The Role of Inflammation in the Initiation and Progression of Myeloid Neoplasms

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ABSTRACT Myeloid malignancies are devastating hematologic cancers with limited therapeutic options. Inflammation is emerging as a novel driver of myeloid malignancy, with important implications for tumor composition, immune response, therapeutic options, and patient survival. Here, we discuss the role of inflammation in normal and malignant hematopoiesis, from clonal hematopoiesis to full-blown myeloid leukemia. We discuss how inflammation shapes clonal output from hematopoietic stem cells, how inflammation alters the immune microenvironment in the bone marrow, and novel therapies aimed at targeting inflammation in myeloid disease.

Significance: Inflammation is emerging as an important factor in myeloid malignancies. Understanding the role of inflammation in myeloid transformation, and the interplay between inflammation and other drivers of leukemogenesis, may yield novel avenues for therapy.

INTRODUCTION

Inflammation is a hallmark of cancer (1), and in recent years, the effects of inflammation on the composition of the tumor microenvironment (TME) in solid malignancies have been thoroughly characterized. Inflamed tumors are associated with specific immune subsets, which can affect prognosis and response to treatment (2). In tumors such as colorectal cancer, melanoma, and lung cancer, chronic inflammation caused by autoimmune diseases (3), obesity (4), UV exposure (5), or cigarette smoke (6) drives tumorigenesis. Furthermore, alterations in the microbiome have recently been characterized as a hallmark of cancer and specific bacterial populations are associated with tumors (7, 8) and can affect prognosis and response to therapy (9), possibly through modulation of immune responses in the TME. Finally, the advent of immunotherapies has shed light on the role of inflammation in modulating the response to therapy.

In hematologic malignancies, the role of inflammation has only recently gained attention. Patients with autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and inflammatory bowel disease are at higher risk to develop myeloid neoplasms (10), suggesting that systemic inflammation can promote myeloid malignancies. The recently described VEXAS syndrome also links autoimmunity with myeloid transformation. In VEXAS, male patients with mutations in *UBA1* experience recurrent skin and lung inflammation, accompanied by vacuolization of myeloid and erythroid cells and bone marrow (BM) dysplasia (11). In age-related clonal hematopoiesis (ARCH), blood cells contain clones with recurrent genetic lesions, which expand with age. Several mutations recurrent in ARCH (*TET2*, *ASXL1*, *DNMT3A*) can trigger inflammatory responses. ARCH is associated with a number of pathologic conditions (12), suggesting that these inflammatory responses can have a systemic impact and are not localized to the BM. In addition, inflammation is associated with progression from myelodysplastic syndrome (MDS) to acute myeloid leukemia (AML; ref. 13), and can affect the composition of the immune microenvironment in the leukemic BM (14). In 2009, inflammation was introduced as the seventh hallmark of cancer, added to the six hallmarks defined by Hanahan and Weinberg in 2000 (15, 16). The 2022 update on cancer hallmarks added emerging hallmarks such as tumor plasticity, nonmutational epigenetic remodeling, and the role of microbiota (17). The connection between different hallmarks reflects the complexity of tumor initiation and progression, and inflammation can serve as a link connecting different hallmarks across tumor types (Fig. 1).

In this review, we will describe the role of inflammation in myeloid malignancies. We will discuss how inflammatory signals regulate normal hematopoiesis, explore the association between common AML driver mutations and dysregulated immune responses, examine the role of the BM niche in maintaining and responding to inflammatory signals, and describe the contribution of inflammation to ARCH, progression to MDS and AML, and vice-versa. Finally, we will discuss the role of epigenetic memory in maintenance of an inflammatory phenotype in AML. We will also shed light on the effect of inflammation on response to therapy in AML.

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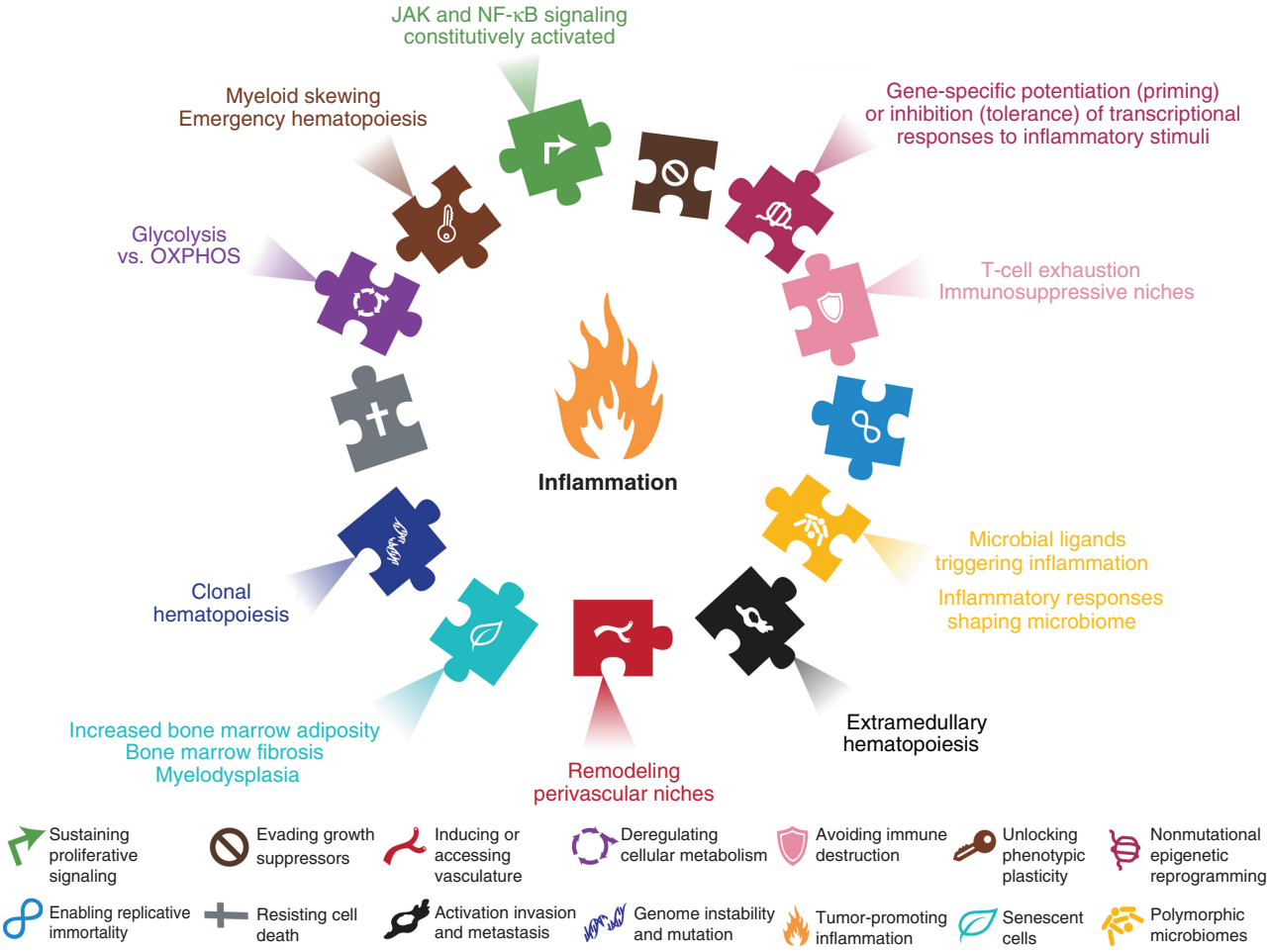

Figure 1. Inflammation: a tumor kernel. Inflammation has been described as a hallmark of cancer since 2009. Here, we present inflammation as an instigator of cancer that affects (directly or indirectly) all other tumor hallmarks.

HEMATOPOIESIS IS SHAPED BY INFLAMMATORY SIGNALS

Hematopoiesis produces billions of immune and blood cells on a daily basis. Hematopoiesis starts in the BM, when hematopoietic stem cells (HSC) abandon their quiescent state to give rise to mature myeloid and lymphoid populations in a highly orchestrated differentiation process. In the BM, several accessory cells work together to create sanctuaries to safeguard quiescent cells, while other cellular neighborhoods coordinate proliferation and differentiation programs (18). Thus, the BM microenvironment is composed of well-characterized niches strategically distributed to ensure functional hematopoietic factories that continously supply blood. Under physiologic conditions, the balance of myeloid/lymphoid production is maintained; however, this balance is disrupted under stress conditions. Bleeding, nutrient starvation, oxygen saturation, toxicity, infection, and other sterile stressors may affect hematopoiesis efficiency and induce emergency hematopoiesis (19). During this transient program, proliferation and cell differentiation occur faster in a short window of time to replenish the lineages that have been consumed during the response to the insult. Emergency hematopoiesis may exhaust HSC (20), and fine-tuning circuits are activated to resolve the stress and protect HSC from exhaustion.

Inflammation is an evolutionarily well-conserved response against tissue damage or dysfunction. Consequently, it recruits professional immune cells to protect against potential aggressors and repair local damage. Tissue damage is sensed by immune and nonimmune cells by direct recognition of the aggressor or detection of early inflammatory elements secreted by other cells. Direct detection is mediated by a wide repertoire of evolutionarily conserved, germline-encoded pattern recognition receptors (PRR) that include the family of Toll-like receptors (TLR), RIG-I-like receptors (RLR), leucine-rich repeat-containing receptors (NLR) and others sensors (21). PRR can respond to endogenous damage-associated molecular patterns (DAMP), which can be released following stress or injury to the tissue (22), or to microbial pathogen-associated molecular patterns (PAMP), which are present during infections or breach of epithelial barriers (23). Recently, a third group, which includes lifestyle-associated molecules patterns (LAMP) such as LDL cholesterol was suggested (24); those LAMP differ from DAMP because they

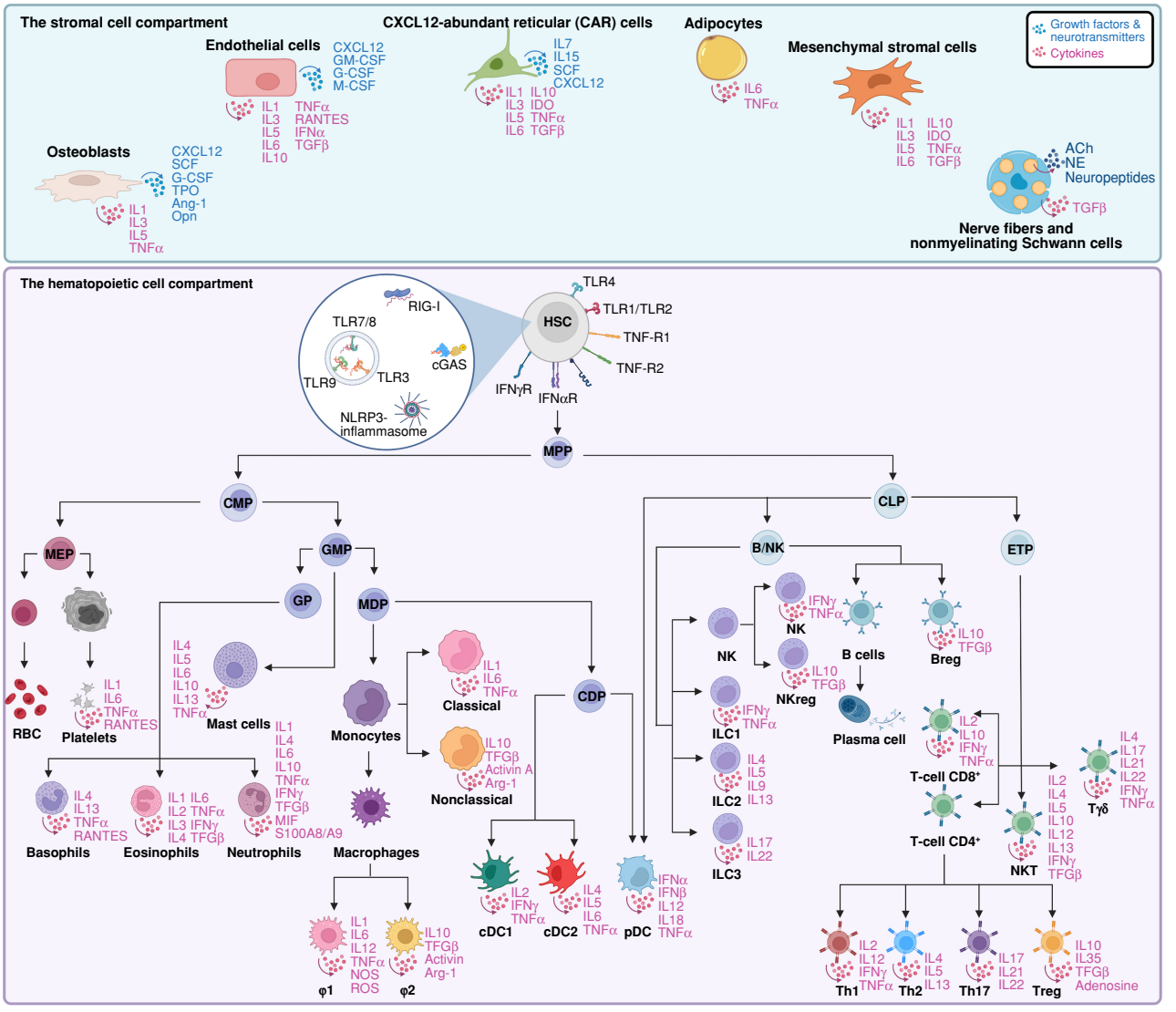

Figure 2. Inflammatory pathways in the hematopoietic system and BM niche components. Within the BM, HSC, progenitors, and committed precursors express a battery of cytoplasmic and cell-surface receptors to detect microbial components and inflammatory cytokines. Similarly, the BM niche and accessory cells possess several sensors and can release high levels of inflammatory cytokines to shape immune responses at the central level of hematopoiesis. Here we highlight the major cytokines and sensors expressed in the BM stromal compartment and throughout hematopoietic differentiation (Created with BioRender.com). Ach, acetylcholine; Ang-1, angiopoietin 1; CAR, CXCL12-abundant reticular; CDP, common dendritic progenitor; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; DC, dendritic cell; ETP, early T-cell precursor; G-CSF, granulocyte colony stimulating factor; GMP, granulocytic-monocytic progenitor; GP, granulocytic precursor; HSC, hematopoietic stem cell; ILC, innate lymphoid cell; MDP, monocytic-dendritic precursor; MPP, multipotent progenitor; MEP, megakaryocytic and erythroid progenitor; MSC, mesenchymal stromal/stem cell; NE, norepinephrine; NK, natural killer; Opn, osteopontin; SCF, stem cell factor; TPO, thrombopoietin.

cannot be easily cleared and remain longer, inducing sterile chronic inflammation.

The signaling pathways activated downstream of PRR in normal and leukemic cells have been extensively reviewed elsewhere (22–26), but briefly, signaling through various kinases, adaptor proteins, and transcription factors results in cytokine release and cell-fate decisions. Although these signaling pathways were discovered and described in mature immune cell populations, hematopoietic stem and progenitor cells (HSPC) also serve as immune sentinels by sensing inflammatory cues by the functional expression of a wide receptor repertoire on their cell surface to shape hematopoiesis at expediency (refs. 27, 28; Fig. 2). In fact, emergency hematopoiesis is a central mechanism crucial to better combat infections. Thus, emergency hematopoiesis accelerates myeloid cell generation to replenish the cells that have been quickly consumed due to the current infection, as well as support pathogen clearance. Long-term HSCs (LT-HSC) and short-term HSCs (ST-HSC) express TLR4 and TLR1/TLR2 and their ligation results in transcriptomic and epigenomic remodeling, consistent with increased cell cycling and myeloid commitment. *In vivo* TLR ligation induces emergency hematopoiesis (28), but chronic exposure compromises the HSC compartment (29, 30). This accelerated hematopoiesis requires the proliferation of HSC, the

abandonment of the niche, exit from quiescence, and myeloid commitment as features of the emergency programs, whereas committed progenitors proliferate in abundance to produce new progeny in a shorter window of time. Thus, the high abundance of emerging myeloid inflammatory cells is controlled by switching off the emergency programs concomitant with the short half-life of the newly-produced innate cells. However, the precise mechanisms that resolve inflammation and restore the steady-state hematopoiesis in HSPC remain poorly understood. Mature myeloid cells respond differently upon secondary TLR stimulation, which is the basis for LPS tolerance. Similarly, several groups have demonstrated that HSPC can be imprinted with long-lasting epigenetic memory to modulate emergency hematopoiesis and protect the organism against secondary encounters. Specifically, following an inflammatory stimulus, the chromatin landscape of the cells is altered, resulting in increased accessibility in loci associated with myeloid lineage commitment and increased responsiveness of immune genes upon secondary stimulation (31, 32). The concept of epigenetic memory in hematopoiesis is intriguing, as many mutations involved in myeloid malignancies are in epigenetic regulators. Thus, it is possible that in cells carrying AML driver mutations, inflammatory stimuli result in defective epigenetic rewiring. This may contribute to the inflammatory phenotype observed for some of these mutations, as well as contribute to impaired myeloid differentiation upon secondary stimulus.

A key question is the nature of the mechanisms of induction and recovery from emergency hematopoiesis at the level of the HSPC in the BM. We have recently identified the ubiquitin ligase SPOP as the enzyme that binds and degrades MYD88 leading to inactivation of Myddosome function (33). Indeed, the combination of TLR stimulation and SPOP inhibition leads to uncontrolled emergency response, leading to lethal neutrophilia. Another mechanism regulating emergency hematopoiesis is the ubiquitination of histone H2B. During infection, H2B monoubiquitination increases in loci of interferon response genes, leading to increased expression of these genes. Deubiquitination of H2B by the deubiquitinating enzyme USP22 leads to termination of the inflammatory response, and, similarly to SPOP inhibition, deletion of USP22 leads to uncontrolled emergency hematopoiesis (34). On the flipside, mechanisms of induction are also ill studied. Studies of patients with VEXAS syndrome or ARCH-induced atherosclerosis would suggest that the genes UBA1 (11) or TET2/DNMT3A, which are frequently mutated in these conditions (35), could control emergency hematopoiesis at the posttranslational and epigenetic levels, respectively, although direct experimental proof is missing.

Upregulation of inflammatory cytokines such as interleukin 1β (IL1β), IL6, and TNF is a hallmark of aging, also referred to as inflammaging (36). Concomitant with reduced lymphopoiesis and the myeloid-bias that is observed during aging (37), the immune landscape is remodeled during this natural transition with the accumulation of inflammatory senescent cells (38). The functional differences between young versus aged HSPC have been extensively reviewed (39–41), but changes in metabolism, chromatin architecture, and incomplete DNA repair may compromise their stem cell function. The high levels of reactive oxygen species (ROS) and heightened proliferation increase the risk of acquiring mutations. As a means to eliminate damaged cells, HSPC can constitutively present antigens in the context of MHC class II to induce T-cell responses (42). Interestingly, neoantigens direct the elimination of potentially malignant HSPC by instructing their differentiation (42). Considering that immunosurveillance may decay over time, a reduction in antigen presentation capacity could play a role in the preservation of aberrant clones generated by inflammation. Proinflammatory cytokines can also dictate hematopoietic cell fate and differentiation. For instance, TNF-α signaling promotes survival and myeloid differentiation via activation of NF-kB (43). Similarly, acute stimulation of HSC with IL1 enhances their proliferation and promotes myeloid differentiation, whereas chronic simulation causes a complete HSC regenerative exhaustion (44). Therefore, some of the mechanisms driving myeloid expansion may be hijacked during clonal and malignant hematopoiesis to sustain aberrant myelopoiesis. Type I and II IFNs are the major soluble factors produced during infections and can also activate HSPC. The role of IFNs in HSPC is multifactorial, as some reports suggest a proapoptotic role (45, 46), whereas others have documented enhanced proliferation in human HSPC (47–49). In nonpathologic conditions, quiescent HSC can be awakened when they are simulated *in vivo* with IFNα in a STAT1/AKT1-dependent manner (49). Notably, IFNα signaling via STAT1 induces upregulation of stem cell antigen-1 (Sca-1; refs. 49, 50), which is widely used to define murine HSPC; thus, the definition of phenotypic HSPC in mice can be controversial in studies focused on inflammation. Acute stimulation with IFNα does not affect HSC function; however, chronic stimulation may alter their reconstitution capacity. Functional exhaustion of HSC can be prevented by termination of the inflammatory signal, for instance, activation of IFN regulatory factor 2 (IRF2) suppresses IFN signaling (51). Likewise, IFNγ was originally associated with HSPC expansion, primarily by activation of dormant HSC during chronic infections and enhanced myelopoiesis. Another important cytokine that regulates HSPC function is the inflammatory cytokine IL6. IL6 deficiency leads to impaired HSPC self-renewal (52). IL6 is one of the first cytokines to be produced after TLR ligation in different contexts, is commonly upregulated in aging (53), and its role has been highlighted in ARCH, particularly in the context of TET2 mutations (54, 55). Another member of the IL6/IL12 family is IL27, which is associated with regulation of the immune response. During emergency hematopoiesis, IL27 is a crucial intermediate that promotes HSPC expansion by promoting myelopoiesis in synergy with SCF.

FREQUENT MUTATIONS IN MYELOID MALIGNANCY AND THEIR INFLAMMATORY PROFILE

The unique nature of leukemia, as a cancer of immune precursor cells, has drawn particular attention to the role of leukemia driver mutations in the modulation of immune responses. Mutations in genes involved in the regulation of immune pathways, such as *JAK2, TRAF6, FLT3, MYD88*, and *NOTCH1*, as well as dysregulation of HLA molecules, are common in leukemias, suggesting that there may be a link between dysregulated immune activation and

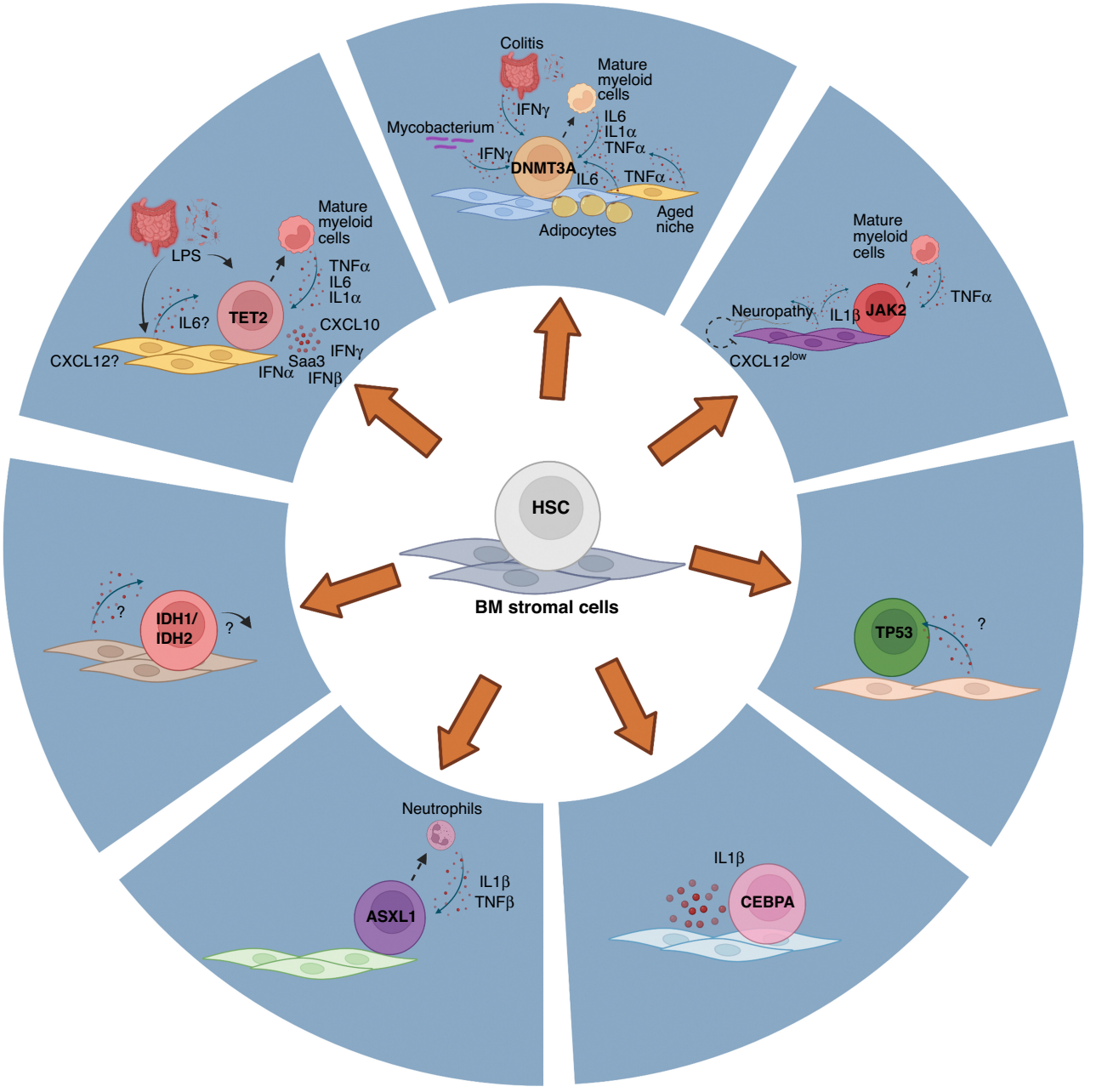

Figure 3. Common myeloid mutations and their inflammatory milieu. Normal HSC are supported by the BM stroma. Mutated HSC (or their progeny) produce inflammatory factors that can activate the BM niche to produce more inflammatory factors creating positive feedback loops. Clinical observations and experimental data have shown that mutations observed CH have different inflammatory profiles and are better sustained by different context niches (Created with BioRender.com).

leukemogenesis in both myeloid and lymphoid malignancies. AML is associated with a unique set of driver mutations, in epigenetic modifiers and splicing factors, occurring in HSPC. HSPC give rise to all mature hematopoietic lineages, yet in AML their differentiation is blocked, leading to the accumulation of immature blasts. The role of HSPC in controlling cellular output at steady state and during infection, and in maintaining epigenetic memory of inflammatory events, has led to specific interest in the role of AML driver mutations in the modulation of HSPC response to inflammatory stimuli. Mutations in epigenetic modifiers, such as TET2, ASXL1, and DNMT3A, are common in CH, MDS, and AML (56, 57). These mutations were associated with specific inflammatory responses, which may contribute to leukemogenesis (Fig. 3).

TET2 is a methylcytosine dioxygenase that catalyzes the conversion of methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), leading to DNA demethylation (58, 59). In murine models, loss of Tet2 is associated with the expansion of myeloid cells and results in a myeloproliferative neoplasm

(60, 61). *Tet2* transcription has been shown to be induced by inflammatory signals, such as LPS, and loss of *Tet2* in macrophages has been associated with increased production of inflammatory cytokines such as IL1β and IL6 (62). Further work has demonstrated that increased IL6 production in *Tet2* knockout (KO) mice is associated with activation of the IL6–STAT3–*Morrbid* pathway, which enhances the cell survival of Tet2 KO HSPC. Inflammatory signals in Tet2 KO mice result in increased expansion of Tet2 KO HSPC and myeloid progenitors, which can be rescued by the blockade of IL6 signaling (54). It has also been shown that in macrophages, TET2 specifically represses IL6 transcription via the recruitment of histone deacetylase 2 (HDAC2) to the *IL6* locus (55). In mast cells, *Tet2* loss of function cooperates with oncogenic mutations in Kit to induce chronic inflammation. TET2 is required to resolve inflammatory phenotypes and hypermethylation of genes associated with resolving the inflammatory phenotype contributes to continued inflammation and transformation of mast cells (63). IL1 signaling was also shown to be important for the clonal expansion of cells carrying *Tet2* mutations. In murine models, IL1 levels in the BM serum correlated with the clone size of $Tet2^{+/-}$ cells (64), and deletion of the IL1 receptor rescued the competitive advantage and clonal expansion of Tet2-deficient cells (64, 65). Moreover, increased IL1β levels were also identified in people with ARCH carrying *Tet2* mutations (66). Interestingly, chronic IL1β exposure also leads to expansion of C/EBPα-mutant cells (67), suggesting this might be a common mechanism for the expansion of mutated clones. Taken together, these results indicate that *Tet2* loss of function is associated with a chronic inflammatory response. Although these reports are in murine models, it is possible that in people carrying TET2 mutations, similar inflammatory processes contribute to leukemogenesis.

Mutations in *Tet2* are also associated with dysbiosis and an impaired intestinal barrier (68, 69). In mice, constitutive or hematopoietic-specific loss of *Tet2* resulted in bacterial translocation to the spleen and mesenteric lymph nodes. This translocation led to increased IL6 production, which contributed to leukemic transformation in these mice (68). Furthermore, Tet2 deficiency in hematopoietic cells was shown to trigger liver dysbiosis, resulting in autoimmune hepatitis. This was due to increased activation of *Tet2*-deficient $CD8^+$ T cells (69), in line with additional reports demonstrating a role for TET2 in controlling the differentiation of T and B cells (70–72). Importantly, mutations associated with ARCH were shown to disseminate to all mature hematopoietic lineages (73), suggesting that defects in lymphoid cell function could also contribute to AML development, possibly by impaired immune response to transformed myeloid cells.

Tet2 mutations can affect the inflammatory profile of malignant cells. In mice carrying an oncogenic *Tet2* mutation (H1881R (in human), H1794R (in mouse), $Tet2^{HR}$), disease progression was associated with an enhanced inflammatory response and emergence of an abnormal inflammatory monocyte population (74). $Tet2^{HR}$ monocytes exhibited elevated levels of IFN response genes, as well as increased production of inflammatory cytokines in the BM. Emergence of $Tet2^{HR}$ inflammatory monocytes was accelerated by inflammatory stimuli and could be blocked by the administration of anti-inflammatory drugs or antibiotics. Notably, similar inflammatory monocytes could be detected in AML patients carrying the same mutation in *TET2*, and expression of the inflammatory gene signature associated with these monocytes was associated with poor prognosis in AML. Intriguingly, although mutations in IDH1/2 are also common in myeloid malignancies (56), and IDH1/2 mutations are thought to be at least partially mechanistically similar to TET2 mutations (75), similar inflammatory phenotypes have not been reported for patients with IDH mutations. Moreover, in glioblastoma, mutations in IDH1 are associated with a reduction in inflammatory markers (76).

DNMT3A is a DNA methyltransferase that catalyzes the conversion of 5hmC to methylcytosine (5mC), which is associated with gene silencing (77). Thus, DNMT3A is thought to function inversely to TET2. However, DNMT3A is the most frequently mutated gene in ARCH, MDS, and AML, and the hotspot R882 mutation confers poor prognosis in AML (77). Mutations in DNMT3A are also associated with inflammation, and murine models demonstrated that they can contribute to the inflammatory profile associated with several pathologies, including osteoporosis and heart failure (78–80). In addition, in mice, chronic infection resulted in the preferential expansion of hematopoietic cells deficient in one or two copies of *Dnmt3a*, effectively mimicking ARCH (81), and in patients with ulcerative colitis high serum levels of IFNγ correlated with clone size of DNMT3A-mutant cells (82). Furthermore, Dnmt3a-deficient HSC were more resistant to stress-induced apoptosis and less susceptible to exhaustion, providing a potential explanation for their accumulation in the blood. Similarly, the deletion of DNMT3A from human $CD34^+$ cells resulted in resistance to IFNγ-mediated differentiation. Thus, cells carrying *Dnmt3a* mutations can generate an inflammatory milieu, which is beneficial to their expansion, effectively generating a positive feedback loop for the expansion of *Dnmt3a*-mutant cells.

ASXL1 is a member of the polycomb repressive complex (83). Mutations in *asxl1* frequently cooccur with *Tet2* mutations in MDS and AML (56, 57, 84). In zebrafish, mutations in *asxl1* were shown to induce the expression of inflammatory genes in mature myeloid cells (85). Intriguingly, *asxl1*-mutant HSC were shown to express anti-inflammatory genes, suggesting that mutant HSC maintain fitness by abrogating the inflammatory response generated by their progeny. Indeed, the deletion of anti-inflammatory genes in the *asxl1*-mutant background rendered the mutant HSC less fit and prevented their clonal expansion. These findings are reminiscent of the inflammatory monocytes identified in $Tet2^{HR}$ mice (74), and suggest that inflammatory cells provide a niche for leukemic stem cells.

Mutations in established inflammatory pathways are also associated with AML. In myelofibrosis, a preleukemic condition, $JAK2^{V617F}$ and other JAK2-activating mutations drive aberrant activation of its downstream signaling pathway (86). Recently, single-cell assay for transposase-accessible chromatin using sequencing (ATAC-seq), coupled with genotyping, in myelofibrosis patients carrying the $JAK2^{V617F}$ mutation revealed differing inflammatory programs in mutant HSPC subsets, with early HSPC displaying increased activation of the NF-kB signaling pathway and megakaryocyte progenitors having increased activation of JUN and FOS transcription factors, which can also mediate inflammatory response and fibrosis (87). Furthermore, DNMT3A mutations were shown

to cooperate with JAK2 mutations to enhance inflammatory signaling and drive myelofibrosis (88). Overall, this demonstrates that driver mutations in myeloid malignancies can have a context-dependent inflammatory profile, driving different inflammatory responses in different cell types.

AML is often preceded by MDS, and mutations in *TET2*, *ASXL1*, *DNMT3A* and a number of RNA splicing genes are common in MDS (89). Notably, MDS has an inflammatory profile, and progression from MDS to AML is linked to increased inflammation (13, 90–92). Many inflammatory cytokines are upregulated in the serum of MDS patients, and may contribute to disease progression. Of note, alterations in immune pathways can cooperate with specific mutations to drive disease progression. One such example is downregulation of TRAF6, which cooperates with TET2 mutations to drive myeloid malignancy. We have recently shown that TRAF6 downregulation results in activation of MYC target genes, resulting in increased proliferation. TRAF6 mediates MYC ubiquitination following TLR activation, which is increased upon TET2 loss. Therefore, silencing of TRAF6 in TET2 mutant cells prevents MYC suppression in inflammatory conditions (93). Overall, the inflammatory phenotype observed in ARCH provides a selective advantage to clones carrying mutations, and enables progression through different stages of premalignant and malignant transformation, culminating in full-blown AML. While ARCH is a risk factor for developing AML, only a small minority of people with ARCH develop a myeloid malignancy (94). Further characterization of the role of inflammation in the progression of ARCH, as well as elucidation of the inflammatory profile of other mutations implicated in myeloid malignancies, may help identify individuals more at risk for transformation, as well as novel therapeutic strategies.

BM NICHE REMODELING BY INFLAMMATION

Hematopoiesis is highly dependent on the BM microenvironment, which provides hematopoietic niches that allow for maintenance and development of different hematopoietic populations, at steady state and in stress conditions. The BM niche also provides a regulatory unit that maintains hematopoietic stem cells. The BM houses several nonhematopoietic populations, such as osteoblasts, endothelial cells, mesenchymal stromal cells (MSC), adipocytes, nerves, as well as mature hematopoietic populations such as macrophages, regulatory T cells and megakaryocytes. Several Cre-Lox systems have allowed the functional elucidation of almost all stromal populations while murine fluorescent reporters have been vastly used to map their distribution. By combining those systems with state-of-the-art single-cell technologies, our lab and others have demonstrated that the architecture of the hematopoietic niche is cellularly heterogeneous but exquisitely dynamic during stress conditions (95, 96). The adaptability of the BM niche to stress suggests that there may be premalignant niches, which could support the expansion of clones with certain growing advantages. Indeed, in a model of MPN, osteoblast lineage cells were increased, accompanied by thickening of the trabecular bones and emergence of BM fibrosis (96). Coculture experiments demonstrated that this phenotype was driven both by direct contact between MSC and leukemic myeloid cells and by secretion of CCL3 and TPO from leukemic cells.

The niche-secreted factors SCF and CXCL12 have been extensively studied, due to their essential role in HSC maintenance (97–99). In mice, the highest levels of CXCL12 and SCF are produced by a MSC subpopulation, CXCL12-abundant reticular cells (CAR), which also expresses Leptin receptor (LepR) and overlaps with Nestin (Nes) expressing MSC (99). CAR niches are affected during infection (100) and other stress conditions, including physiological aging (101). The loss of niche factors such as CXCL12 may be a hallmark of the leukemic niche (102, 103). The BM niche plays a major role in protecting HSC pool integrity during emergencies. Thus, the BM stem cell niches maintain immune privileged zones in order to safeguard HSC against insults or attack by immune cells. One of these mechanisms is through the recruitment of $CD150^+$ Treg cells via CD39 activity (104). Another unique mechanism to safeguard the HSC pool is via MHC-II-dependent antigen presentation by HSC to $CD4^+$ T cells, which can eliminate damaged HSC presenting aberrant peptides (42). Interestingly, AML cells downregulate the antigen presenting machinery including HLA molecules (105, 106).

BM MSC can play both inflammatory and anti-inflammatory functions. MSC derived from healthy donors can suppress T-cell proliferation and NK cytotoxicity by the expression of PD-L1, IL10, IDO1 and TGFβ (107). However, these properties are dysregulated in MSC derived from pathological conditions, such as myeloproliferative neoplasms (MPN). Leukemic cells can reeducate the BM niche to create sanctuaries which protect leukemic stem cells (LSC) from chemotherapy and immunotherapy, sustain massive cell proliferation and favor LSC at the expense of healthy HSC (108). Similarly, transcriptomic studies on MSC derived from leukemia patients suggest that niche cells contribute to the inflammatory microenvironment (109).

The inflammatory microenvironment in myeloid malignancies is also accompanied by changes in the immune microenvironment in the BM. In MDS, regulatory T cells are expanded, suppressing immune activation against malignant cells (110). Recently, we described inflammatory programs in adult and pediatric AML patients that were associated with specific B- and T-cell populations in the BM (14). Inflammation was associated with an accumulation of atypical B cells, a subset of memory B cells associated with chronic inflammation and recurrent infections. Atypical B cells are thought to have reduced antibody production capacity, suggesting they are less efficient in targeting malignant cells in the BM. Furthermore, inflammation was also associated with an enrichment in regulatory T cells and in $GZMK^+$ $CD8^+$ T cells in pediatric patients. Furthermore, $GZMK^+$ $CD8^+$ T cells were found in several chronic inflammatory conditions (111, 112), and GZMK was shown to induce the release of inflammatory cytokines (112), suggesting these cells may act to perpetuate the inflammatory profile in AML. Interestingly, $GZMK^+$ $CD8^+$ T cells were previously reported to be associated with increased response to immune-checkpoint blockade in AML (113), and share transcriptional similarity to the recently described progenitor-exhausted T cells (114), suggesting patients with an inflammatory profile may benefit from immune-checkpoint blockade therapy.

Unlike its well-characterized role in AML, the role of the BM niche in ARCH remains unknown. The correlation of

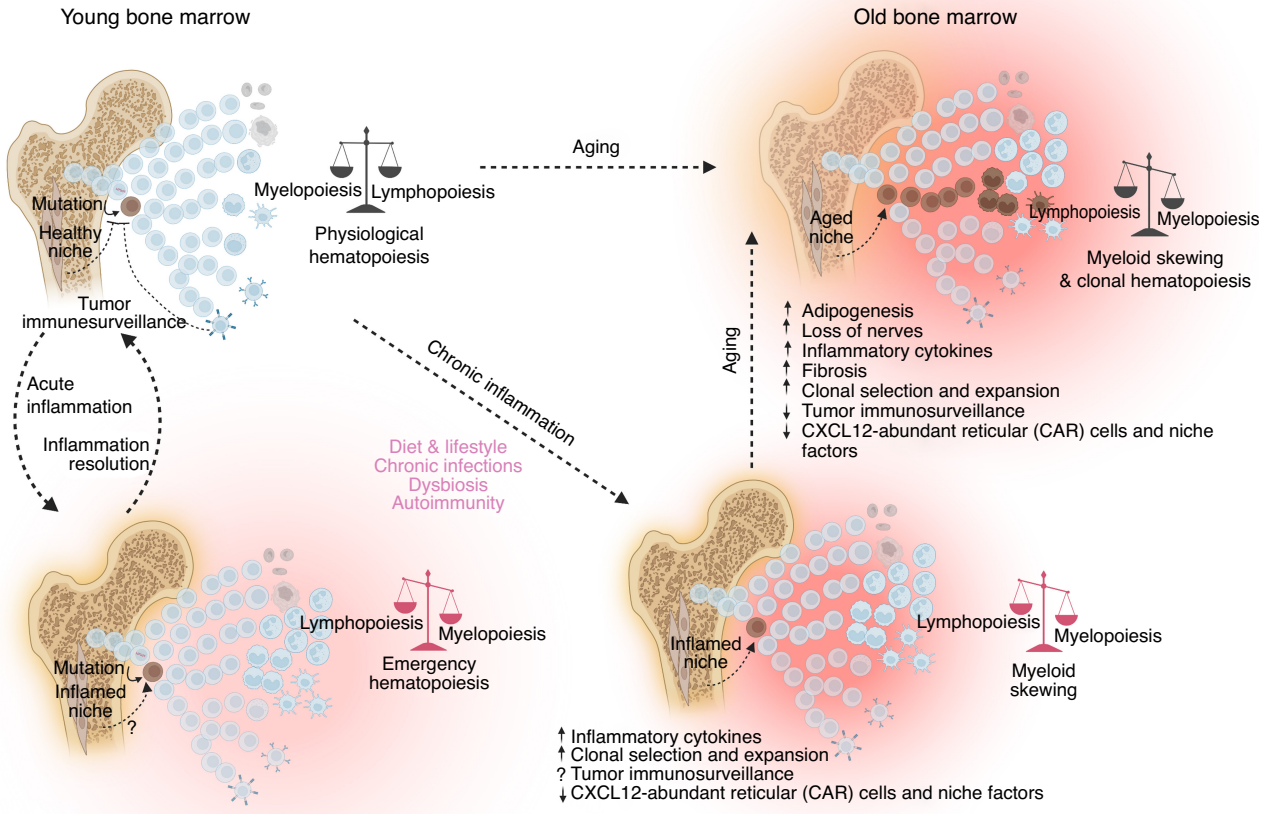

Figure 4. BM dynamics during aging and acute/chronic inflammation. Homeostatic hematopoiesis occurs in the BM and maintains a balance of mature immune cells. Emergency hematopoiesis is a process of rapid proliferation of HSPC due to an insult, which is rapidly resolved. The increased levels of ROS and inflammation may induce silent mutations in HSPC, which can be restricted by different checkpoints (repair mechanisms, immunosurveillance, repressor niches). Sustained inflammation may reprogram HSPC and the BM niches. Physiologic aging is associated with increased systemic levels of inflammatory cytokines (inflammaging) and erosion of the BM niche. The protumoral niches sculpting during chronic inflammation or aging and immune editing may create neighborhoods that allow the selection of mutant clones (Created with BioRender.com).

ARCH and hematological decline in elderly patients suggests that an aged/inflamed niche is a universal prerequisite for the loss of clonal diversity observed in older individuals (115). The erosion of the BM niche during physiological aging is characterized by the loss of niche factors and the acquisition of inflammatory phenotypes, including high production of IL6 and other factors that promote myelopoiesis and suppresses lymphopoiesis, concomitant with increased adipogenic potential and the loss of sympathetic nerves (Fig. 4) (99, 116). Thus, an aged and inflamed niche might impair hematopoiesis, and the HSC must adapt to this harsh microenvironment. Remarkably, infections can also cause a transient erosion of the BM niche (117). Additionally, CXCL12-abundant reticular cell (CAR) niches sustain irreparable damage following chronic viral infections, due to $CD8^+$ T cell activation and type I and II IFN secretion (118), providing a possible explanation for high correlation between ARCH, chronic viral infections (119) and niche remodeling during aging (101). Similar to the accelerated aging of HSC during chronic inflammatory conditions, aged stroma transcriptional signature overlaps with LPS- or poly(I:C)-treated BM stromal cells. Thus, infection-induced or aged CAR cells upregulate IFN-related chemokines such as CXCL9, CXCL10, CXCL11 and the inflammatory IL6 (101). Indeed, CAR cells are the major source of IL-6 in the BM during LPS or poly(I:C) challenge (120). Although both hematopoietic and non-hematopoietic cells contribute to local BM IL6 secretion, systemic IL6 levels are niche-dependent via TLR4 and Myd88 signaling. Other niche cells such as endothelial cells produce high levels of G-CSF that enhances granulopoiesis during LPS sensing, concomitant with their activation state which is necessary to allow HSC mobilization.

It is still controversial whether mutations and chromosomal aberrations in MSC contribute to the aberrant phenotypes in MPN. The study of human BM niches has been challenging due to lack of models that allow to mimic human-human cell interaction as occurs *in vivo*. Moreover, the low frequency of stromal cells in BM aspirates as well as the absence of *bone-fide* phenotypic markers has delayed their study. Novel tools such as humanized mice and human BM organoids will be useful as they can partially mirror the BM topology and niche function as observed *in vivo*. Nevertheless, it has been demonstrated that in some cases, lesions in BM niche components can promote MPN. For example, the loss of

Dicer1 in osteoprogenitors causes MDS and more importantly, BM transplantation demonstrated that MDS in this model is determined by the BM niche (121). Transcriptomics have shown an upregulation in some inflammatory cytokines and stress response genes. Similarly, the inactivation of retinoic receptor gamma (RARγ) in the niche induces TNF expression by T cells and induces a myeloproliferative-like disorder (122). Mutations in the WNT pathway in niche cells were also shown to induce myeloid neoplasms (123), however the inflammatory component of this phenomenon remains unknown.

In the context of ARCH, TET2 mutations in MSC alter self-renewal and their proliferation capacity, in addition with an osteoblast-bias differentiation potential which promotes the *in vitro* expansion of myeloid colonies. Interestingly, the loss of *Tet2* in MSC has more impact in cooperating with preleukemic *Tet2*-KO cells in disease progression compared with the loss of *Tet2* in the endothelial or osteoblastic niches (124). Furthermore, similar observations have been published for ASXL1 mutations in the stem cell niche (125). Several groups have documented the differences between young and aged BM niche. Early changes that can be detected in the BM of middle-aged mice confer selective advantage for *DNMT3A*^{R878H} clones through TNF signaling (126). Similarly, fatty BM can better sustain DNMT3A-mutant clones, presumably due to secretion of IL6 (127). In the context of JAK2 mutations, the genetic depletion of CXCL12 in Nes^{+} niche cells enhanced the expansion of JAK2-mutant clones. Mechanistically, JAK2-mutant hematopoietic cells can remodel the BM by reducing CXCL12 expression and the number of MSC to create a CXCL12$^{low/neg}$ niche, which sustains the tumor cells (128).

Besides fibrosis, neuropathy and loss of nerves have also been documented during BM aging (116) and in MPN (129, 130). In the context of JAK2 mutations, apoptosis of sympathetic nerves is mediated by IL1β produced by Nes^{+} cells (128). Neurotransmitters such as acetylcholine (ACh) have also been implicated in emergency hematopoiesis. In the BM, B cells are the main source of choline acetyltransferase (Chat) required for the ACh synthesis. During physiologic conditions, Chat is produced by a subset of BM-residents mature B cells; interestingly, the loss of Chat in B cells induces an increased hematopoietic activity due to the reduced ACh in the BM niche (8). Mechanistically, ACh stimulates MSC in the niche to downregulate the niche factors *Cxcl12* and angiopoietin-1 (*Angpt1*), which are critical factors for the retention and quiescence of HSC. During myocardial infarction, the loss of ACh in B cells leads to an exacerbated inflammatory response and stimulates myelopoiesis revealing its anti-inflammatory secondary role as an emergency hematopoiesis limiting factor. In aging or leukemic settings, B-cell lymphopoiesis is critically reduced, and the limited source of ACh leads to a hyperinflammatory response during inflammatory circumstances such as cardiovascular disease highly correlated with ARCH (130). These findings highlight the delicate balance between the BM niche, mature immune cells, and HSC.

FUTURE PERSPECTIVES

Inflammation is emerging as a novel regulator of myeloid malignancy development and progression. Understanding the role of inflammation in shaping the BM microenvironment in myeloid malignancies and ARCH could open up novel avenues for therapy for myeloid malignancies (Fig. 5), which have seen very little progress over the past decades. Furthermore, as novel regulators of inflammation emerge, novel targets for therapy are being discovered. One such example is the loss of mitochondrial function during HSC aging. Loss of NPM1 can recapitulate the activated state of mitochondria frequently observed in aging. Thus, NPM1 loss activates the NLRP3 inflammasome leading to the secretion of IL1β (131). Interesting findings in the field have suggested a major role for mitochondria driving inflammation, as they can serve as cellular stores of DAMP such as ATP and mtDNA. Thus, mitochondria dysfunction and/or inefficient mitophagy can affect ROS balance and promote the release of different mitochondrial DAMP. In mature macrophages, loss of TET2 or DNMT3A imprints a type I IFN signature due to loss of mtDNA integrity and the consequent activation of the cGAS–STING signaling pathway, opening new roads for therapy (132). Another factor that may contribute to inflammation in myeloid malignancy is chronic viral infections. Patients with HIV have a higher incidence of ARCH (119), suggesting a link between the immune response to infection and expansion of aberrant clones. Although IFN have been shown to promote ARCH, IFNα also has a therapeutic role in CML.

Targeting of inflammation is emerging as a novel strategy in the treatment of MDS, and there are several clinical trials targeting specific inflammatory pathways currently under way (NCT04239157, IL1; NCT04278768 and NCT05178342, IRAK4; NCT04245397, CXCR1/2). Targeting the IL6/JAK/STAT3 pathway by pharmacologic inhibitors is being explored in both hematologic and solid tumors, and monoclonal antibodies such as siltuximab or sirukumab (anti-IL6) and tocilizumab (anti-IL6R) are still under clinical trials for different types of tumors. Targeting inflammation could also be a strategy to prevent progression from CH to myeloid malignancy. Current clinical trials examining anti-inflammatory agents for other indications often include patients with CH, and recently it has been reported that patients with TET2 CH have fewer cardiovascular events following treatment with canakinumab, an IL1β blocking antibody (133). It would be interesting to examine whether the rate of progression to myeloid malignancy in these patients is also reduced. Combination of anti-inflammatory drugs with established therapies for AML could prove beneficial for high-inflammation patients, both via intrinsic effect on the malignant cells and via remodeling of the immune microenvironment. Consideration of the inflammatory state of patients could affect treatment decisions and allow better stratification of patients to appropriate treatment regimens. In addition, understanding the specific inflammatory profile associated with specific mutations can enable targeted anti-inflammatory treatments for patients carrying these mutations. Finally, it is possible that induction of inflammation, for example, by the intense chemotherapy currently offered to some AML patients, can reshape the immune microenvironment, affecting the immune response to AML. In line with this, identifying novel regulators of inflammation in AML can help pinpoint novel therapeutic targets. One such example is IRF2BP2, which is highly expressed in AML (134). IRF2BP2 depletion leads to a dysregulated inflammatory phenotype, which results in differentiation and cell death (135).

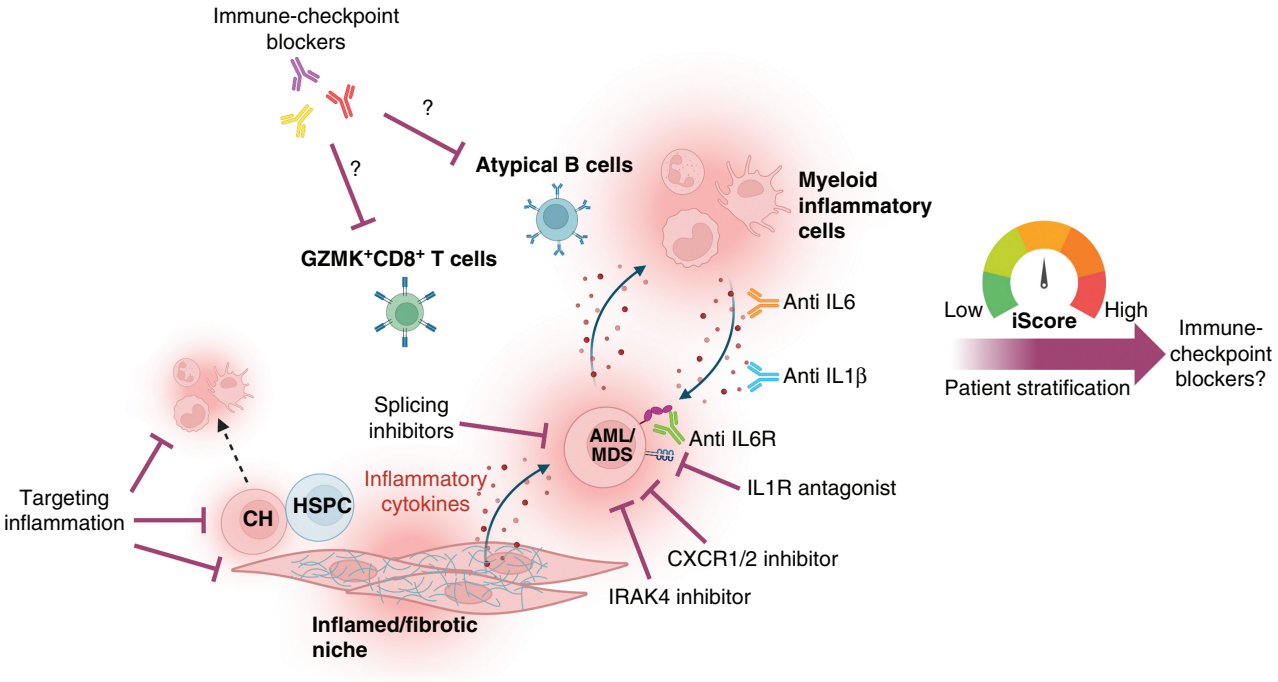

Figure 5. Targeting inflammation in myeloid neoplasms. Novel therapeutic strategies are emerging to target inflammatory pathways in myeloid neoplasms. Ongoing clinical trials include small molecules and neutralizing antibodies. Novel studies are suggesting the efficacy of some splicing inhibitors or the use of immune-checkpoint blockers in patients with high levels of inflammation showing exhausted and/or anergic immune cells. Future therapeutic options may include targeting the inflamed niche and eliminating fibrosis; achieving repression of CH will be a major milestone for preventative medicine (Created with BioRender.com).

Characterization of inflammation-mediated effects on the BM immune microenvironment could provide additional therapeutic strategies. Identification of specific B- and T-cell populations that are associated with inflammation in AML is one such example. In AML patients, inflammation is associated with the enrichment of atypical B cells, an inhibitory memory B-cell population observed also in chronic or recurrent infections. In pediatric patients, inflammation is also associated with an increase in regulatory T cells and progenitor-exhausted GZMK$^+$ CD8$^+$ T cells, which may be more responsive to immune-checkpoint blockades (14). It is possible that targeting these populations would improve outcomes in high-inflammation patients. Another subset of common mutations in ARCH, MDS, and AML occurs in genes involved in RNA splicing. Interestingly, pharmacologic targeting of splicing using drugs against the accessory splicing factor RBM39 or PRMTI rendered cells more susceptible to T-cell killing, by generation of neoantigens due to aberrant splicing (136). These findings raise the possibility that mutations in splicing-related genes in AML similarly increase the immunogenicity of mutant cells, suggesting that patients with splicing mutations may be more susceptible to T cell–stimulating therapies. It would be interesting to examine how splicing inhibition affects the immune response in an inflamed microenvironment. In AML, progenitor-exhausted T cells, which are more likely to respond to immune-checkpoint blockade (ICB), are enriched in high-inflammation AML patients. It is, therefore, possible that the combination of splicing inhibitors with ICB in this subset of patients will prove particularly potent.

Authors' Disclosures

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