrow cells that contained 10% TET2 deficient cells (TET2-/-) and 90% TET2 replete cells (TET2 is a common clonal hematopoiesis mutation and the 10:90 ratio was used to mimic the ratio of abnormal to normal seen in CH patients). This clonal hematopoiesis mouse model mimicked clonal hematopoiesis in humans, with increased HSC self-renewal and a normal CBC. When fed a high cholesterol diet this mouse model had significantly increased atherosclerosis compared to the control ldlr -/- mice that did not have TET2 deficient bone marrow cells.

The investigators then deleted TET2 specifically in monocytes and observed that the proneness to atherosclerosis persisted. They found that these monocytes/macrophages had increased production of IL1β over normal and detected increased IL1β in the atherosclerotic plaques. They then repeated the experiment, this time using an inflammasome inhibitor, and found that this led to both decreased IL1β production and decreased development of atherosclerosis.

Thus, these data support a model of clonal hematopoiesis-exacerbated atherosclerosis in which the atherosclerosis is worsened by monocytes/macrophages which are excessively activated because of CH mutations.

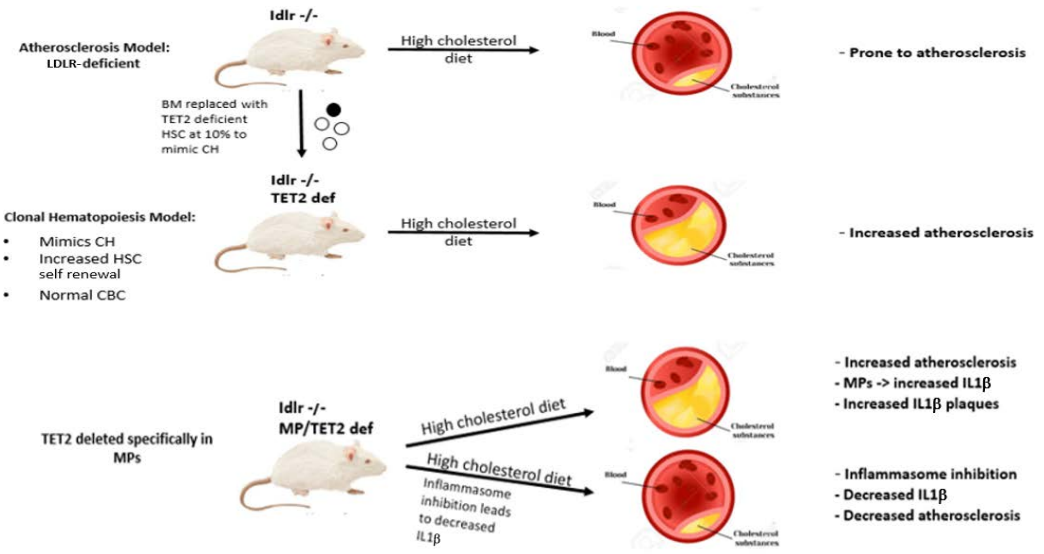

Figure 7. Mouse model of TET2-deficient macrophages results in accelerated atherosclerosis. Inflammasome inhibition decreases IL1β production and this is associated with decreased atherosclerosis. (See text above and reference 16 for additional explanation.)

Clonal Hematopoiesis in the Clinic

The clinical and pathologic description of clonal hematopoiesis is still evolving (4-6). The following terms are in common usage to define different potential clinical presentations.

Clonal Hematopoiesis of Indeterminate Potential (CHIP)

- Definition. CHIP is defined as the presence of a clonal mutation with a VAF of at least 2% and a gene associated with hematologic malignancy. The CBC is essentially normal and the person does not meet criteria for a WHO-defined neoplasm (e.g. MDS). When associated with older age this condition is sometimes called "age-related clonal hematopoiesis (ARCH)."
- Presentation: The patient has had detection of a mutation in the blood or bone marrow in a next-generation sequencing (NGS) panel that has been done for some other reason, e.g. when blood is used as a germline control in solid tumor sequencing.
- CBC: usually normal. May have mild increase in MCV or RDW. May have minimal, clinically insignificant cytopenias.
- Mutation: the most common mutations are in DNMT3A, TET2, ASXL1, TP53, JAK2, SF3B1, GNB1, CBL, SRSF2, and GNAS. By definition the VAF is ≥ 2%
- Implications: increased risk of hematologic malignancy—MDS or AML – 0.5-1% per year. The risk is higher with high VAF (≥10%), with certain mutations (e.g. TP53), and if there is more than one mutation. Patients have a significantly increased risk of MI or stroke, on the order of the risk from smoking or uncontrolled hyperlipidemia.

- Management: regarding the blood cancer risk, patients should have periodic monitoring of their CBC, more frequently if higher risk. Regarding the cardiovascular risk, patients should have assessment and optimal management of other cardiac risk factors.

Clonal Cytopenias of Undetermined Significance (CCUS)

- Definition. Clonal mutation in a patient with one or more clinically meaningful, otherwise unexplained cytopenias. Does not meet requirements for a WHO-defined hematologic neoplasm
- Presentation. One or more clinically significant cytopenias.
- Bone Marrow. Lack of dysplasia. Does not meet criteria for MDS
- Work up. No other cause for cytopenias found
- Mutation. Picked up on NGS panel – typically done as part of bone marrow evaluation. Same mutations as listed under CHIP. VAF at least 2%.
- Implications. Increased risk of hematologic neoplasm and cardiovascular disease. Risk of hematologic neoplasm is greater with higher VAF and more than one mutation. The overall risk appears to be significantly higher for CCUS than for CHIP.
- Management. Same principles as CHIP (probably even closer follow up).

Idiopathic Cytopenias of Undetermined Significance (ICUS)

- Definition: single or multiple blood cytopenias that remain unexplained after a full work-up including a bone marrow exam. No clonal mutations.
- Presentation: Cytopenia or cytopenias with negative workup – negative bone marrow, no clonal mutations. No evidence of other causes, e.g. nutritional, autoimmune, enlarged spleen, T-LGL, lymphomas, etc.
- Outcome:
    - Some develop MDS or other myeloid neoplasms with time
    - Some will have another malignant or non-malignant cause become apparent with time
    - Some will persist for years without diagnosis and without worsening
    - Some will have resolution of abnormalities

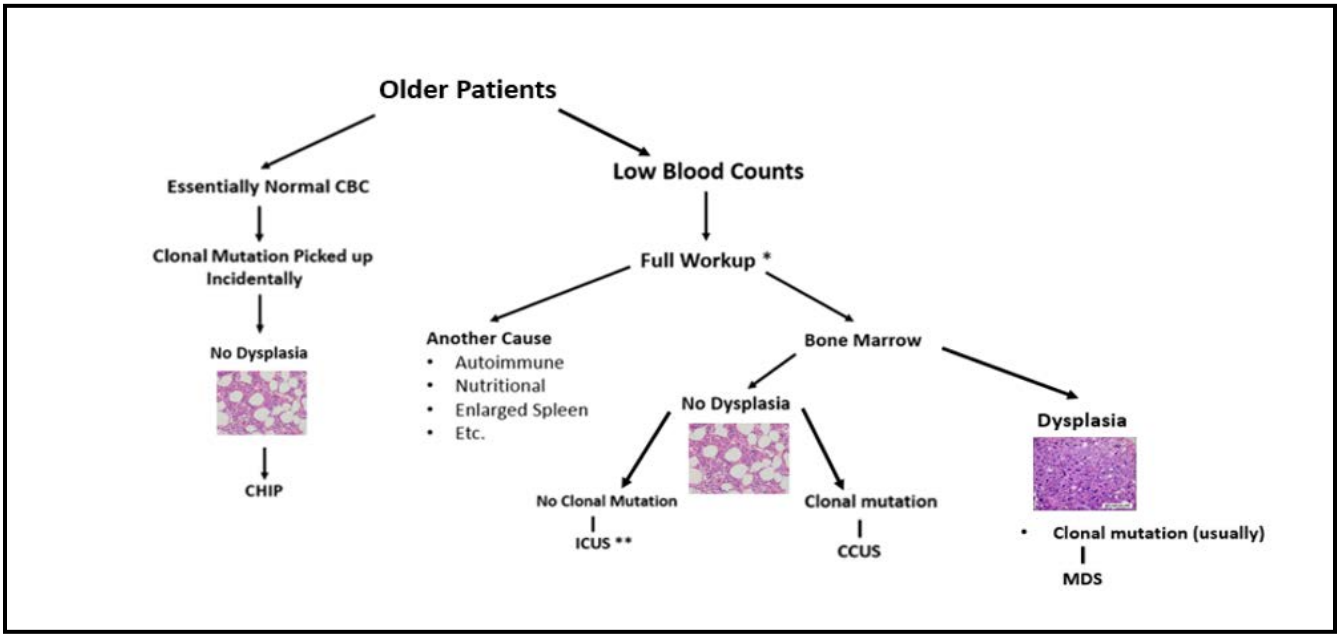

Figure 8. CHIP, ICUS, CCUS, MDS. Workup of older patients with cytopenias. Note: patients can have "ticks and fleas", i.e. a patient can have clonal mutations but have the cytopenias be due to something else. *Full workup may include: B12, folate, iron studies, retic count, epo level, ANA, TSH, drugs, spleen size, etc. **Idiopathic cytopenias of undetermined significance.

Other Clonal Hematopoiesis Scenarios

- After chemotherapy for non-hematologic tumors (17). 25% of non-hematologic cancer patients have evidence of clonal hematopoiesis. It is associated with increased age, prior radiation therapy, and tobacco use. TP53 and PPM1D mutations are associated with prior chemotherapy exposure and are associated with a markedly increased risk of evolution to therapy - related MDS/AML compared to other clonal hematopoiesis patients. Patients have an increased risk of mortality, which is mainly due to increased risk of progression of the primary tumor.
- After autologous stem cell transplantation for non-Hodgkin lymphoma (18). At least 10% have mutations in hematologic malignancy genes. These patients have poor graft function and cytopenias and a higher risk of developing secondary MDS/AML.
- After allogeneic stem cell transplantation (19-21). Recipients of allogeneic stem cell transplants from older donors have worse outcomes. It is possible that this is due in part to clonal hematopoiesis in the older donors, which has been associated with poor bone marrow function in the recipient, increased graft-vs.-host disease, and donor-derived leukemia. Transplant centers are increasingly choosing younger donors over older donors even when the younger donor has a greater degree of HLA mismatch with the recipient.
- In aplastic anemia patients (22). Aplastic anemia is caused by an autoimmune attack against HSCs. MDS and AML develop in 15% of these patients over time. Clonal hematopoiesis is detected in 47% of aplastic anemia patients, with the majority having mutations in myeloid cancer genes. Certain mutations, such as DNMT3A and ASXL1, tend to increase with time and are associated with worse outcomes. Other mutations, such as PIGA, BCOR, and BCORL1, are associated with better responses to immunosuppressive therapy; clones containing these mutations decrease or remain stable with time and are associated better outcomes.

Testing for Clonal Hematopoiesis

Most people diagnosed with clonal hematopoiesis are diagnosed "by accident". Patients with normal CBCs don't generally have a reason for NGS panels to be done on the peripheral blood.

One can argue that older patients should have NGS panels done on a routine basis to test for clonal hematopoiesis: the entity is very common and it is a major risk factor for cardiovascular disease and for hematologic malignancy. One might be able to use detection of clonal hematopoiesis to diagnose MDS or AML at an earlier and more manageable stage. Certain patients are at particularly high risk of hematologic disease, such as solid tumor patients who have had chemotherapy and who have high-risk mutations such as P53 and PP1MD at high VAF levels—these patients would warrant particularly close follow-up. In addition, one could envision managing a newly diagnosed patient's cardiovascular risk factors more aggressively.

However, the approach of wide-scale testing would certainly be very expensive and there is no evidence that more aggressive management of cardiovascular risk factors would help. One might argue that at this point we wouldn't have anything to do with such a patient except watch and wait (watch and worry).

Additional recent insights into clonal hematopoiesis

The incidence of CH is actually much higher when looked at with more sensitive techniques

All of the initial studies looking at clonal hematopoiesis looked at VAFs of ≥2%, the lowest limit of detection with standard NGS techniques. A recent study using error-corrected sequencing, which can detect VAFs at a level of 0.03%, looked at AML mutations in 20 healthy participants from the Nurses' Health Study who were between 50-60 years old (23). This study showed CH mutations in 95% of the individuals, at a median VAF level of 0.2%. Most mutations were in DNMT3A and TET2, the 2 most common mutations in studies of CHIP using less sensitive methods. VAF levels remained at relatively low levels over 10 years and were not associated with adverse outcomes. Thus, in individuals age 40-60, clonal hematopoiesis at low levels seems to be nearly ubiquitous.

Clonal Hematopoiesis with and without *typical myeloid leukemia mutations is very common in the elderly*

Early studies of CH focused on panels of known cancer-related genes. However, this approach might miss cases of clonal hematopoiesis caused by other genes or associated with other mechanisms. To address this issue, investigators in Iceland performed whole-genome sequencing on peripheral blood of 11,262 Icelanders (24). They were able to approach the issue of clonal hematopoiesis by a technique involving "barcodes of mosaic somatic mutations" (see the reference for explanation). This approach determined clonality without bias as to whether the clonality was caused by one of the myeloid malignancy genes. This group found that clonality is very common in the elderly, tending toward inevitability. They confirmed that genes such as DNMT3A, TET2, ASXL1, and PPM1D were frequently associated with CH, but strikingly, they found that mutations in such genes were evident in only a fraction of CH cases. Thus, the great majority of CH cases were *not* associated with the usual myeloid leukemia genes. Nevertheless, regardless of whether CH was associated with a myeloid gene mutation or not, it was still associated with an increased mortality rate.

The cause for the clonal hematopoiesis in the absence of mutations in candidate driver genes in unclear but includes possibilities such as non-coding DNA mutations, heritable epigenetic changes in promotor regions or mutations in genes which could contribute to clonality but have not yet been identified. Another, and more likely, possibility is that random clones attain dominance due to chance-driven genetic drift rather that mutation-driven selection advantage (24). This phenomenon is more likely to occur when the overall number of stem cells is decreased, as is expected to be the case in age-related stem cell attrition.

A Model for CH

To summarize certain observations about CH:

- CH caused by typical myeloid mutation is nearly ubiquitous in individuals age 50-60 but at a low level (VAF 0.2%) and with a low likelihood of progression.
- CH caused by typical myeloid mutations detectable at high levels (median VAF 10%) is very common in individuals age 70 and older.
- CH is extremely common in older individuals using non-biased assessments of clonality (not depending on myeloid mutations).
- CH is common in other settings where stem cell numbers are decreased: radiation, chemotherapy, stem cell transplantation, and autoimmune attack against stem cells.

HSC numbers are known to decrease with age and a model of CH centered around stem cell attrition is consistent with these observations (see figure 9 below).

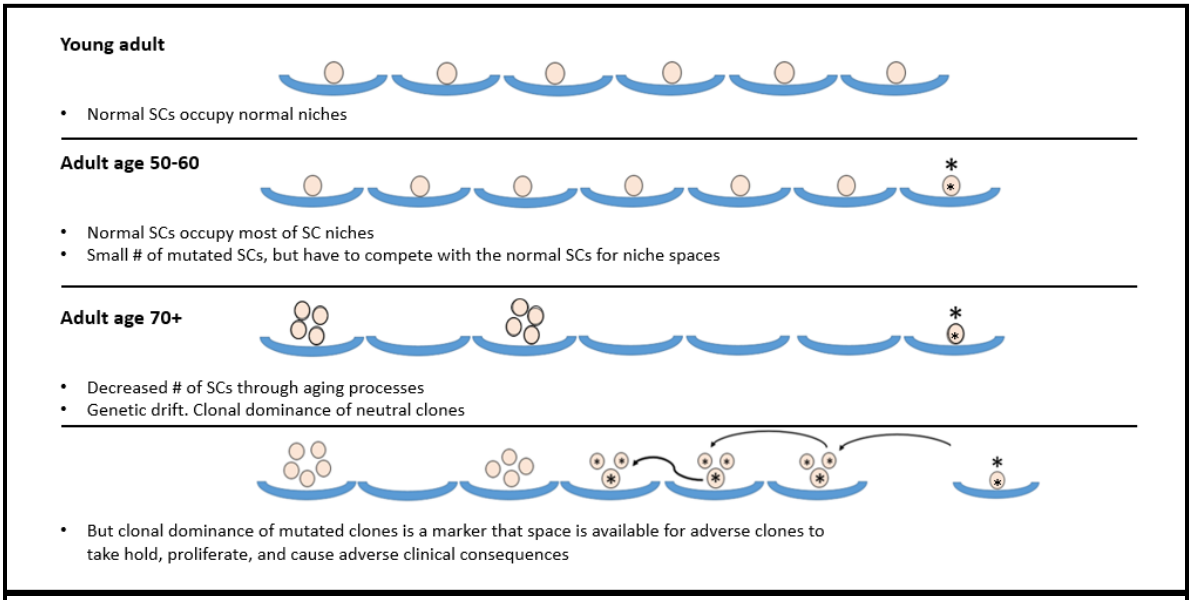

Figure 9. A model for age-related CH. *-mutated stem cell

Stem Cell Aging

Thus, the question of why stem cells age is a crucial question for understanding age-related clonal hematopoiesis. Recent advances serve as a framework for further research in this area (reviewed in reference 25).

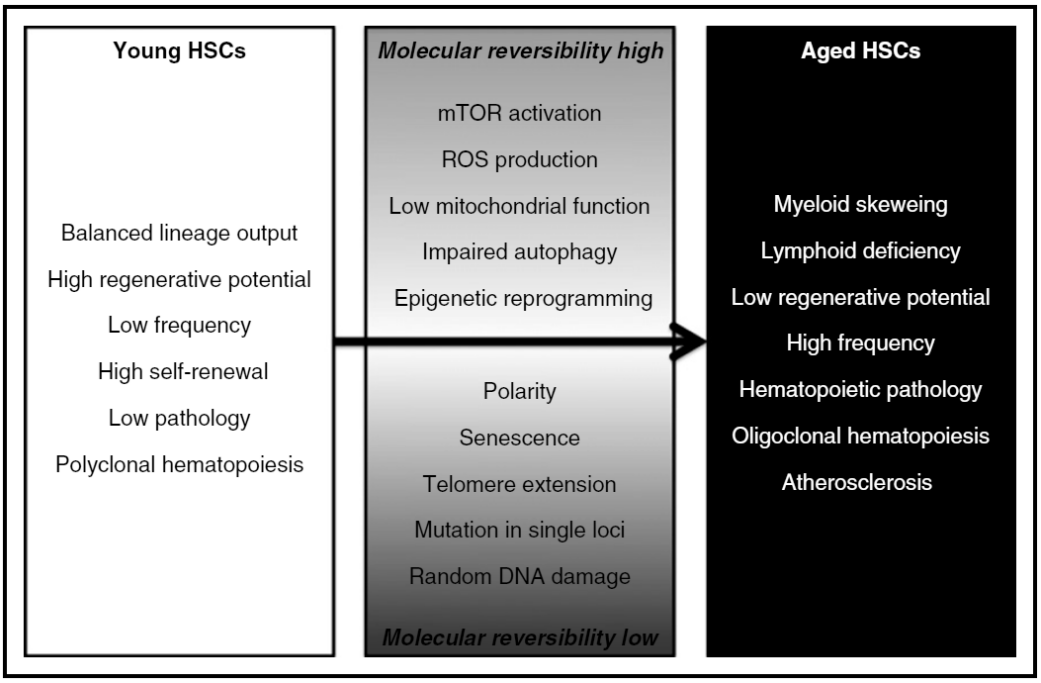

Figure 10. Cell-intrinsic mechanisms that contribute to stem cell aging. (From reference 25)

Glimpsing the future of clonal hematopoiesis: Could prediction and early detection lead to early intervention?

Early prediction/detection of AML

Several groups have recently looked more deeply into the question of whether abnormal hematopoietic clones may be detectable long before the development of AML (26,27). Abelson et al. analyzed peripheral blood cells from 95 individuals at a time point an average of 6.3 years before the subsequent diagnosis of AML (26). They performed deep-sequencing for AML-associated genes on the pre-AML cases and compared the results to those from 414 age- and gender-matched controls who did not develop AML. They showed that the pre-AML cases had a higher frequency of mutations, had more mutations per sample, and had higher VAFs, in addition to involvement of different genes. They used these parameters to develop a genetic model that accurately predicted future risk of AML. Such a model would be impractical for large-scale screening because of the rarity of AML, the cost of screening, and the problem of false positives. Therefore, the investigators used a large electronic health record (EHR) database of over 3.45 million persons which contained 875 cases of AML to develop an AML predictive model that had 25.7% sensitivity and 98.2% specificity. Such an EHR-based AML predictive model could be used to identify individuals at risk for more in-depth sequencing analysis.

Early intervention

One can imagine treating CH patients preemptively to prevent development of hematologic malignancies or cardiovascular complications. Several UTSW investigators are working in research with potential relevance to this area.

TET2 and Vitamin C. TETs is one of the most frequently mutated genes in CH. This protein causes hydroxylation of methyl groups on DNA, leading to their removal; this demethylation of DNA leads to transcription of genes involved in normal proliferation and differentiation of early hematopoietic progenitor cells. Mutations of TET2 lead to loss of function and impaired demethylation of target genes. Drs. Michail Agathocleous and Sean Morrison at UTSW have recently shown that vitamin C is a critical cofactor for TET2 function (28). Vitamin C deficiency is observed in approximately 25% of the population, and by impairing TET2 function could worsen patients who already have a mutant TET2 allele (and thus are particularly reliant on a vitamin C as a TET2 cofactor) or could lead to TET2 dysfunction even in the absence of a mutation. Thus, vitamin C supplementation may play an important role in preventing CH-related disease (28,29).

Targeting Spliceosome Mutations. Mutations of components of the spliceosome are common in CH and in patients who subsequently develop MDS and AML. Dr. Deephak Nijhawan at UTSW has discovered that an anti-cancer sulfonamide, indisulam, targets the splicing machinery by acting as a "molecular glue" between an E3 ligase and a key spliceosome component, RBM39; this leads to ubiquitination of RBM39 and proteasomal degradation (30). Cells with splicing factor mutations are particularly dependent on a degree of residual spliceosome function and thus may be particularly susceptible to targeting by this agent.

Targeting Stem Cell Translation. Normal and malignant stem cells have relatively low levels of protein translation and thus may be particularly susceptible to any further decrease in levels of translation. Dr. Stephen Chung at UTSW has discovered that the cell surface molecule, CD99, is preferentially expressed by leukemic stem cells (31). He is investigating a strategy of using anti-CD99 antibodies conjugated to ribosomal toxins to preferentially target the translational machinery of CD99 positive malignant stem cells.

Targeting monocyte/macrophages. As outlined above, CH-derived monocyte/macrophages appear to play a major role in exacerbating atherosclerosis. Thus, targeting monocyte/macrophages might play a role in inhibiting atherosclerosis. Dr. Alec Zhang at UTSW has identified a cell surface molecule, LILRB4, which is overexpressed by premalignant and malignant monocytes, and has developed both monoclonal antibodies and CAR T cells which target the molecule (32). These drugs are in clinical development.

Targeting the monocyte/macrophage mediator, IL1β. As outlined above, CH-derived monocyte/macrophages may mediate their effect through IL1β. Several antibodies target IL1β and one of these, canakinumab, has been evaluated in patients at high risk of cardiac disease in a randomized clinical trial. Patients receiving canakinumab had a marked improvement in key outcomes, including myocardial infarction, stroke, readmission for unstable angina, and death (33). DNA sequencing studies are currently ongoing to determine if the patients who benefitted may have had CH mutations.

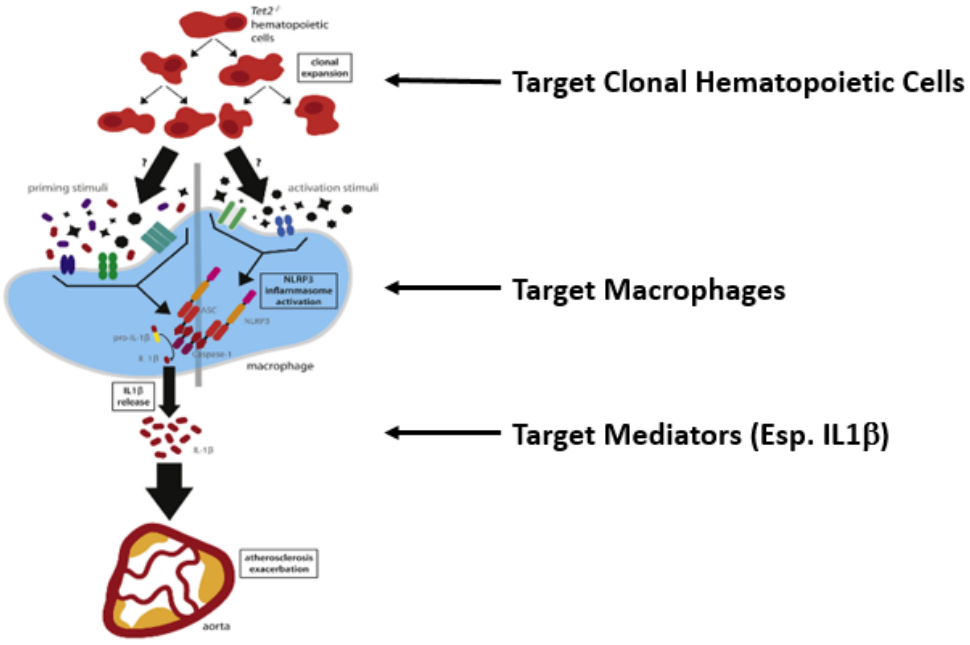

Figure 11. Approaches to clonal hematopoiesis and its adverse consequences. See text above for examples of approaches to (A) target clonal hematopoietic cells; (B) target macrophages; (C) target mediators, especially IL1β. (From reference 15)

Might clonal hematopoiesis be playing a role in a broader range of diseases? (4)

The apparent linkage between ASCVD and inflammatory mediators released by CH-derived monocyte/macrophages raises the question of whether such cells could play a role in diseases of other tissues as well. Along these lines, TET2 knockout mice have increased lung damage and gut damage after exposure to inflammation-promoting compounds, and clinical studies have associated the presence of TET2 mutant clones with COPD/asthma, and CH mutations with development of type 2 diabetes mellitus. In addition, clinical and laboratory observations suggest that CH-derived monocyte/macrophages might promote solid tumor growth by impairing the local anti-tumor immune response. Studies have also identified mutations in tumor-infiltrating lymphocytes, raising the question of CH-related effects on tumor immunity through additional mechanisms.

The broad disease implications of clonally expanded stem cells of the hematopoietic system raise the question of whether similar expansions of *non*-hematopoietic tissue stem cells may be implicated in diseases of other tissues as well.

Clearly, the field is just in its infancy, with far more questions raised than answered and the hint of many more interesting and unexpected findings to come.

UT Southwestern Clonal Hematopoiesis Clinic

UTSW is establishing a Clonal Hematopoiesis Clinic that will be under the direction of physician-scientist Stephen Chung, MD. This program will serve as a site for both clinical care and translational research in this rapidly developing field.

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