REVIEW SERIES

Pathogenesis and inflammaging in myelodysplastic syndromes

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Abstract

Myelodysplastic syndromes (MDS) are a genetically complex and phenotypically diverse set of clonal hematologic neoplasms that occur with increasing frequency with age. MDS have long been associated with systemic inflammatory conditions and disordered inflammatory signaling is implicated in MDS pathogenesis. A rise in sterile inflammation occurs with aging and the term “inflammaging” has been coined to describe this phenomenon. This distinct form of sterile inflammation has an unknown role in the pathogenesis of myeloid malignancies despite shared correlations with age and aging-related diseases. More recent is a discovery that many cases of MDS arise from clonal hematopoiesis of indeterminate potential (CHIP), an age-associated, asymptomatic pre-disease state. The inter-relationship between aging, inflammation and clonal CHIP is complex and likely bidirectional with causality between inflammaging and CHIP potentially instrumental to understanding the pathogenesis of MDS. Here we review the concept of inflammaging and MDS pathogenesis and explore their causal relationship by introducing a novel framing mechanism of “pre-clonal inflammaging” and “clonal inflammaging”. We aim to harmonize research on aging, inflammation and MDS pathogenesis by contextualizing the current understanding of inflammaging and the aging hematopoietic system with what is known about the etiology of MDS via its progression from CHIP.

Introduction

Myelodysplastic syndromes (MDS) are a diverse group of clonal hematologic neoplasms characterized by ineffective hematopoiesis, morphological dysplasia in one or more cell lineage and risk of transformation to acute myeloid leukemia (AML).[1] Approximately 50-60 recurring somatic gene mutations and structural chromosomal abnormalities contribute to the pathogenesis of MDS and are readily discoverable with standard-of-care, next-generation sequencing and classic karyotyping; starting to comprehend the mechanisms of these aberrations has aided in the development of an understanding of MDS. Still, the disease is difficult to treat, and hematopoietic stem cell (HSC) transplantation remains the only curative therapy.

For this reason, efforts towards detecting mutational changes, and hopefully, intervening at an earlier phase of disease, are of great clinical interest. A revolution towards this end has been the increased understanding of clonal hematopoiesis of indeterminate potential (CHIP), which is an age-associated, asymptomatic outgrowth of somatically mutated clones in HSC, which harbor the potential for evolution to MDS or other hematologic malignancies.[2] CHIP cells are most commonly characterized by mutations in the transcriptional regulators *DNMT3A*, *TET2* and *ASXL1*; all genes frequently observed in myeloid malignancies and hallmarked by gradual outgrowth, which often occurs with the onset of a clinical phenotype of MDS. Somatic mutations occur in many tissues at a linear rate with age and the hematopoietic system is no exception.[3] Only a small portion of these mutations occur in exons and an even smaller portion are thought to provide a growth advantage to the stem cell, which is a defining characteristic of CHIP.[4] CHIP is infrequently detected in individuals under 40 years of age but its prevalence gradually increases to >10% at the age of 70 years, making clonal hematopoiesis a signature of aging and offering one explanation as to why MDS is primarily seen in older patients.[5-7] More recent analysis

of the phylogenies of clonal hematopoiesis in human marrow samples has suggested that there is an abrupt loss of polyclonality in the bone marrow with aging. Samples from otherwise healthy donors under the age of 65 years had 20,000 to 200,000 genetically distinct HSC clones contributing to blood pool production while most blood cells derived from only 10-20 HSC clones in older individuals.[8]

Aging and MDS share another key feature: inflammation is inextricably linked to both processes. "Inflammaging" has become a term popular in the field of geroscience to describe repeatedly observed changes in metrics of inflammation in an aging immune system. In this context, inflammaging implies a sterile, chronic, low-level inflammatory state associated with advanced age. It has been implicated as one of the seven pillars of aging, contributing to gradual global organ dysfunction and frailty seen in the elderly.[9,10] Observations of increased MDS prevalence in cohorts of patients with chronic inflammatory disease, including vasculitis,[11] rheumatoid arthritis,[12] Crohn's disease,[13] Bechet's disease[14] and others, have been noted for decades.[15] A recent retrospective case-control study re-demonstrated those associations while also identifying a higher prevalence of gout and fatty liver disease in MDS patients than in an age-matched cohort of patients with solid tumors.[16] These observations have prompted questions of a causative relationship between inflammation and MDS, but the exact nature and relative direction of this causation remain incompletely understood. Although the field is rapidly advancing, the majority of existing work in humans is exploratory and correlates myeloid neoplasms or aggressivity of these neoplasms with high titers of inflammatory cytokines.[17] This review seeks to harmonize some of the research on aging, inflammation and MDS pathogenesis by contextualizing the current understanding of inflammaging and the aging hematopoietic system with what is known about the etiology of MDS via its progression from CHIP.

Throughout this review, we will highlight data generated by retrospective analysis of large biobanks along with laboratory modeling of CHIP, MDS and aging. This work is either correlative in nature or uses model systems when discussing relationships between inflammation, aging and MDS in the context of human disease. Causative links between CHIP-associated inflammation and disease remain less clear; nevertheless, reviewing these data in the context of inflammaging and MDS may inspire discussion or new lines of inquiry in the study of MDS pathogenesis.

Clonal and non-clonal inflammaging models

Inflammaging as a concept was developed as a heuristically powerful framework over two decades ago to describe a pro-inflammatory cytokine profile in aged human and mouse peripheral blood in the absence of infection, specifically tumor necrosis factor (TNF)-α[18] and interleukin (IL)-6,[19] in initial reports.[20] The concept has grown to encompass a list of observations about the aging immune system, including a reduced capacity to mount an effective inflammatory response in some cases,[21] a pathological over-response in others,[22] and increased organ infiltration by immune cells[23] that are increasingly autoreactive.[24] The precise etiology of inflammaging and the biological mechanisms of how it contributes to organ dysfunction are incompletely understood and it remains possible that inflammaging is only a non-causal biomarker of the aging process. It is crucial to note that much of the original geroscience research into inflammaging occurred before the discovery of CHIP and CHIP's own possibly causal relationship with age-related diseases, including MDS.

The irreversible loss of proliferative capacity seen globally with aging, termed cellular senescence, is also true of immune cells and is hypothesized to lead to a loss of ability to detect and eliminate cancer cells via a process known as immunosenescence.[25] Immunosenescence is linked to inflammaging and hypothesized to contribute to MDS pathogenesis. A recent study identified 115 cases of CHIP that subsequently evolved to a myeloid neoplasm in the UK Biobank and found that a key predictor of progression from CHIP to hematologic malignancy was an increased level of pro-inflammatory serum proteins.[26] Additionally, correlative work with human samples from patients with ulcerative colitis suggests that *DNMT3A* CHIP clones may have a competitive growth advantage in this inflammatory condition.[27] Multiple mouse models have demonstrated that increases in inflammation in the HSC microenvironment may provide a selective pressure favoring disease-initiating clones, including microbial signals or cytokines such as IL-1 or TNF-α promoting *TET2* CHIP competitiveness,[28-30] interferon-γ promoting *DNMT3A* CHIP[31] and obesity-associated inflammation driving both.[32]

This hypothesis is reminiscent of the immune escape mechanism proposed to explain the outgrowth of paroxysmal nocturnal hemoglobinuria clones in aplastic anemia, which resist inflammatory damage from T-cell-mediated destruction of wild-type HSC due to their loss of essential surface proteins.[33] These reports are highly supportive of the hypothesis that inflammation may promote the development of clonal hematopoiesis, and subsequently MDS, but questions remain about the relative contribution of non-clonal inflammaging and CHIP itself to this inflammatory milieu. Our proposed models inter-relating CHIP and inflammaging in MDS pathogenesis are provided in Figure 1.

Inflammaging as a driver of age-related diseases and clonal hematopoiesis

In one conceptual model (Figure 1A), lifelong exposure to various antigens and stressors to the immune system gradually produce the pro-inflammatory phenotype seen in inflammaging.[34] These stressors could include a variety

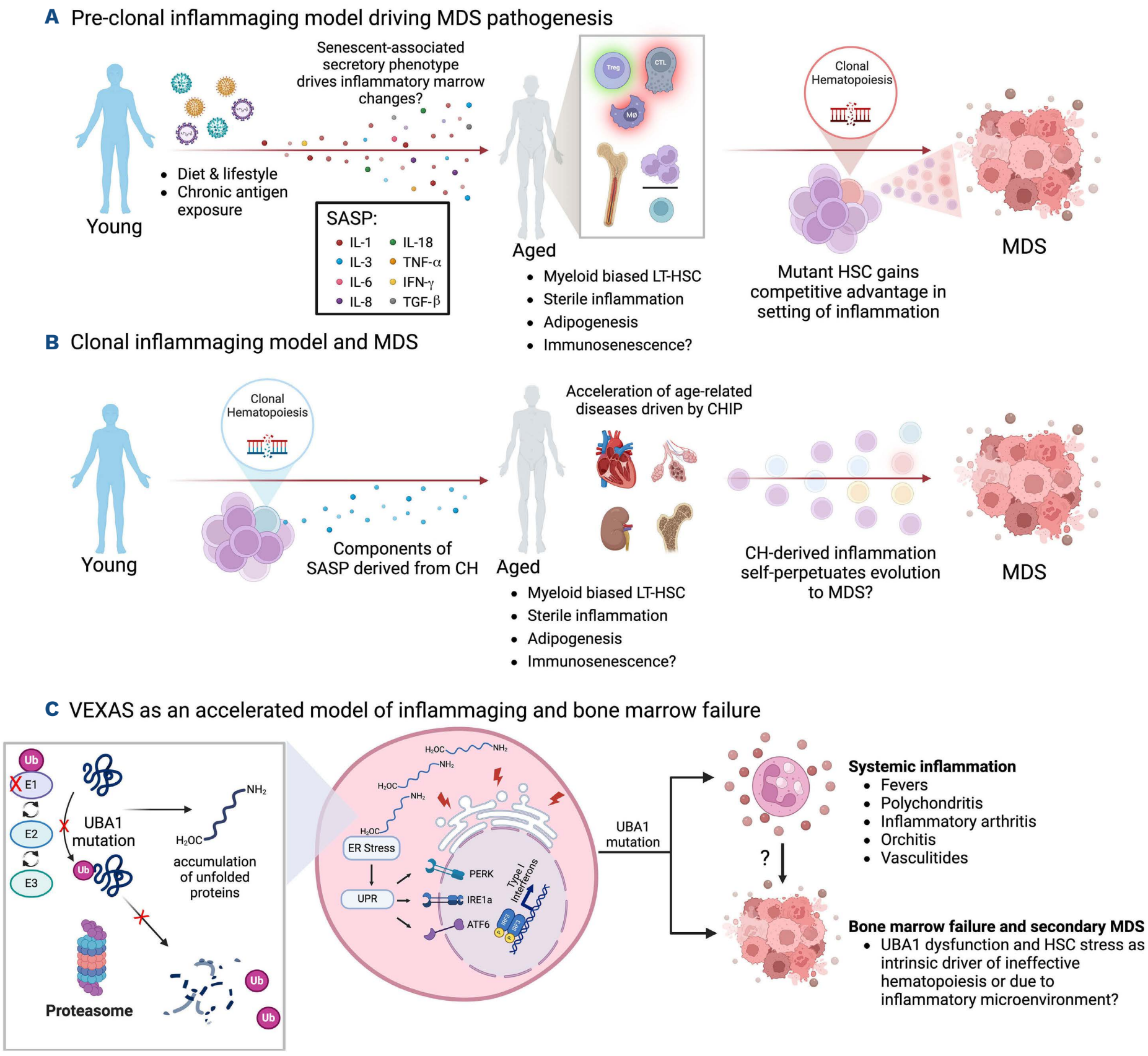

Figure 1. Inflammaging in the era of clonal hematopoiesis of indeterminate potential: understanding causal relationships between aging, inflammation and myelodysplastic syndromes. (A) The 'pre-clonal inflammaging' model: inflammaging arises prior to the development of clonal hematopoiesis of indeterminate potential and leads to the pro-inflammatory phenotype of the aged bone marrow microenvironment which facilitates the subsequent development of clonal hematopoiesis and progression to myelodysplastic syndrome (MDS). (B) The 'clonal inflammaging' model: clonal hematopoiesis arises as a stochastic event of aging and drives the inflammaging phenotype. Clonal hematopoiesis-derived pro-inflammatory cells contribute to various age-related diseases while mutant hematopoietic stem cells clonally evolve until the MDS clinical phenotype develops. (C) The VEXAS syndrome as a model of the inter-relationship between inflammation and MDS development. Loss of the functional *UBA1* gene product E1 prevents the first step in ubiquitin activation and subsequent ligation to target proteins by E2 and E3 to mark them for degradation via the proteosome. This leads to an accumulation of unwanted and unfolded proteins which triggers the unfolded protein response and its effectors PERK, IRE1α, and ATF6 which culminates in an increased production of type I interferons. Created with BioRender.com. SASP: senescent-associated secretory phenotype; IL: interleukin; TNF: tumor necrosis factor; IFN: interferon; TGF: transforming growth factor; LT: long-term; HSC: hematopoietic stem cells; CH: clonal hematopoiesis; CHIP: clonal hematopoiesis of indeterminate potential; VEXAS: vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic; UBA1: ubiquitin-like modifier activating enzyme 1; ER: endoplasmic reticulum; UPR: unfolded protein response; PERK: PRKR-like endoplasmic reticulin kinase; IRE1a: endoplasmic reticulum to nucleus signaling 1; ATF6: activating transcription factor 6.

of factors such as diet, exercise, and even climate, but are commonly related to perturbing factors including tobacco, toxins or secondary to chronic viral infections.[35,36] This phenotype has been suggested to arise from dysregulation of unmutated immune cells and is characterized by the senescent-associated secretory phenotype, which is a more complete compendium of cytokine changes associated with aging beyond those described in the initial reports on inflammaging.

In this conceptual model, the pro-inflammatory milieu has grave effects on the bone marrow microenvironment which becomes the fertile ground for the expansion of clonal hematopoiesis both by driving intrinsic changes in HSC and by providing an environment by which clonal hematopoiesis gains a competitive growth advantage. While causal evidence defining how an aged or inflammatory bone marrow microenvironment induces or promotes clonal hematopoiesis in humans is lacking, chronic sterile inflammatory stimuli can induce somatic mutations in mouse HSC, which is hypothesized to result from repeated activation of the HSC out of their dormant non-cycling state.[37] Additionally, in a mouse model of the pre-malignant CHIP state, HSC clones with genetic deletion of *TET2* were found to have a repopulation advantage, over wild-type cells, after acute inflammatory insults.[38] Another recent study using single-cell multi-omics techniques showed that both mutant and wild-type HSC taken from patients with clonal hematopoiesis showed increased signatures of inflammation and aging when compared to samples from patients without clonal hematopoiesis.[39] However, the same study showed that mutant HSC had decreased responses to this inflammation when compared to wild-type HSC from the same human sample, leading the researchers to suggest that the mutant HSC could have a competitive advantage under inflammatory conditions. Aging cells outside of the hematopoietic compartment may also induce intrinsic changes in HSC, as seen in a study of mouse and human fibroblasts. These reports show that aged stromal cells themselves could produce a pro-inflammatory microenvironment that drives a myeloid bias in HSC via changes in Notch signaling in vascular endothelium.[40,41]

In the pre-clonal inflammaging model (Figure 1A), the initial insult to the immune system is not the acquisition of a mutation in the HSC compartment, but rather an inflammatory stimulus which could increase the rate of mutagenesis.[37] There are multiple hypotheses for how this could arise. One is that the organ tissue senescence and fibrosis seen with aging lead to increased tissue permeability and increased release of damage-associated molecular pathogens (DAMP) from injured tissue and pathogen-associated molecular pathogens (PAMP) from invasive pathogens via weakened external tissue barriers.[29] This chronic immune insult leaves a lasting imprint or "immunobiography" which may be epigenetic in nature.[42] Increasing visceral adipose tissue, which has an epidemiological correlation with sterile inflammation and MDS,[43,44] may also trigger inflammaging, with mouse models suggesting that this is driven by macrophage accumulation in adipose tissue and activation of the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome.[45,46] Other hypothesized aging-related inflammatory triggers include changes to gut microbiota and permeability,[47] cell stress from decreased autophagy and macromolecule recycling[48] and accumulation of senescent cells, which are known to produce a senescent-associated secretory phenotype in mice.[49] A fascinating study using a mouse model of atherosclerosis demonstrated that vascular plaques themselves may promote HSC proliferation, increase clonal hematopoiesis several-fold and specifically promote *Tet2* knockout clone expansion.[50] This is an important study to emphasize as it highlights a possible reverse-causal relationship from the more popular hypothesis of CHIP-driven atherosclerosis discussed in more detail below.

Clonal hematopoiesis as a driver of inflammaging

In an alternative model that we have termed "clonal inflammaging" (Figure 1B), clonal hematopoiesis arises prior to, or independent of an aging inflammatory environment, whether from an age-associated mutation related to replication error,[6] germline predisposition,[7] or facilitated by an immune or genotoxic insult, such as has been described for everything from simple upper respiratory tract infections to exposure to carcinogens such as cigarette smoke,[51] or dust from the collapsed World Trade Center towers.[52,53] We propose that, in this case, these CHIP clones subsequently become the driving force of the chronic sterile inflammation described as inflammaging. The pro-inflammatory mutant HSC clones produce dysregulated mature immune cells which directly contribute to aging-related disease in various organs. The local niche of the CHIP clones becomes a positive feedback system via paracrine inflammatory signaling, which further reprograms the bone marrow stroma and immune niche and accelerates selection for clones with a growth advantage in inflammatory settings, eventually leading to MDS. In this model, inflammaging is a phenotype that arises from the outgrowth of CHIP populations.

There is strong human and murine evidence of an inflammatory predisposition in immune cells derived from CHIP HSC. Initial studies primarily found changes in *Tet2*-deficient mouse macrophages and demonstrated that these macrophages expressed higher levels of lipopolysaccharide-induced genes and produced more IL-1β and IL-6.[54,55] This potentially occurred due to loss of *Tet2*-mediated histone deacetylation of the IL-6 promoter preventing the resolution of the inflammatory response.[56] Recent work using single-cell analysis of peripheral blood samples isolated from *TET2*-mutated CHIP patients revealed increased cytokine expression in *TET2*-mutated monocytes, but not in *TET2* wild-type cells from the same patient, as well as gene signatures of impaired differentiation in both mono-

cytes and T cells.[57] In another study, human *TET2*-, and *DNMT3A*-mutant macrophages were found to have impaired mitochondrial DNA integrity and activation of cGAS signaling triggering a type I interferon response.[58] Loss of *Dnmt3α* in murine macrophages prevents suppression of the type I interferon response and loss in mouse mast cells predisposes to IgE hypersensitivity.[59] Patients with *JAK2* V617F CHIP have increased levels of IL-6 and IL-18.[7] In mice, this form of clonal hematopoiesis produces mutant neutrophils with abnormal neutrophil extracellular traps and similar findings can be reproduced in neutrophils isolated from patients with myeloproliferative neoplasms.[60,61] The effects of other common CHIP mutations on inflammation have received less study; however, patients with *SF3B1*-mutant CHIP have increased levels of IL-18 in their serum,[62] and pre-clinical models have established a causal connection between spliceosome mutations seen in MDS (*SF3B1*, *SRSF2*, *U2AF1*) and disordered inflammatory signaling.[63]

Clonal inflammaging, cardiovascular and other age-related diseases

Whereas non-clonal inflammaging arises alongside, and perhaps as a consequence of, age-related diseases, CHIP, via the process of clonal inflammaging, may drive gradual organ dysfunction over years through dysregulated immune cell damage of visceral tissue. There is an ever-growing list of disease states associated with CHIP with both epidemiological studies and experimental models supporting their relationship.

Central to the epidemiological study of how CHIP and systemic diseases are related is the growing list of large-scale cohorts that provide rich lifestyle data and health outcomes along with genomic data. The UK BioBank is the most studied of these massive datasets; it has the longest follow-up time with sample collections dating as far back as 2006 and now has whole genome sequencing for 500,000 participants. It has some key limitations, the most important being that the population is predominantly of European descent, limiting its ability to provide data about groups traditionally underrepresented in research settings. Existing work has identified a potential decreased frequency of CHIP in people who self-identify as Hispanic[2,7] or East Asian[7] but high-quality published studies of clonal hematopoiesis in diverse populations are limited and have largely used self-identified categorizations of race and ethnicity, which are difficult to apply to issues of genetic ancestry. A genome-wide association study of the Trans-Omics for Precision Medicine (TOPMed) dataset identified a germline *TET2* variant that is associated with an increased incidence of CHIP which was only identified in people of African ancestry, further highlighting the need for enrollment of diverse populations in these cohorts.[7] The All of Us cohort began enrollment in 2018 with a pre-specific goal of enrolling a more diverse population.[64] While follow-up health data for this cohort are less mature, they are likely to offer unique advantages to study historically underrepresented groups.

Heart disease is the most common cause of death in patients suffering from MDS.[65] The connections between anemia and ischemic cardiac disease, as well as transfusion-related iron overload and non-ischemic cardiomyopathy are long studied and key tenets to care of patients with MDS. However, recent research has implicated a pro-inflammatory environment in CHIP patients as a driver of heart disease unrelated to tissue oxygen delivery, alone. Research demonstrating increased expression of inflammatory genes in innate immune cells derived from clonal hematopoiesis has made popular the idea that clonal hematopoiesis may be a mechanism linking aging, inflammation, and cardiovascular disease. Initial reports linking CHIP and coronary artery disease were based on unplanned secondary analyses but the findings have since been replicated in additional cohorts.[2,66] Subsequent identification of a dose-response relationship between CHIP burden and coronary artery disease and a germline IL-6 signaling deficiency as protective against coronary artery disease in the setting of CHIP only further supports this hypothesis.[62,66] In both *Tet2*-deficient[67] and *Jak2*-mutated mice, clonal macrophages with increased inflammasome activity were found to accumulate in atherosclerotic plaques and plaque stability could be increased by disrupting inflammasome activity.[68] Ischemic cardiomyopathy[69] and aortic stenosis[70] have also been associated with CHIP. Most experimental work was done in mice which were exposed to additional cardiac stress to accelerate the desired cardiac phenotype; nonetheless, one *Tet2*-mutant CHIP mouse model was shown to be predisposed to age-related cardiac dysfunction even under homeostatic conditions, further supporting a causal relationship between CHIP and cardiac disease.[71]

There is growing evidence to suggest that specific CHIP mutations variably correlate with cardiovascular disease, with a recent analysis of the UK Biobank showing that *TET2* and spliceosome CHIP associate more strongly with atherosclerotic disease than does *DNMT3A* CHIP, emphasizing that all CHIP must not be treated the same when discussing age-related diseases.[72] Another analysis of the UK Biobank again showed that *DNMT3A* CHIP had a weaker association with vascular disease but interestingly also found that DNA damage repair genes (*TP53* and *PPM1D*) and *JAK2* had a stronger correlation than *TET2*.[73] One possible explanation for this difference is that *DNMT3A*-mutated macrophages uniquely promote cardiac fibrosis which could theoretically result in a different cardiac disease phenotype (heart failure *vs*. atherosclerosis) despite both contributing to cardiac-related morbidity.[74]

Studies of large national prospective cohorts have revealed associations between most age-related diseases and CHIP. Analyses of a cohort of CHIP patients in the UK Biobank identified associations between *TET2* and *JAK2* CHIP, but not *DNMT3A*, and both chronic kidney disease and acute

kidney injury.[75,76] Researchers were able to recapitulate this predisposition to acute kidney injury in mice CHIP models and showed increased inflammatory macrophage infiltration as a potential mechanism. A similar cohort was used to identify an association between *DNMT3A* CHIP and osteoporosis; experimental replication of this phenomenon was again done with a CHIP mouse model and, interestingly, this process could be reversed by IL-20 neutralization.[77] The COPDGene cohort, with its associated spirometry data, was used to elucidate the relationship between chronic obstructive pulmonary disease and CHIP and mice with *TET2* CHIP exposed to cigarette smoke had worse emphysematous changes than wild-type controls.[78] The same study attempted to quantify a possible confounding relationship where smoking drives both chronic obstructive pulmonary disease and CHIP independently through a genotoxic effect on HSC and found that, while there was a statistically significant association between CHIP and cumulative cigarette use at high exposures, smoking was only a weak risk factor for CHIP and a multivariate analysis including smoking history retained a significant association between chronic obstructive pulmonary disease and CHIP. CHIP may even have a causative role in the development of solid organ malignancies, specifically lung, prostate, and non-melanoma skin cancers.[79]

Efforts to collect the sorts of prospective cohorts needed to understand the relationship between CHIP, inflammaging and MDS are inherently costly and painstaking due to the prolonged time needed for the hypothesized precipitating events to lead to a detectable phenotype. Despite these challenges, efforts are under way that could provide high quality evidence in the future.[57,80]

VEXAS syndrome as an accelerated model at the interface of inflammation and clonal hematopoiesis

A potentially useful model to understand causality in such cases is the newly identified syndrome termed VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic), which is a novel hemato-inflammatory condition we frame here as an accelerated and exaggerated version of the relationship between inflammation and MDS (Figure 1C). E1 ubiquitin-like modifier activating enzyme 1 (UBA1) mutations play a pivotal role in the VEXAS syndrome, characterized by systemic inflammation and a predisposition to develop MDS.[81] This syndrome is a unique opportunity to study inflammatory pathogenesis in the bone marrow, unraveling the intricate interplay between somatic mutations, innate immunity dysregulation and hematologic malignancies.

Central to the pathophysiology of the VEXAS syndrome is dysfunction of the ubiquitin-proteasome system in HSC, triggered by loss of the cytoplasmic form of UBA1, which allows the accumulation of unfolded proteins in the cytoplasm due to decreased ubiquitination of the usual targets for proteosome-mediated degradation. The innate immune response depends on the ability to rapidly remodel signaling networks and is mainly organized by the ubiquitin-proteasome system. Ubiquitin-mediated protein recycling has emerged as a key factor in fine-tuning the strength and duration of the inflammatory response and loss of function at each step of the ubiquitin-proteasome system has been implicated in inflammatory conditions.[82] In the case of the VEXAS syndrome, accumulating cytoplasmic protein overwhelms the processing ability of the endoplasmic reticulum, which triggers the unfolded protein response and downstream signaling through PRKR-like endoplasmic reticulin kinase (PERK), endoplasmic reticulum to nucleus signaling 1 (IRE1a), and activating transcription factor 6 (ATF6) all of which are implicated in inflammatory signaling, specifically nuclear factor-κB (NF-κB), and the increased production of type I interferons. Recently, monocytes isolated from patients with VEXAS syndrome were shown to have disordered expression of chemokine receptors, increased TNF-α and NF-κB signaling and increased inflammasome activity, all highly reminiscent of observations of monocytes in CHIP patients described above.[83] The disordered ubiquitin-proteasome system produces a striking systemic inflammatory phenotype characterized by fevers, polychondritis, inflammatory arthritis, vasculitis, dysregulated proinflammatory neutrophil activation and increases in inflammatory cytokines such as IL-6, TNF, and interferon-γ.[81]

Patients with VEXAS syndrome have notable hematologic abnormalities, including peripheral cytopenias (macrocytic anemia, lymphopenia, monocytopenia), and a hypercellular and myeloid skewed bone marrow.[84] MDS is the most common hematologic neoplasm seen in VEXAS patients. MDS cases occurring in VEXAS syndrome present later in the disease course and are enriched for a lower risk profile, as per the Revised International Prognostic Scoring System, and, interestingly, the majority have normal karyotypes and a low mutational burden. Few of the initially reported VEXAS cases had a variant allele frequency >5% in recurrently mutated genes, compared to more than 80% of cases in a typical MDS population.[84] This lack of clonal complexity supports a theory that inflammation may be the primary driver of MDS pathogenesis in the VEXAS syndrome or that *UBA1* itself can drive dysfunctional hematopoiesis. Ongoing debate about the relative contributions of intrinsic HSC dysfunction from somatic *UBA1* mutation or extrinsic inflammatory disruption of HSC function mirrors our competing models of clonal and non-clonal inflammaging and makes it a ripe ground for future studies seeking to understand inflammation and MDS. Better understanding the consequences of inflammaging on the hematopoietic system, both extrinsic changes occurring in the HSC niche and those intrinsic to the HSC itself, will benefit the scientific community in its efforts to restore the regenerative capacity of HSC and intervene earlier in the pathogenesis of MDS. The next section offers comparisons between what is known about aging HSC and MDS-initiating clonal HSC, with a special emphasis placed on inflammation.

Intrinsic changes of hematopoietic stem cells in aging and myelodysplastic syndromes

Early histological and functional observations of the aging human bone marrow revealed a consistent pattern of decreased cellularity and increased adipocytes, a decline in lymphopoiesis with an increase in myeloid differentiation potential, a seemingly paradoxical increase in long-term HSC but which have reduced replicative capacity and finally an increased predisposition to myeloid neoplasms.[85] The mechanisms which drive these changes are incompletely understood and difficult to study in human tissue. The concomitant rise in clonal hematopoiesis seen with aging and the potential for these clonal cells to accelerate aging-related inflammation confound our understanding of the aging wild-type HSC. There are intrinsic changes of HSC in aging and MDS pathogenesis and understanding their similarities and differences is crucial to begin unraveling the relationship between inflammaging and MDS (Figure 2).

Non-clonal aging of the hematopoietic system: focus on hematopoietic stem cell senescence and inflammation

Epigenetic dysregulation is implicated as a driver of cellular aging and in HSC these changes are felt to promote self-renewal at the expense of differentiation. Transcrip-

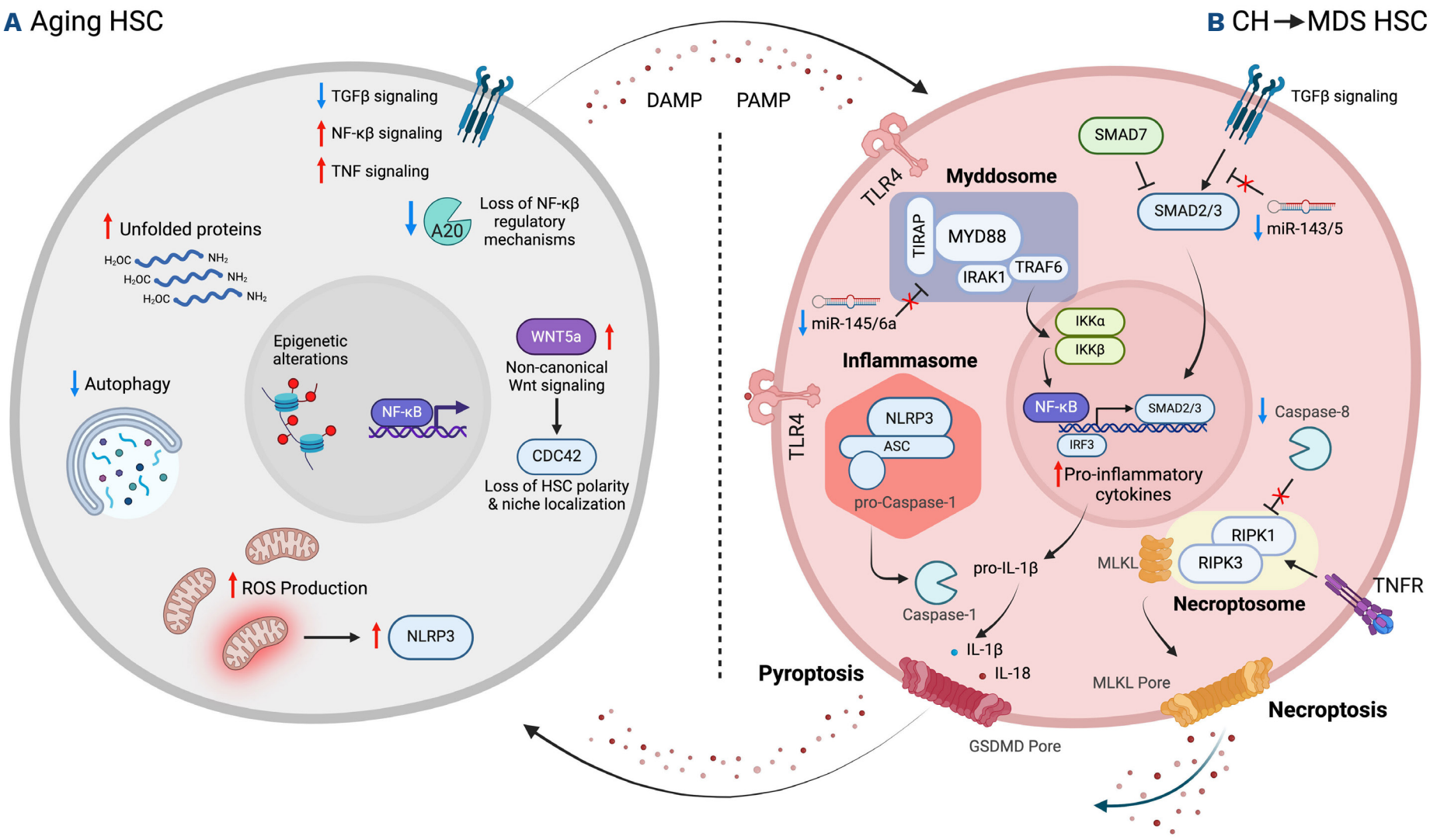

Figure 2. Intrinsic defects of hematopoietic stem cells in aging and myelodysplastic syndromes. (A) Intrinsic defects in aging hematopoietic stem cells (HSC) include a deficiency in autophagy and unfolded protein processing, an increase in mitochondrial oxidative stress which can trigger the NLRP3 inflammasome, increased NF-κB activity via increased inflammatory signaling and decreased regulatory mechanisms and increased non-canonical WNT signaling. (B) Intrinsic defects in myelodysplastic HSC include dysregulated innate immune signaling via TLR receptor triggering of NF-κB through the myddosome and increased TGF-β signaling via SMAD proteins due to loss of the miR-143/5 negative regulators. Increases in programmed inflammatory cell death mechanisms, such as necroptosis and pyroptosis, also characterize myelodysplastic HSC and produce a pro-inflammatory positive feedback cycle further triggering inflammatory pathways in neighboring HSC. Created with BioRender.com. CH: clonal hematopoiesis; TGF: transforming growth factor; NF-κB: nuclear factor-kappaB; TNF: tumor necrosis factor; ROS: reactive oxygen species; NLRP3: NOD-, LRR- and pyrin domain-containing protein 3; DAMP: damage-associated molecular pathogens; PAMP: pathogen-associated molecular pathogens; TLR: toll-like receptor; TIRAP: toll/interleukin-1 receptor domain-containing adapter protein; IRAK1: interleukin 1 receptor associated kinase 1; TRAF6: TNF receptor-associated factor 6; IKK: IκB kinase; ASC: apoptosis-associated speck-like protein; SMAD: small mother against decapentaplegic; IRF3: interferon regulatory factor 3; IL-1β: interleukin-1beta; MLKL: mixed lineage kinase domain like pseudokinase; RIPK: receptor-interacting serine/threonine-protein kinase; GSDMD: gasdermin-D; TNFR: tumor necrosis factor receptor.

tional level changes are thought to affect self-renewal; for example, aged mice HSC demonstrate an inflammatory transcriptional memory that is myeloid-biased and persists for months after an inflammatory insult.[86] Seminal research on aging mouse HSC revealed that, while the DNA methylome is broadly unchanged with age, specific loci associated with differentiation potential are hypermethylated in old compared to young mice.[87] Specifically, hypermethylation occurred at loci marked by H3K27, which are key targets of polycomb repressive complex 2 (PRC2). It was found that these epigenetic changes may be driven by decreased levels of PRC2, which is heavily implicated in aging and senescence in other tissue types, as evidenced by decreased gene expression of a core PRC2 component, *EZH2*, which is also occasionally mutated in MDS.[88] Another study in aged mice showed more trimethylation at H3K4-marked loci, which is associated with increased transcription of stem cell self-renewal effectors and loss of differentiation capacity; similar findings have been reproduced in human HSC.[89]

Changes in mitochondrial health and function are also tightly linked to HSC age and may be linked to an inflammatory phenotype, which has been reviewed more extensively elsewhere.[90] Young and healthy HSC are enriched for a quiescent cell state with a low metabolic rate and low levels of mitochondrial reactive oxygen species. Aged mice have an increasing proportion of HSC with a high content of reactive oxygen species, which may be due to age-related loss of FOXO3 and SIRT3, increases in AKT/mTOR signaling, accumulation or mitochondrial DNA mutations or loss of mitochondrial quality control via a decreased mitochondrial unfolded protein response.[90] Accumulating mitochondrial reactive oxygen species can trigger the NLRP3 inflammasome, potentially through epigenetic remodeling by SIRT2, linking mitochondrial health, epigenetics and a pro-inflammatory cell death process.[91] Interestingly, aged mouse HSC seem to rely on increased basal autophagy to maintain their state of low oxygen metabolism, and in so doing preserve their self-renewal capacity, but an increasing proportion of HSC lose this enhanced autophagic capability with age.[92] Finally, there seems to be loss of protein homeostasis in aging mouse HSC with gradual age-related decreases in SIRT7 leading to accumulation of unfolded proteins in mitochondria and subsequent compromised regenerative capacity.[93]

Inflammatory signaling is also dysregulated in aged mouse HSC. Age-related chromatin and transcriptional changes lead to an upregulated interferon response.[42] These HSC also lose the ability to appropriately downregulate NF-κB signaling after an inflammatory insult, potentially via two mechanisms. Increased RAD21 activity, which typically increases NF-κB binding to its target genes in response to inflammation, results in hypersensitivity to NF-κB which limits the self-renewal capacity of HSC.[94] Aged mouse and human HSC also have decreased levels of A20, a negative regulator of NF-κB, and decreased A20 levels have been linked to myeloid proliferation, B-cell apoptosis, anemia and overproduction of inflammatory cytokines.[95]

Other key signaling networks have defined differences in aged mice HSC. TGF-β signaling in HSC is bidirectional, with elevated levels favoring quiescence, via SMAD2/3 activity, and low levels favoring myeloid differentiation. Aging of mouse HSC is associated with decreased TGF-β signaling, likely due to loss of TGF-β receptor expression, and this may contribute to myeloid skewing and predisposition to myeloproliferative disease.[96,97] WNT signaling may be at the heart of another key observation regarding aging HSC, loss of the asymmetric distribution of cellular components, termed cell polarity. A shift from the canonical β-catenin-mediated WNT signaling to CDC42-mediated, non-canonical WNT signaling occurs with age in mouse HSC, driven by predominate expression of *Wnt5a*.[98] The non-canonical WNT5a/CDC42 axis may exert some of its aging phenotype via crosstalk with the NOTCH1 pathway, which leads to an overabundance of quiescent HSC in mice with reduced engraftment potential.

Disordered innate inflammatory signaling in clonal hematopoiesis and myelodysplastic stem cells

A majority of MDS initiating molecular events lead to, paradoxically, a loss of proliferative advantage when studied in typical *in vivo* and *in vitro* model systems. A key feature proposed to explain mutant clone outgrowth in the bone marrow of MDS patients is a dysregulated innate inflammatory system, which is not recapitulated in those typical models.[99] While the previous section highlighted how this local inflammatory milieu could derive from non-clonal aged HSC, this section will highlight how clonal hematopoiesis could initiate and self-perpetuate this inflammatory advantage.

For example, in MDS patients' samples, an inflammatory bone marrow microenvironment with increased TNF-α levels has been observed; this can induce apoptosis in healthy HSC while providing a selective advantage for particular pathogenic clones. Stem cells from MDS patients without excess blasts show predominate expression of the pro-apoptotic TNF receptor 1 whereas MDS stem cells from patients with excessive blasts show increased levels of the anti-apoptotic TNF receptor 2. Dysregulated TGF-β signaling is also implicated in MDS pathogenesis though it is increased, rather than decreased as seen in aged HSC. This increase is thought to occur via loss of negative feedback components, SMAD7 and miRNA-143/5 in some cases. In mice and *ex vivo* human models, the loss of negative regulators then in turn leads to upregulated SMAD2/3 which inhibits erythropoiesis and triggers inflammation via crosstalk with the NF-κB pathway.[100]

Central to disordered immune signaling in MDS are the toll-like receptors (TLR). These receptors recognize DAMP and PAMP and initiate an inflammatory response important

to innate immunity and are known to be increased in MDS patients' samples.[101] The primary effector via which TLR trigger myeloid differentiation is the myeloid differentiation primary response gene 88 protein (MYD88). MYD88 forms a complex with interleukin-1 receptor associated kinases (IRAK1 and IRAK4), termed the "myddosome", in response to TLR or IL-1 receptor activation.[102] The myddosome, in turn, triggers increased NF-κB and MAPK activity through TNF receptor-associated factor 6 (TRAF6) and IκB kinases (IKK) leading to increased expression of inflammatory cytokines (IL-1β, IL-6, IL-9, TNF-α). Multiple TLR proteins are overexpressed in MDS patients' HSC, including TLR2, TLR4 and TLR6. Additionally, genes encoding modulators of the TLR pathway, miRNA145/6a, exist on the short arm of chromosome 5, the loss of which defines the entity MDS-del(5q). Loss of the inhibitory effects of these non-coding RNA leads to upregulated TLR signaling which drives a MDS-del(5q) phenotype in mouse models.[103] Other examples linking MDS-related gene mutations to TLR signaling include both *SF3B1*[104] and *U2AF1*[105] mutations leading to IRAK4 signaling.

Programmed necrotic cell death pathways in the pathogenesis of myelodysplastic syndromes

As opposed to apoptosis, an immunologically silent programmed cell death process, there are alternative pro-inflammatory cell death processes that share a common lytic cell death mechanism that leads to the release of DAMP and other pro-inflammatory cytokines. Thought to be a component of the innate immune system, these cell death processes create a highly inflammatory local milieu which recruits the innate immune system to combat infection or repair tissue injury. A shared inflammatory cell death process is one explanation for how a common phenotype of cytopenias and morphological dysplasia can be seen across the myriad of MDS-related gene mutations.

Pyroptosis can be initiated either through binding of DAMP, such as S100A8/A9, to cell surface receptors including TLR4 and CD33 or by increased intracellular reactive oxygen species.[106] NF-κB activation downstream of TLR4 increases expression of pro-IL-1β and NLRP3 inflammasome components, which are assembled and further activated in the cytosol in the presence of reactive oxygen species. NLRP3 polymerizes apoptosis-associated speck-like protein (ASC) which participates in the recruitment of caspase-1 to generate the inflammasome. The NLRP3 inflammasome responds to cytosolic DAMP and, when activated, caspase-1 converts pro-IL-1β to IL-1β and triggers gasdermin-D (GSDMD) membrane pore formation which leads to release of IL-1β and IL-18 outside of the cell, triggering further TLR signaling and pyroptosis in neighboring cells.

Inflammasome components (NLRP3, CASP1), but not apoptotic machinery, are increased in MDS bone marrow samples at the transcript and protein levels and increased inflammasome activity is supported by observed increases of ASC polymers.[107] This pattern was recapitulated in MDS mouse models across a wide range of driver mutations including epigenetic regulators (*ASXL1*, *TET2*) and splicing machinery (*SF3B1*, *SRSF2*, *U2AF1*), further supporting pyroptotic cell death as a common driver of the MDS phenotype.[107] However, a recent study of a large cohort of MDS patients suggests that increased inflammasome activity may be more specific to low-risk MDS.[108]

Necroptosis is an analogous programmed lytic cell death pathway with alternative triggers and effectors.[106] It is initiated downstream of death domain receptors (FAS or TNFR) or TLR by the intermediaries FADD, TRADD and TRIF. First, RIPK1 is deubiquitylated which allows recruitment of RIPK3. RIPK1 and RIPK3 function as a complex that recruits and phosphorylates MLKL which oligomerizes, thus completing the necroptosome.[109] Cell membrane pores composed of these MLKL oligomers cause the uncontrolled release of cellular material and DAMP. In one cohort of low-risk MDS cases, bone marrow mononuclear cells had consistently increased necroptosome components when compared to those from age-matched healthy donors or AML patients. Apoptotic pathways are known to antagonize necroptosis and a mouse model which lacks apoptotic machinery was shown to favor necroptosis and lead to a MDS-like phenotype without typical MDS-related gene mutations. Given prior observations of apoptosis-resistance in advanced cases of MDS, the causal link between alternative inflammatory cell death processes and MDS pathogenesis remains intriguing.[110]

Immune evasion in clonal hematopoiesis

The evolution of MDS from CHIP due to an intrinsic advantage of certain hematopoietic clones to evade immune surveillance remains a speculative hypothesis though one with interesting correlatives. A strong association between germline HLA polymorphisms and CHIP prevalence suggests dysfunctional immune surveillance may allow the outgrowth of micro-clones which would otherwise be removed by HLA-recognizing cells.[111] PD-L1 expression is increased in a substantial minority of HSC isolated from MDS patients, which could aid in immune evasion.[112] PD-1H has been shown to drive T-cell evasion in AML blasts and is also upregulated in samples from MDS patients.[113] Recent work studying CHIP in solid organ transplant recipients identified an increased incidence of *TET2* CHIP in these transplant recipients. This correlation was only true for those far removed from their transplant date, suggesting that it may be the transplant and/or subsequent immunosuppression leading to CHIP rather than the reverse.[114] An increased prevalence of CHIP was mostly observed in patients who received anti-thymocyte globulin as a part of their immunosuppressive regimen, implying that the expansion of clonal hematopoiesis may depend on how and to what degree the immune landscape is altered.

The bone marrow microenvironment in inflammaging and the pathogenesis of myelodysplastic syndromes

The bone marrow microenvironment has significant implications for both the aging process and clonal hematopoiesis, including the mesenchymal stroma and resident immune cells which compose the HSC niche (Figure 3). An aged bone marrow niche seems to accelerate epigenetic markers of aging in healthy transplanted HSC in humans more quickly than does a young recipient's niche.[115] The aged niche of mice also exerts a selection pressure on mutant HSC, facilitating their evolution towards oligoclonality , which is reminiscent of the evolution of CHIP into MDS in humans.[116] Inflammation plays a key role in the relationship between the niche and HSC. Niche cells themselves are involved in promoting myelopoiesis in response to systemic inflammation and become increasingly pro-inflammatory with aging in mice, with increased secretion of IL-1β and CCL5 contributing directly to the aging phenotype of myeloid skewing.[40,117] Exactly which components of the niche contribute to decreased support for normal hematopoiesis is unknown, but this section offers a brief overview of recent observations and hypotheses on the contributions of

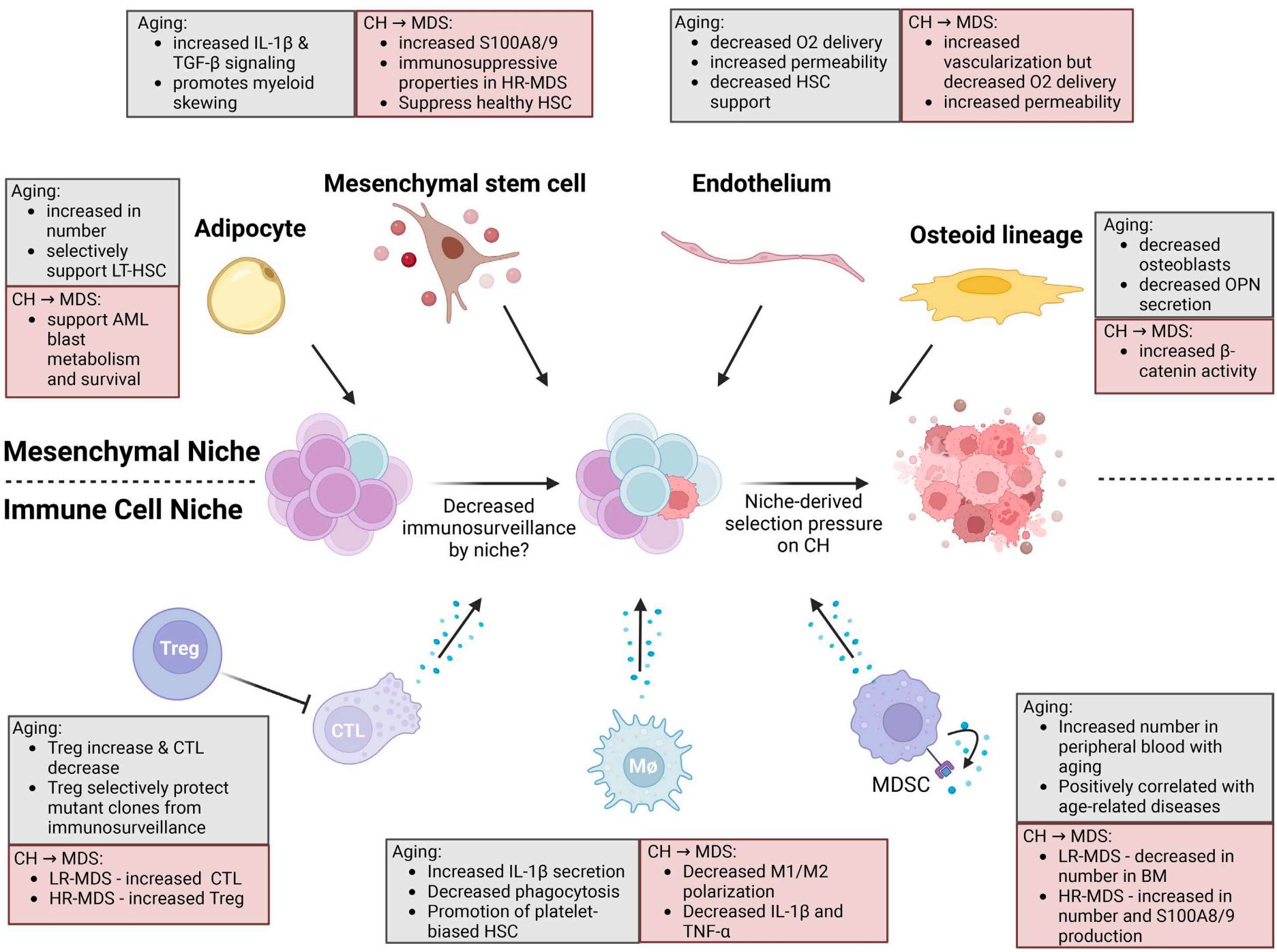

Figure 3. Comparing changes in the bone marrow microenvironment in aging and myelodysplastic syndromes. Descriptions of changes observed in the aging bone marrow (gray boxes) and clonal hematopoiesis/myelodysplastic syndromes (red boxes) for the mesenchymal marrow niche including adipocytes, mesenchymal stem cells, endothelial and osteoid lineage cells and for the immune cell marrow niche including regulatory and cytotoxic T cells, macrophages and myeloid-derived suppressor cells. Created with BioRender.com. IL: interleukin; TGF: transforming growth factor; HR: high-risk; MDS: myelodysplastic syndrome; HSC: hematopoietic stem cells; CH: clonal hematopoiesis; LT: long-term; AML: acute myeloid leukemia; OPN: osteopontin; Treg: regulatory T cells; CTL: cytotoxic T lymphocytes; LR: low-risk; TNF: tumor necrosis factor; MDSC: myeloid-derived suppressor cells; BM: bone marrow.

various niche cell subsets to aging and the pathogenesis of MDS.

Mesenchymal stromal cells are perivascular bone marrow-resident cells that play an essential role in HSC maintenance. With aging, mesenchymal stromal cells decrease in number, differentiate primarily into adipocytes at the expense of the osteoid lineage and lose HSC supportive capacity. In low-risk MDS patients' samples, mesenchymal stromal cells secrete increased levels of S100A8/A9 and in high-risk MDS they obtain an immunosuppressive secretory profile driven by TGF-β.[118] Bone marrow adipocytes increase with age in humans and even more so with obesity and impair short-term repopulating ability of HSC in mice via secretion of DPP4. They also simultaneously promote the maintenance of more quiescent long-term HSC, which correlates with the phenotype of an increased number but decreased repopulating ability of HSC observed in the aged bone marrow.[119] Little is known about the contribution of bone marrow adipocytes to MDS pathogenesis, but AML blasts are able to induce lipolysis in neighboring adipocytes to facilitate their own metabolism.[120] Osteoblasts, but not osteoclasts, decrease with age and loss of their secretory component, osteopontin, may contribute to HSC aging through loss of senescence.[121] One third of samples from a cohort of MDS/AML patients showed β-catenin accumulation in osteoblasts and a mouse model with an activating mutation of β-catenin could induce AML.[122] Bone marrow endothelial cells in aging lose their ability to support HSC function, potentially due to decreased vascular oxygen delivery and decreased secretion of supportive factors such as stem cell factor and CXCL12.[123] Whether derived from inflammatory paracrine signaling from clonal hematopoietic cells or the systemic inflammation of inflammaging, chronic exposure to inflammation is hypothesized to initiate or exacerbate most of these highlighted changes in the mesenchymal niche.

Changes in circulating immune cells with inflammaging and clonal hematopoiesis have already been discussed but the bone marrow niche's immune cell repertoire has an important, but largely unclear, role in both these processes. Analogous to the better characterized solid tumor-infiltrative immune cells, bone marrow-resident immune cells can have a pro-inflammatory or immunosuppressive phenotype which varies with age and associated hematopoietic malignancy.[124]

Bone marrow macrophages have an increasingly pro-inflammatory secretory profile in aging mice, with increased IL-1β production, but a reduced phagocytic capacity which allows the outgrowth of senescent neutrophils in the marrow. This pattern directly caused a platelet bias in HSC which mirrored differentiation abnormalities seen in humans and this bias could be reversed by eliminating the defective macrophages.[125] Macrophages have received considerable attention in MDS given the promising preclinical and then recent disappointing clinical performance of the anti-CD47 antibody magrolimab. Magrolimab leads to cell death in leukemic mouse models by blocking anti-phagocytic signaling on disease cells. Unfortunately, MDS marrow-resident macrophages, just like tumor-associated macrophages, are polarized towards an immunosuppressive M2 phenotype instead of the phagocytic M1 phenotype which may explain its lack of clinical efficacy of the anti-CD47 antibody.[126,127]

Myeloid-derived suppressor cells are an immunosuppressive component of the bone marrow microenvironment which increase in number with aging in the peripheral blood and are also found in excess in organs afflicted by age-related diseases.[128] Like the functionally-similar regulatory T cells, they are increased in low-risk MDS but decreased in high-risk MDS and uniquely secrete S100A8/A9, suggesting an ability to drive the pathogenesis of MDS. Recent work has elucidated a bidirectional relationship between clonal hematopoiesis and regulatory T cells, with mutant HSC promoting regulatory T-cell expansion through presentation of neoantigens on major histocompatibility complex class II molecules, and regulatory T cells decreasing apoptotic priming of the mutant clones in return.[129] A more detailed description of all the changes in the immune microenvironment in aging and MDS pathogenesis is beyond the scope of this review and has been expertly reviewed recently.[118]

Conclusions and future therapy directions

The relationship between hematopoiesis, aging and inflammation is the undercurrent of age-related disease, particularly MDS. The emergence of clonal hematopoiesis as either a harbinger or a driver of age-related organ dysfunction and inflammation (i.e., pre-clonal *vs*. clonal inflammaging) marks the path from normal hematopoietic function to the dysregulated immune response in CHIP and MDS. Striving to understand these intricate mechanisms not only sheds light on the etiology of MDS but also offers potential avenues for therapeutic intervention aimed at restoring hematopoietic homeostasis in the aging population and potentially even preventing some of the most feared pathologies of aging.

Unsurprisingly, numerous therapeutic strategies, inspired by some of the preclinical research highlighted here, which target inflammation in MDS are currently being explored, including inhibitors of most of the inflammatory mediators discussed in this review. While immune checkpoint inhibitors have thus far been disappointing in myeloid disease, studies of the bone marrow T-cell repertoire are seeking to identify biomarkers of subsets of patients who may benefit from traditional immune checkpoint inhibitor therapy and new targetable immune checkpoints continue to emerge.[113] Similarly, in an AML mouse model, failure of CD47 blockade to clear bone marrow-resident blasts was revealed to be due to a lack of M1 polarized bone marrow macrophages,

a deficit overcome with adjuvant TLR3 agonism, which promoted M1 macrophage polarization and phagocytosis, and restored CD47 inhibitor activity in the bone marrow.[127] Direct interruption of cytokines (IL-1, IL-6), blockage of inflammatory receptors (IL1R, IL6R, CXCR1/2) and mediators (JAK1, IRAK4, NLRP3) are all strategies being pursued to various extents in MDS.[104,130] As examples, an IRAK4 inhibitor has demonstrated clinical activity in patients with spliceosome mutations[131] and a TLR2 antagonist has shown the ability to induce improvements in hematologic parameters in MDS patients.[132] However, designing interventions for a pre-disease state, such as CHIP, has numerous additional nuances as drug toxicities that are acceptable in a MDS population would not be in an asymptomatic condition that may never progress to a true hematologic disease. Novel therapeutic technologies beyond any currently undergoing clinical investigation will likely be required for tertiary or secondary prevention studies in CHIP, but we highlight some of the most interesting forays into this space here.

A fascinating story has emerged from the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS), which sought to study the effects of IL-1β inhibition on cardiac complications in patients after a myocardial infarction. While cardiac events were modestly reduced in the overall population, a subsequent subgroup analysis suggested that this effect was derived entirely from patients with *TET2* CHIP who had an over 60% reduction in the composite cardiac endpoint.[133] Further study also showed that only the CANTOS patients with CHIP saw improvements in anemia, which was associated with multiple markers of decreased inflammatory signaling.[134] Ongoing prospective efforts to disrupt IL-1β in MDS pre-disease states are already underway, highlighted by the IMPACT study of canakinumab in patients with clonal cytopenia of undetermined significance (NCT05641831).

Table 1 provides an overview of therapeutic strategies, at various stages of preclinical or clinical development, which specifically target either aging or clonal hematopoiesis. They are provided here only to offer an overview of areas of active research and to prompt comparisons between interventions targeted at aging and those targeting clonal hematopoiesis. Strategies to reverse clonal hematopoiesis include those seeking to restore normal driver mutation function, including by pharmacological intervention[135] and by gene editing.[136] Others seek to identify vulnerabilities that are specific to mutant HSC such as through pharmacological inhibition of mutant proteins themselves (NCT05102370) or by exploiting metabolic differences between mutant and wild-type HSC.[137] Another novel approach is the antibody-mediated depletion of myeloid-biased HSC, which was found to reverse age-related deficiencies in immune response.[138] Finally, age-related changes in HSC could also be reversed by genetically inducing pluripotency in aged cells which subsequently adopted a young HSC phenotype when transplanted back into mice.[139] Efforts looking to reverse the aging process directly have spawned the development of senolytics, the selective eradication of senescent cells which accumulate with age and have been implicated in

Table 1. Sample of therapeutic strategies in clonal hematopoiesis and aging.

Strategy	Study description	Reference
Clonal hematopoiesis		
Anti IL-1β antibody in CCUS (NCT05641831)	Randomized phase II	134
High dose vitamin C in CCUS (NCT03418038)	Randomized phase II	135
IDH2 inhibitor in CCUS (NCT05102370)	Non-randomized phase II	N/A
Metformin in *DNMT3A* CHIP	Preclinical	137
CRISPR-Cas9 gene editing to repair SNV in HSC	Preclinical	136
Antibody cocktail to deplete myeloid-biased HSC	Preclinical	138
Induced pluripotency in aged HSC	Preclinical	139
Aging		
Senolytics		
TKI (dasatinib) + BCL-2 (quercitin) inhibition	Phase I and phase II	141
MDM2 inhibition in osteoarthritis	Randomized phase II	N/A
Anti BCL-xL small molecule	Preclinical	142
SASP inhibitors		
Metformin	Preclinical	145
NF-κB inhibition	Preclinical	143
Rapamycin	Preclinical	144

IL-1β: interleukin-1β; CCUS: clonal cytopenias of undetermined significance; IDH2: isocitrate dehydrogenase 2; *DNMT3A*: DNA methyltransferase 3 alpha; CHIP: clonal hematopoiesis of indeterminate potential; CRISPR: clustered regularly interspaced short palindromic repeats; SNV: single nucleotide variant; HSC: hematopoietic stem cells; TKI: tyrosine kinase inhibitor, N/A: not applicable; BCL-2: B-cell lymphoma-2; MDM2: murine double minute 2; BCL-xL: B-cell lymphoma extra-large; SASP: senescent-associated secretory phenotype; NF-κB: nuclear factor-kappaB.

age-related diseases of every organ.[140] Initial pharmacological senolytic candidates were selected based on their ability to target senescent cell anti-apoptotic pathways that were identified in transcription analysis of mouse senescent cells. The combination of dasatinib (a tyrosine kinase inhibitor used in chronic myeloid leukemia) and quercetin (a flavonoid with numerous proposed chemical targets, including BCL-xL) emerged as the most potent and selective senolytics in the original drug screen. The combination of these drugs or the related flavonoid fisetin are being studied in at least 18 phase I and II clinical trials to reverse cellular senescence in numerous age-related diseases (examples NCT02874989, NCT02848131) although only changes in biomarkers of cellular senescence have been reported.[141] Navitoclax has also been proposed as a senolytic agent.[142] A potential senolytic targeting MDM2, UBX0101, did not reduce osteoarthritic pain more than placebo in a phase II randomized study (NCT04349956).

An alternative strategy to counteracting senescent cells is to attempt to suppress the pro-inflammatory senescent-associated secretory phenotype cytokines which they secrete. In pre-clinical mouse models, NF-κB inhibition,[143] rapamycin analogues[144] and metformin[145] have all been shown to decrease the senescent-associated secretory phenotype and alleviate age-related conditions in mouse models. Parallels can be seen between treatment strategies for MDS, clonal hematopoiesis and aging, sometimes with identical agents being pursued (metformin) and other times with common pathways such as inflammatory modulators (canakinumab and NF-κB inhibition). The identification of navitoclax as a potential senolytic is reminiscent of the recognition that BCL-2 family protein inhibitors may potentially be effective in eradicating the senescent leukemia stem cell population.[146]

The discovery of CHIP as an immunologically active precursor to myeloid disease has quickly expanded our understanding of the pathogenesis of both MDS and inflammaging. Improved understanding of the non-hematologic implications of CHIP has exponentially expanded the cohort of scientists and physicians studying the hematopoietic system and now spans experts from genomics to cardiology to geriatrics. The discoveries and terminology coined in the literature on aging (e.g., "inflammaging") contextualize the effects of somatic mutations on hematopoiesis, and help in understanding mechanisms and manners with which to effectively treat and improve the lives of those suffering from MDS.

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Contributions

MTV and MRS designed, conceived and wrote the manuscript and figures.

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