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Flaming and fanning: The Spectrum of inflammatory influences in myelodysplastic syndromes

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Abstract

The myelodysplastic syndromes (MDS) represent neoplasms derived from the expansion of mutated clonal hematopoietic cells which often demonstrate aberrant differentiation potential with resultant cytopenias and a propensity to evolve into acute myelogenous leukemia. While multiple mutations have been identified which may serve as drivers of the MDS clone, there is accumulating evidence that MDS clones and subclones are subject to modulation by the marrow microenvironment and its inflammatory milieu. There is also a strong link between autoimmune disorders and MDS. In this review, we examine the role of inflammatory cytokines, toll like receptors, pyroptosis, stromal cells, and cellular inflammatory mediators in MDS initiation, propagation, and progression. These contributions in a background of mutational, epigenetic, and aging changes in the marrow are also reviewed. Such inflammatory mediators may be subject to therapeutic agents which will enhance suppression of the MDS clone with potential to improve therapeutic outcomes in this disease which is usually incurable in aged patients not eligible for stem cell transplantation.

Keywords

Myelodysplasia; Autoimmunity; Cytokines; Inflammasome; Microenvironment

1. Introduction to myelodysplastic syndromes

Myelodysplastic syndromes (MDS) are neoplasms derived from the expansion of mutated clonal hematopoietic cells. The cell of origin in MDS is not clear; whether a true hematopoietic stem cell or an early multipotential progenitor cell [1]. MDS is characterized by varying degrees of cytopenia(s) which result from aberrant differentiation manifested as abnormal cell morphology. In addition, chromosomal abnormalities and gene mutations are

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found in a high percentage of cases, and there is a variable prognosis primarily related to severity of cytopenias and a variable rate of transformation to acute myelogenous leukemia. These disorders increase in incidence with age, and median age of onset is about 70 years [2]. The incidence is cited at 3–4 per 100,000, but this is thought to be an underestimate.

The commonly seen mutations in MDS involve epigenetic modulators of DNA methylation and histone modifications, RNA splicing genes, transcription factors, and growth factor signaling pathways [3]. High risk MDS is often treated with hypomethylating agents, and allogeneic stem cell transplantation is considered the only curative treatment for MDS, but only a fraction of MDS patients are appropriate for this based on age, co-morbidities, or in rare cases, donor availability. The only three treatment modalities currently approved in MDS include lenalidomide in those who demonstrate deletion of chromosome 5q on karyotype or fluorescence in situ hybridization analysis, 5-azacitidine, and decitabine. No treatment has been approved by the United States FDA for MDS since 2005.

Despite the heterogeneity within the MDS syndromes, the variation in degree of marrow cellularity, and the variable prognosis ranging from a median survival of a few months in MDS-EB-2 (MDS with excess blasts 10–19%) to > 5 years in MDS-RS-SLD (MDS-ring sideroblasts-single lineage dysplasia) [4], there are recurring themes amongst the MDS subtypes including ineffective normal hematopoiesis and increased apoptosis of hematopoietic precursors in the marrow. With disease progression, apoptosis lessens and proliferation of clonal progenitors becomes the predominant feature [5]. Mutational analysis of MDS patients demonstrates a propensity for complex subclones super-imposed on clones which possess driver mutations. These clones can persist after treatments and could affect the clinical course [6].

It has been appreciated for some time that disease emergence, manifestations, and progression in MDS are influenced by the marrow microenvironment and its inflammatory milieu [7–9]. Where in the MDS stem and progenitor cell hierarchy inflammatory modulation might have the greatest effect has not been worked out, since in these syndromes, certain clonal populations can encompass cells such as monocytes which themselves mediate immune responses. In this review we explore immunologic changes which have been reported in MDS in an attempt to understand how these influence disease progression and how they might be targeted for therapeutic benefit.

Since MDS occurs at a median age of 70 years, it is also important to understand how age related alterations in the marrow microenvironment might contribute to a changed milieu in which MDS could be promoted. Changes which occur with aging in the marrow micro-environment include increased adipogenesis, decreased bone formation, increased CCL5 (CC-chemokine ligand 5), and increased CXCL12 (C-X-C chemokine ligand 12) (reviewed in [10]). How these environmental changes impact changes in the aging hematopoietic stem cell are also incompletely understood. Such changes involve impaired lymphopoiesis (myeloid skewing), increased senescence markers, and increased susceptibility to pyroptosis [11]. The increased incidence of clonal hematopoiesis in aging also relates to pro-inflammatory effects in marrow and to the increase in risk of myocardial infarction and stroke due to effects of clonally derived immune mediators on vascular endothelium [12].

It is unknown whether MDS is the result of stochastic mutations in hematopoietic stem and progenitor cells (HSPCs), influences from the microenvironmental aging process on HSPCs, other inflammatory environmental influences, or a combination of pressures. In any case, the MDS HSPC gains a survival advantage over normal counterparts. It is this survival advantage that leads with time to suppression of normal hematopoiesis with consequent risk for infections, anemia, and bleeding as well as to increased proliferation of the MDS clone with propensity for transformation to an acute leukemia. These processes represent the causes of demise in the MDS patient, so understanding the influences of an inflammatory marrow environment on this process has potential clinical significance.

1.1. The Link of MDS with autoimmune disorders

One compelling observation suggesting a link between MDS and immune dysfunction is the frequency at which MDS and autoimmune diseases coexist. Epidemiologic studies of various sizes have suggested a 10–30% incidence of autoimmune disorders co-occurring with MDS. Most commonly, these diseases are vasculitis, seronegative polyarthritis, and skin diseases such as Sweet's Syndrome [13]. As one study across 26 French Medical Centers showed, the diagnosis of these autoimmune diseases occurs with near-equal frequency before (37%), with (31%), and after (32%) the diagnosis of MDS [14]. Moreover, patients already diagnosed with autoimmune disorders are more likely to develop MDS than the general population [13]. These findings suggest that therapies related to either disease are unlikely to be the cause of the co-occurrence of these diseases. Rather, there may be a common pathophysiologic mechanism leading to both. Some connections between the two diseases are clear. For example, the abnormal release of cytokines has been associated with both MDS and autoimmune disorders (See Table 1). Autoimmune diseases are often a result of dysfunctional T regulatory cells (Tregs), and these cells have been shown to be dysregulated in MDS as well. In high-risk MDS, for example, increased T reg cells suppress autoimmune activity of cytotoxic T cells against malignant clones and thereby favor disease progression [15]. Ongoing studies are looking at more specific molecular pathways that may lead to both diseases. The NLRP3 inflammasome, discussed in detail below, has recently gained attention as a targetable anomaly in MDS. Interestingly two variants of the NLRP3 pathway genes, V200 M and Q750K, are known to be associated with autoimmune disorders [16]. One study, albeit small, showed that these variants were found in higher frequencies in MDS patients with autoimmune disease than in the general population [16].

Grignano et al. [17] divided the immunologic disorders associated with MDS into systemic vasculitis, connective tissue disorders, auto-immune phenomena, immune mediated hematologic abnormalities, and asymptomatic serological abnormalities. Noninfectious fever with systemic symptoms can occur also. Some co-occurring autoimmune diseases may respond to 5-azacytidine [18]. Whether the concurrence of MDS with an autoimmune process has any ill effects on outcome of MDS therapy is uncertain [18]. Infection is often higher in these patients with associated inflammatory conditions, often limiting use of cytotoxic therapies. It is uncertain if there is a common genetic pre-disposition or rather a chronic inflammatory state which alters marrow stem and progenitor cells leading to mutation acquisition and concurrent propensity for an autoimmune disease. While there is more work to be done in both elucidating the pathways leading to MDS and the connection

with auto-immune disorders, current work suggests that there may be a common source in a dysregulated immune system.

1.2. Immune system aberrations in MDS

1.2.1. Inflammatory cytokines and growth factors—Many studies have noted abnormal levels of cytokines, chemokines, and growth factors in MDS. Of the cytokines that are noted to be altered amongst various studies, tumor necrosis factor- α (TNF α), interferon γ (IFN γ), transforming growth factor β (TGF β), interleukin-6 (IL-6), and IL-8 are the most consistently noted to be over-expressed [19]. Table 1 lists some of the cytokines overexpressed in MDS. While the exact role of these cytokines in MDS is still under study, the increase in expression is enough to suggest that there is dysregulated immune signaling. One possible link between these particular cytokines and MDS is the activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B) pathway. The NF κ B pathway is important for HSPC proliferation and differentiation, and it is postulated that the deregulation of this pathway may lead to the HSC abnormalities seen in MDS [20]. It is also possible that the cytokine changes observed in MDS may be related to dysregulation of this pathway. Beyond the change in cytokines, findings of overexpressed myeloid and other growth factors also reflect the dysregulated cell differentiation seen in MDS [20].

At least one group has attempted to use the cytokine profiles in MDS to gauge prognosis [19,21]. Some studies have shown that TNF α is a poor prognostic indicator in high-risk MDS, however overall, cytokine profiles have not yet been useful for determining prognosis. This is largely due to the heterogeneous nature of MDS and the varying qualities of MDS subtypes which lead to inconsistencies amongst studies. For example, it is reasonable to see a different cytokine profile in low-risk MDS where there is a high apoptotic index than in high-risk MDS where there are more blast cells, clonal expansion and evasion from self-immunity. Indeed, in low-risk MDS, higher levels of TNF α , IFN γ , and IL6 are often observed as these cytokines are closely linked with apoptosis. In contrast, IL10, an immunosuppressive cytokine, is more likely to be elevated in high-risk MDS [8].

1.2.2. Toll-like Receptors (TLRs)—The innate immune response is activated by interaction of PAMPs (pathogen-associated molecular patterns) with pattern-recognition receptors (PRRs). Toll-like receptors are PRRs that are an integral part of this system. They mediate multiple cellular processes including immune regulation and cell differentiation. There are 10 total toll-like receptors in humans. All except for TLR3 require the adaptor protein MYD88 for signal transduction [25]. Signaling via toll-like receptors eventually leads to the activation of NF-KB, activation of protein kinases such as mitogen-activated protein kinases (MAPKs), and induction of inflammatory cytokines. Recent studies have proposed that TLR signaling may be involved in the development of MDS and the resulting alterations in cytokines therein [7].

TLR signaling has been shown to be directly related to hematopoietic stem cell development [26]. As such, it is reasonable that alterations in TLR signaling can lead to HSC dysfunction and the disruption of normal hematopoiesis. Indeed, studies have found many of the TLRs to be upregulated in MDS. Of these, the upregulation of TLR2 and TLR4 appear to be the most

consistent findings. TLR2 specifically has been shown to be higher in low-risk MDS as compared to high-risk MDS [26]. Not only have TLR2 and TLR4 been shown to be upregulated in MDS, it has been shown that they are able to induce cytokine secretion leading to a positive feedback loop that allows that signaling to self-maintain. For example, TLR4 has been linked with elevated TNF α , which further leads to TLR4 activation [27]. Similarly, TLR2 is linked with NF κ B activity [21]. Consistent with the upregulation of TLR4 in MDS, many of the downstream signaling mediators of the pathway have been shown to be abnormally elevated as well [21,28]. For example, the adaptor protein MYD88 is upregulated in CD34+ cells [29]. 5q deletion is the most common cytogenetic abnormality that occurs in MDS. In 5q deletion, the loss of micro RNA 145 (miRNA 145) and miRNA 146a leads to overexpressed TRAF6 (TNF-receptor-associated factor 6), which again, is an intermediate in the TLR to NF κ B pathway [30]. IRAKI (interleukin-1 receptor-associated kinase 1), further upstream from TRAF6 is also overexpressed in MDS [31].

Multiple studies have shown that TLR2 and TLR4 and their associated pathway intermediates are upregulated in MDS. The question then remains – what causes TLR4 upregulation? The answer may lie in a cellular process called pyroptosis.

1.2.3. The role of pyroptosis vs. apoptosis in MDS—Previous work has attributed the cytopenias in MDS to increased apoptosis. Apoptosis was thought to be induced by the interaction of Fas and Fas ligand (FasL) (CD95) [32]. CD34+ cells express Fas after interaction with TNF α . Studies have shown that TNF α , Fas and FasL are overexpressed in MDS and furthermore that this expression is correlated with apoptosis [21]. Apoptosis via Fas and FasL interaction is mediated by Caspase 8 and Caspase 3. However, more recent work suggests that apoptosis does not fully explain the cell death seen in MDS. For example, when healthy subjects are compared to MDS patients based on 93 chosen apoptotic genes, there is no difference in expression noted [33]. Furthermore, Caspase 3 levels, associated with apoptosis, are normal in MDS. In contrast, caspase 1 is elevated [34]. Caspase 1 is involved in an inflammatory mediated cell death pathway called pyroptosis.

Pyroptosis is a caspase 1 mediated process that occurs via formation and activation of an inflammasome. An inflammasome is a protein complex with a nucleotide binding domain with a leucine-rich repeat pattern. The most well characterized inflammasome associated with MDS is the NLRP3 (nucleotide-binding domain, leucine-rich repeat family pyrin domain containing 3) inflammasome [34]. Interestingly, NLRP3 has also been linked with various autoimmune disorders [35]. NLRP3 is activated by reactive oxygen species (ROS).

ROS may be generated by a few different pathways. In MDS it is thought to be generated from either somatic mutations or the danger-associated molecular proteins (DAMPs) such as S100A8 or S100A9 [36]. DAMPs or alarmins are molecules released by injured cells during pyroptosis which affect the innate immune system via TLR signaling. They serve as danger signals, and when activated, they promote and propagate an inflammatory response. S100A9 homodimers and S100A8 and S100A9 heterodimers function via TLR4 which is also known to be overexpressed in MDS [34]. Both the TLR4 signaling and somatic gene mutations lead to the production of ROS by activation of NADPH oxidase-1 (Nox1) [34]. Driven by ROS, NLRP3 recruits the apoptosis-associated speck-like protein (ASC) and induces ASC

polymerization. This leads to caspase 1 recruitment, cleavage, and activation. Caspase 1 activation then leads to many downstream effects including activation of the cytokines IL-1 β and IL-18 as well as cellular pore formation. Pore formation leads to an influx of cations, cell swelling, and eventually the lysis that is characteristic of MDS [26]. This entire process leads to the production of further DAMPs and ROS, feeding back into the pathway. Knockdown of NLRP3 or caspase-1, neutralization of S100A9 by a high-affinity chimeric decoy receptor, or inhibition of NLRP3 or Nox1 pharmacologically has been shown to suppress pyroptosis and to have ability to improve hematopoiesis in MDS [34].

The ROS generated by the above pathways also feeds into the Wnt/ β -catenin pathways which are known to promote stem cell proliferation [34]. Thus, the same pathway that leads to increased pyroptosis and cell death can also help explain the MDS clonal proliferation. It is hypothesized that this proliferated clone propagates the inflammatory environment leading to further pyroptosis. Fig. 1 illustrates some of the main components of the inflammasome pathway which may be activated in MDS.

1.2.4. Mutation effects on inflammation in MDS—Given its heterogeneity, no consistent genetic profile has been linked with MDS nor with an inflammatory state in MDS. Various individual somatic mutations have been shown to produce ROS and activate the NLRP3 inflammatory pathway to pyroptosis as noted above. The most common chromosomal abnormality in MDS is the 5q deletion. 5q deletion causes the haploinsufficiency of many genes including important regulators of inflammatory signaling like the ribosomal proteins miRNA 145 and miRNA 146a [30]. As previously described, loss of miRNA 145 and 146a leads to overexpression of TRAF6, an intermediate in the pathway from TLR4 to NF κ B. NF κ B is a contributor to the inflammasome NLRP3 formation. One group did a large genome-wide study with more than four thousand participants focusing on regions linked with immune-mediated diseases to look for single nucleotide polymorphisms (SNPs). They were able to identify one variant that passed the genome-wide significance threshold and another just below. These were variants on the *RTEL1* and *MTHFR* genes, respectively [37]. Both genes are involved in the maintenance of genomic stability. The *RTEL1* gene also plays a role in telomere-length regulation. Together, they suggest a scenario of increased genomic instability in MDS. Other common mutations found in MDS involve the *TET2*, *DNMT3a*, *RUNX1* and *TP53* genes [26]. These mutations have been studied for prognostic implications, but no clear relationship to an inflammatory microenvironment in MDS has been determined. *TET2* has been associated with damping of inflammation and suppression of IL-6 effects [38]. *TET2* has also been found to restrain inflammatory changes in macrophages as related to IL-1 and IL-6, so loss of function mutations in *TET2* might be expected to contribute to an inflammatory environment with suppression of normal hematopoiesis [39,40]. Spliceosome mutations may activate ROS/ β -catenin pathways and activate inflammasomes. [34,41]. This could be overcome by *N*-acetyl cysteine. Mutations of *ASXL1* can also result in activation of inflammasomes, and *DNMT3a* mutations have been linked to alterations in IFN- α/β levels which might also have an influence on the inflammatory environment [42].

2. Cellular inflammatory mediators in MDS

2.1. Stromal cell contribution to inflammatory states in MDS

The marrow stroma is a complex array of cellular, humoral, and matrix components. Cellular composition of marrow stroma classically encompasses mesenchymal stromal cells (MSCs), endothelial cells, osteoblasts, osteoclasts, macrophages, and adipocytes. The contribution of these cells to both normal and malignant hematopoiesis is incompletely understood, and their contributions in MDS are also incompletely studied both in terms of hematopoietic support and immune modulation.

2.2. Mesenchymal stromal cells (MSCs)

MSCs are multipotent cells capable of differentiation into various cell lineages including osteoblasts, adipocytes, fibroblasts, chondrocytes, and myoblasts. MSC differentiation and fate decision are regulated by RUNX2 (Runt-related transcription factor-2) which influences osteoblast and chondrocyte differentiation, and peroxisome proliferator-activated receptor γ 2 (PPAR γ) as a regulator of adipogenesis [43]. During aging or osteoporosis, osteoblast numbers decline, and there is an increase in Wnt inhibitors such as sclerostin and dickkopf-1 with a reduction in bone mass [44]. Since MDS has predominance in older individuals, it is possible that some of these microenvironmental changes could influence the emergence and progression of MDS clones. MSCs are not part of the MDS clonal process. MSCs from MDS patients often lack chromosome alterations in favorable risk cases, but in high risk cases, they often express aberrations which are distinct from the MDS HSPC mutations [45]. Because MSCs secrete cytokines and exosomes, participate in lodgment and adhesion of stem cells, and have immunomodulatory properties, they are an important component of the inflammatory milieu in MDS.

2.2.1. Involvement of MSCs in MDS/AML pathogenesis—There are mouse models which suggest the direct involvement of MSCs in the evolution of MDS and leukemia. These include: 1) An activating mutation in *CTNGB1* (encodes beta-catenin) in murine osteoblasts can promote clonal myeloid disease [46]. In this model, increased expression of Jagged 1, a Notch ligand, was found. 2) Decreased expression of *Sbds* (encodes a ribosome maturation protein mutated in Schwachman-Bodian-Diamond syndrome) in immature murine osteoprogenitors results in an MDS phenotype [47]. This involves the genetic disruption of DICER, an RNase III endonuclease involved in microRNA biogenesis and RNA processing. Gene expression analysis of DICER mutated osteoblasts showed down regulation of the *Sbds* gene. This MDS mutation results in mitochondrial dysfunction, oxidative stress, and activation of DNA damage responses. S100A8/9-toll-like receptor (TLR) signaling was found to be the mediator of these changes, thus linking this phenotype to an aberrant inflammatory state in MDS [48]. 3) Activating mutations in protein tyrosine phosphatase (*Ptpn11*) also known as *Shp2* in murine MSCs and osteoprogenitors can lead to a JMML (juvenile myelomonocytic leukemia) phenotype. This seems related to increased production of CCL3 (C–C motif chemokine)/MIP-1 α (macrophage inflammatory protein-1 α), which recruits monocytes that produce cytokines such as IL-1 β which can contribute to an inflammatory milieu favoring HSPC activation [49]. This mutation effect, like that of

DICER, was not seen when it involved mature osteoblasts or endothelial cells. CCL3 receptor antagonists were able to prevent the myeloproliferative process.

2.2.2. MSC alterations in human MDS—In terms of morphology as well as expression of surface markers and adhesion receptors, many groups have found no differences between MSCs from MDS versus normal subjects. MSCs from MDS secreted more IL-1 β and more stem cell factor (c-kit ligand) in response to TNF- α as compared with those from normal subjects [50]. Other groups have found abnormal CD90, CD104, and CD105 expression on MDS-MSCs [51]. In a systematic look at MSCs derived from 106 patients with newly diagnosed MDS, Geyh et al. [52] found reduced growth capacity as compared with MSCs from age matched controls. Premature senescence was noted as measured by β -galactosidase staining. They demonstrated reduced osteogenic differentiation capacity which was confirmed by reduction in osterix and osteocalcin expression. Analysis of methylation patterns revealed consistent differences between MDS MSCs and MSCs from healthy controls. Altered expression of osteopontin, Jagged 1, c-kit-ligand and angiopoietin were noted, and there was a diminished ability to support CD34+ cells in long-term culture assays [52]. Others have found premature exhaustion, decreased osteogenic differentiation ability (associated with Osterix and Runx2 downregulation) and slower passage rates [53]. Aurora kinases A and B have also been found upregulated in MDS MSCs [54,55] but contribution to MDS support or inflammatory milieu has not been completely studied.

MSCs do respond to signals from other immune cells of the environment which are both soluble and contact-mediated which allow them to assume their immunomodulatory properties (reviewed in [56]). There are also environmental signals such as TLR4 which can result in MSCs demonstrating a pro-inflammatory phenotype which results in the ability to release pro-inflammatory cytokines, recruit other immune cells, and activate B and T lymphocytes [57,58]. TLR3 priming may in contrast lead MSCs to an anti-inflammatory state with upregulation of IL-4 and indoleamine 2,3-dioxygenase (IDO) [59]. Human MSCs use IDO to mediate immune suppression. IDO induces Tregs and inhibits activation of pro-inflammatory monocytes and macrophages. In high grade MDS, the microenvironment is thought to switch the phenotype of MSCs to a tolerogenic phenotype which then allows unfettered tumor growth [60]. Lower expression of co-stimulatory molecules such CD40, CD80, and CD86 on MSCs in MDS and higher levels of TGF β and hepatocyte growth factor contribute to the immunomodulation properties of MSCs in high risk MDS [60]. Whether the immunosuppressive nature of MSCs changes with transition from low to high risk MDS is not completely understood, but high-risk MDS is associated with increased T reg numbers and evidence for enhanced immune suppression during disease progression. The Tregs also secrete IDO and inhibit dendritic cell (DC) maturation from monocytes and reduce expression of T-cell co-stimulatory molecules.

Stromal cells may also be subject to alterations in miRNA expression in the clonal population. For example, in del 5q MDS cases, allelic deletion of miRNA-145 and 146a results in repression of TIRAP (Toll/interleukin-1receptor domain-containing adaptor protein), an adaptor molecule associated with TLRs and TRAF6 (discussed in Sections 1.2.2 and 1.2.4 above). This has potential effects on marrow stromal cells [61].

2.2.3. Changes in MSCs with disease progression—In early stage MDS, there is evidence for existence of a pro-inflammatory state. This state recruits MSCs and other stromal and microenvironment cells and causes them to eventually assume a role in creation of an immune suppressive state that then leads to tumor promotion (reviewed in [56]). The types of cells recruited to accomplish this state of tolerance toward the malignancy include CD4+ and CD8+ T cells, $\gamma\delta$ T cells, NKT cells, NK cells, dendritic cells, and B cells. Myeloid derived suppressor cells (MDSCs) may also be involved [62]. These effects of MSCs can be either contact or non-contact mediated. The soluble mediators include cytokines, miRNAs, and exosomes.

There are few studies which have examined how MSCs change in the course of MDS evolution from low risk to high risk MDS or to AML. CD271+ MSC density was increased in higher grade MDS compared with lower grade MDS and had predictive ability in regard to overall survival [63]. CD271 is the low-affinity nerve growth factor receptor. This population is thought to have direct contact with CD34+ cells. Pathways that involved RNA polymerases I and III and mitochondrial transcription have been found to be down-regulated in RAEB MSCs (high grade) compared to MSCs from refractory cytopenia with multilineage dysplasia (lower risk), although only a small number of cases were examined [64]. Zhao et al. have reported upon differences in the immunoregulatory capacity of MSCs from low and high risk MDS [65]. MSCs isolated from high risk MDS had increased TGF- β 1 secretion and induced T-cell apoptosis more than did those from low-risk cases, although MSCs from all MDS risk groups showed reduced T-cell apoptosis induction as compared with normal MSCs. Also, there was diminished ability to support normal hematopoiesis. The ability to induce Tregs was also divergent with Tregs defined as CD4+ CD25+ Foxp3+ and with high risk MDS MSCs having greater induction rates [65]. This was thought to be mediated by TGF- β 1 as well. Low risk in this study was defined as IPSS score [66] of 1. These results suggested that in high-risk MDS, MSCs may inhibit effective immune responses against the dysplastic clone by inducing Tregs and therefore contributing to disease progression.

2.2.4. Prospects of MDS MSC restoration to Normal—There is some evidence that after hypomethylation therapy, cell cycle and inflammatory changes in MDS MSCs upregulated after exposure to MDS HSPCs can be restored to normal [67]. Also, hypomethylation of MDS-MSCs resulted in better engraftment of normal stem and progenitor cells in immune suppressed murine models [68]. This may also have implications for the engraftment of normal HSPCs after stem cell transplantation and for relapse tendencies which might be fueled by an abnormal microenvironment. This is an area of controversy, however, as other groups have shown that abnormalities in the microenvironmental inflammatory state may persist even after stem cell transplantation. For example, recipient marrow resident macrophages are needed for long-term reconstitution of donor hematopoietic stem cells in marrow, and these could be damaged in MDS [69]. Residual microenvironmental abnormalities could lead to poor engraftment of normal HSPCs and could contribute to relapse of the MDS state.

2.3. Other niche cells potentially involved in the MDS inflammatory state

Niche accessory cells include endothelial cells, sympathetic neurons, adipocytes, osteoclasts, marrow macrophages, Tregs, and other immune cells present in marrow. Hematopoietic cells, both clonal and normal, such as monocytes and neutrophils are also contributors to the niche and the marrow inflammatory milieu.

2.3.1. Adipocytes—Adipocytes are an important component of marrow, and marrow adipose tissue increases with age [70]. Marrow adipocytes have been shown to interact with hematopoietic cells [71], and their contribution to hematopoietic function continues to be examined. They have autocrine, paracrine, and endocrine functions, so it is likely they influence MDS as well as normal hematopoiesis. Marrow adipocytes are distinct from white, brown and beige adipocytes and are therefore thought to arise from a distinct progenitor [70]. While the role of differentiated adipocytes in MDS has not been systematically studied, it has been shown that higher levels of serum fetuin-A and lower adiponectin and free leptin are associated with higher risk of MDS incidence, particularly amongst overweight individuals [72]. Since marrow adipocytes are probably unrelated to body mass or to adipocytes from other sites, how this contributes to a marrow inflammatory state is uncertain.

2.4. Contribution of hematopoietic cells from the MDS clone to inflammation

Mature cells that derive from the MDS progenitor cell(s) can produce pro-inflammatory chemokines and cytokines which could contribute to a microenvironment which favors aberrant vs normal hematopoiesis. They may redirect cell fate toward a myeloid lineage or could have effects on erythropoiesis through cytokine production.

2.4.1. Monocytes—Monocytes from patients with MDS are derived from clonal HSPCs and do not upregulate matrix metalloproteinase-9 [73] in marrow as do normal counterparts. This may contribute to decreased mobilization and to marrow hypercellularity sometimes noted in all MDS subtypes. Monocytes secrete increased TNF- α [5] in MDS, and they may become iron overloaded which may impair their function.

2.4.2. Monocyte—derived macrophages—In a systematic study of monocytes in MDS, when macrophages were induced from blood monocytes by granulocyte-macrophage colony stimulating factor exposure, they demonstrated impaired ability to form macrophages despite increased numbers of monocytes in the MDS cases as compared with normal controls. They had defective phagocytic function, and lower levels of CD206 (mannose receptor) and signal regulatory protein-alpha (SIRP- α) were expressed. Levels of induced nitric oxide synthase (iNOS) were increased [74], suggesting contribution to an inflammatory state. Also, in low-grade MDS, there may be decreased IL-10 secretion from monocytes, and this may lead to activation of the NLRP3 inflammasome and production of IL-1 β [75].

2.4.3. Dendritic cells—Dendritic cells (DCs) are antigen-presenting cells that are important in immune responsiveness. Dendritic cells may be conventional myeloid (monocyte—derived) or plasmacytoid types. Myeloid dendritic cells are part of the MDS

clone, and there is evidence that their impairment can hamper immune responses, especially in high grade MDS [15]. Others have shown that dendritic cells are reduced in marrow samples from untreated MDS patients [76]. Monocyte derived dendritic cells have been found to have reduced co-stimulatory molecule expression but they are able to stimulate autologous T-cells in MDS [77]. In low-risk MDS, apoptotic cells may be taken up by dendritic cells. Pro-inflammatory cytokines such as IL-6 and TNF- α will induce maturation of these DCs. This could induce T-cells to further suppress hematopoietic precursors and contribute to the increased apoptosis noted in low grade MDS cases [78].

2.4.4. Neutrophils—In MDS, blood neutrophils are often decreased, and impaired neutrophil function such as impaired migration and opsonization may exist [79]. In early phase MDS, neutrophils and other myeloid precursors undergo an increased rate of death which may contribute to the marrow inflammatory state.

2.5. Contribution of immune cells not part of the MDS clone

2.5.1. Resident macrophages—Within the marrow niche, macrophages interact with hematopoietic stem and progenitor cells to influence homeostasis and fate decisions. They are also involved in regulation of erythropoiesis. They also produce cytokines such as VEGF and basic fibroblast growth factor which result in increased vascularization in low risk MDS [80]. There are thought to be distinct macrophage populations in the marrow; erythroid island macrophages [81], osteal macrophages, and hematopoietic stem cell niche macrophages [69]. Some of these resident macrophages can survive radiation and chemotherapeutic agent exposure, and how their numbers are affected by an MDS clone and how they in turn might affect MDS hematopoiesis is not known. Because these macrophages are not derived from the MDS clone, they could contribute to malignant hematopoiesis through alterations in the marrow inflammatory state. Based on a reduction in IL-10 in some cases of low-risk MDS, macrophage mitophagy is inhibited, contributing to activation of the NLRP3 inflammasome and increased IL-1 β production [82].

2.5.2. Myeloid-derived suppressor cells (MDSCs)—MDSCs have been found to be increased in the marrow of MDS patients. These cells are Lin[−]/HLA-DR[−]/CD33⁺ and derive from a population distinct from the neoplastic clone. These cells overproduce cytokines that suppress hematopoiesis such as IL-10 and TGF- α as well as nitric oxide, and they enhance apoptosis of autologous hematopoietic progenitors [62]. Their expansion is driven by interaction of S100A9 with CD33, a hallmark of the MDSCs [83]. S100A9 transgenic mice have MDSC increases, and these mice develop dysplasia and pancytopenia. The phenotype can be rescued by inhibition of the immunoreceptor tyrosine-based activation motif-bearing (ITAM-bearing) adapter protein which interrupts the interaction of MDSCs with S100A9 [83]. These cells increase in aging and are thought to be involved in the age-dependent incidence of MDS. GM-CSF and IL-6 may stimulate generation of MDSCs, and both may be elevated in various MDS states. It is speculated that the inflammatory changes resulting from increased MDSCs may in fact induce DNA changes which result in development of MDS clones [83].

2.5.3. Other lymphoid cells—There is evidence in MDS that Tregs, NK/T cells, Th17 cells and cytotoxic T cells may be aberrant. As noted above, MSCs can exert a potent suppression on both innate and adaptive immunity by acting on NK cells, B lymphocytes, dendritic cells, and Tregs [59].

2.5.4. NK cells—NK cells are part of the innate immune system, and they participate in responses against viruses and other infectious pathogens as well as in tumor surveillance. NK cell function can be influenced by IL-2 and IL-15. Decreased killing activity of NK cells has been observed in high risk MDS, and in contrast, NK cells demonstrate cytotoxicity toward clonal precursors in low-risk MDS [84]. Their function may also be affected by enhanced IL-32 levels in MDS [85].

2.5.5. B lymphocytes—It is uncertain whether B-cells are at times involved in the MDS clone, but there is evidence that B-cell signaling may be deregulated in early MDS [86]. These pathways may be involved in some of the autoimmune disorders associated with MDS described above.

2.5.6. Cytotoxic T lymphocytes—Monoclonal and oligoclonal CD8+ T cell proliferation as well as enhanced CD8 negative T cell cytotoxicity has been observed in MDS [87]. This may contribute to elimination of normal hematopoietic progenitors [84,88]. Other studies have shown altered CD4/CD8 ratios in younger, lower risk patients who might respond to immune suppressive therapy. In some cases, CD4 cells may be decreased in lieu of an increase in cytotoxic CD8 cells [89].

2.5.7. Regulatory T cells (T regs)—High risk MDS is associated with high levels of Tregs as compared to low numbers in low-risk MDS [90]. Tregs are generally characterized as CD4+ CD25+ FoxP3+. T regs have immune suppressive functions, and low numbers of Tregs allow activation of T cell clones which would enhance progenitor cell apoptosis in low-risk MDS. Thus, while they can prevent immune activation that could foster autoimmunity, their presence also influences immune surveillance and anti-tumor response (reviewed in [56,90,91]).

2.5.8. Th17 cells—Higher expression of IL-17 and pro-inflammatory cytokines were observed in low-risk as compared to high-risk MDS patients. IL-17 can induce apoptosis [75] and Th17 cells act on macrophages and dendritic cells to produce IL-23, IL-1, IL-6 and TGF- β [92].

2.5.8.1. Effect of epigenetic changes in marrow stromal cells on the inflammatory state: Myelodysplastic syndromes have been treated with epigenetic modifiers for over 20 years [93]. Stromal cells are also subject to epigenetic changes, and there are changes in both methylation status and histone modification that occur with age. These cumulative changes may aid in creating an environment in which certain stem or progenitor cells might have a survival advantage [94]. As in all stem cell—microenvironmental interaction studies, it is difficult to discern causal direction, and it is also possible that somatic mutations in the MDS stem/progenitor cells promote epigenetic changes in the microenvironment which in turn promote survival and evolution of clonal processes [95].

2.6. Means to target inflammatory changes in MDS

2.6.1. Therapy of autoimmune diseases associated with MDS—There are few large series which have systematically examined treatment of inflammatory diseases accompanying MDS. 5-azacitidine may control paraneoplastic manifestations and may spare steroid usage [96]. Steroids can be effective for control of inflammatory states, but there is a high frequency of steroid dependence manifested by flares during taper of medication [97]. Cyclophosphamide, methotrexate, and azathioprine are thought to have restricted value due to concern about cytopenias and enhancing evolution to AML with prolonged exposures [98]. Rituximab has found some activity in systemic vasculitis, and TNF inhibitors have been utilized to treat arthritis manifestations. Tocilizumab, an IL-6 receptor antagonist and anakinra, an anti-IL-1 agent have been utilized but no large series results are available. Other than Rituximab, biologics have had overall poor efficacy with only 44% complete and partial responses in the autoimmune process in one series [97] with infection complications occurring commonly. Treatments approved for MDS such as hypomethylating agents and lenalidomide may treat associated inflammatory conditions by reducing the expression of neoplasia-related inflammatory mediators or they may have direct effects on the inflammatory conditions. Fraison et al., in a retrospective study, found that steroids could be reduced in 96% of cases with MDS and associated immunologic disease when treated with hypomethylating agents, and the rate of response of the MDS to azacitidine was comparable to that of controls [99].

2.7. Cyclosporine

Cyclosporine, a calcineurin inhibitor, suppresses CD4+ T-cells and as such could lead to increased cytotoxic lymphocyte function and suppression of TNF- α in MDS. It is usually used in conjunction with ATG so that all T-cell subsets are suppressed [100]. In low-risk MDS, when ATG is combined with cyclosporine, response rates up to 51% have been noted [101].

2.8. Hypomethylating agents as immune modulators

The two hypomethylating agents approved for use in MDS, azacitidine and decitabine, have direct cytotoxicity and hypomethylating effects. Methylation of the FOXP3 promoter enhances FOXP3 expression in vitro and in vivo [102], which may be beneficial in low-risk MDS where Tregs are suppressed. This could however result in a detrimental effect in high-risk MDS. Upregulation of KIRs (killer cell immunoglobulin like receptors) on NK cells by azacitidine leads to reduced cytotoxicity which may also be of benefit in low-risk disease [103]. Azacitidine treatment also has inhibitory effects on CD4+ T-cell mediated immune responses which may be detrimental in high-risk disease but beneficial in low-risk disease where it may actually alleviate coexisting immune symptoms. HDAC (histone deacetylase) inhibitors may inhibit dendritic cells and T-cell function and may increase FOXP3 expression such that they could have inhibitory effects on the activated immune state in low-risk disease (reviewed in [78]). Many HDAC inhibitors have been examined in MDS but none are approved for use in this indication.

2.9. Lenalidomide

Lenalidomide is an iMiD (immunomodulatory imide drug) approved for treatment of del(5q) MDS. It causes upregulation of RPS14 gene expression and can normalize erythropoiesis. It inhibits angiogenesis and modulates NK cells, monocytes, and dendritic cells, and inhibits Treg cells. It reduces TNF- α , IL-6, and IL-1 β levels [104]. The iMiDs therefore have both pro- and anti-inflammatory effects [105].

2.10. Anti-TNF and anti-apoptotic therapy

Apoptosis of progenitor cells in low-risk disease is mediated through TNF- α , IL-1, IL-6, IL-12, and IFN- γ as well as through a host of other cytokines [8,56,78]. Anti-cytokine therapy or anti-apoptosis agents might therefore have benefit in low-risk MDS. With TNF- α inhibition, there may be activation of cellular immunity and differentiation of CD4⁺ T cells into Tregs. This may have a favorable effect on emergence of normal erythropoiesis. Monoclonal antibodies targeting TNF- α , soluble TNF- α receptors, and chemical inhibitors of TNF such as thalidomide are available means to suppress TNF. Both Infliximab (chimeric monoclonal antibody) [106] and etanercept (soluble TNF- α receptor linked to IgG1Fc) have been utilized in low-risk MDS [107]. These have resulted in low response rates. Stirewalt [108] found that TNF increased expression of IL-6, IL-8 and IL-32 in stroma 1 lines, and it decreased bcl-2 and increased bid expression; thus favoring a pro-apoptotic state. Inhibiting TNF would therefore be anticipated to result in an anti-apoptotic state with greatest effects in low grade MDS with possible improvement in normal hematopoiesis. Pro-apoptotic agents such as venetoclax, a BCL-2 (B-cell lymphoma) inhibitor are being examined in high-grade MDS where there is sometimes anti-apoptotic resistance due to overexpression of BCL-2 [109].

2.11. Other cytokine approaches for potential immune modulation

Erythropoietin—Erythropoietin can improve hemoglobin levels and improve transfusion needs in low risk MDS. There is evidence that S100A9 and TNF- α may suppress erythropoietin effects in MDS, and preincubation with lenalidomide can diminish this suppression [110]. There have been trials of lenalidomide compared with lenalidomide plus an erythroid stimulating agent which have shown greater erythroid responses in the combination arm (discussed in [26] and published only in abstract form).

2.12. Cellular therapies

Treg infusions, NK cell therapy combined with other biologics, and dendritic cell vaccines have all been proposed in MDS. Autologous vaccination with DCs generated from their precursors is an attractive approach for presenting antigens which might elicit an anti-MDS immune response [15]. It is possible, however, that immature monocyte-derived dendritic cells from MDS patients may be dysfunctional themselves as they arise from the MDS clone.

2.13. Anti-CTLA-4 antibodies or antibodies to checkpoint inhibitors

Programmed cell death protein 1 (PD-1) is an inhibitor expressed by T lymphocytes which functions as an immune checkpoint by inducing the apoptosis of antigen specific T

lymphocytes while inhibiting the apoptosis of regulatory T lymphocytes. The PD-1/PD-L1 inhibitors might be anticipated to have benefit in high-risk MDS where there is evidence for immune suppression. Nivolumab has been combined with azacitidine in the therapy of MDS. Several other trials are ongoing with these agents. These include nivolumab with ipilimumab, oral cyclophosphamide, decitabine, or azacitidine [109,111]. Other PD-1 or PD-L1 inhibitors are also being explored in MDS [109].

2.14. Kinase inhibitors

Rigosertib, a multi-kinase inhibitor with polo-1-like kinase inhibitory function has shown some activity in MDS [112]. TLRs can activate MAP kinase activity in MDS, and given that MAP kinase activity has been found to activate apoptosis in low grade MDS, inhibition of MAP kinase might have potential to improve growth of normal progenitors in this state. This has been examined in the form of ARRY-614 [113] which also inhibits Tie2 kinase. In a phase I study in low or intermediate-1 MDS, the response rate was 32%, and the drug was well-tolerated [113].

2.15. Role of iron overload and chelation

MDS patients often have iron overload due to red cell transfusion needs and ineffective erythropoiesis which leads to iron release and oxidative stress (reviewed in [114]) with effects on inflammatory states. Iron can inhibit osteoblast formation [115] and would be expected to affect macrophage phagocytosis activity. Hepcidin levels are quite variable in MDS [116,117] and there is evidence that iron chelation can lead to hematologic improvement in some patients [118].

2.16. Role of inflammasome inhibition

Use of inflammasome inhibitors has not been reported in clinical trials in MDS. Multiple agents have been reported to target components of the inflammasome. These include but are not limited to glyburide [119], methylene blue [120], nucleoside reverse transcriptase inhibitors [121], parthenolide which alkylates caspase-1 [122], and Bay11-7082 which alkylates NLRP3 [122]. Inhibitors of NF κ B may also inhibit the NLRP3 inflammasome. Proteasome and neddylation inhibitors which inhibit NF κ B have been examined in MDS [123]. Other proposed inhibitors include MCC950 which more specifically targets NLRP3 [124], isoliquiritigenin which also inhibits NLRP3 [125], and CRID3, the cytokine release inhibitor drug which disrupts IL-1 β secretion [126]. The cyclopentenone prostaglandin 15d-PGJ2 also inhibits the NLRP1 and NLRP3 inflammasomes [127]. Bruton's tyrosine kinase (BTK) inhibitors can also interfere with NLRP3 activation [128]. Abnormal TLR signaling in MDS leads to hyperactivation of IRAK1. IRAK1 can be inhibited by small molecules [129] which could also have potential effect in low-grade MDS. As noted, most of these agents have not been studied in MDS either alone or in combination [8].

2.17. Toll-like receptor inhibitors

A humanized IgG4 monoclonal antibody against TLR2 (OPN-305) has been examined in healthy subjects in a phase 1 design, and this was well-tolerated. Full TLR2 receptor

blockade was noted on monocytes, and IL-6 release was inhibited [130]. No studies have been completed in MDS as of yet.

2.18. Other Broader Considerations Regarding the Inflammatory Milieu of MDS

2.18.1. Role of the organism's macroenvironment—While inflammation in the marrow microenvironment may have impact on MDS, it is also possible that an organism-wide inflammatory state could have impact on MDS emergence. Recent interest in the intestinal microbiome [131] raises the possibility that interaction between bacteria and circulating hematopoietic cells (monocytes, neutrophils, and others) could enhance pro-inflammatory tendencies. This could further have impact for intramedullary hematopoiesis and influence myeloid stem and progenitor cells. Dietary effects on inflammation would be anticipated to be widespread and could impact the inflammatory state of the marrow microenvironment. Environmental chemicals such as tetrabromobisphenol-A can lead to reduced Thy1 (CD90) expression with resultant increase in adipogenesis. Since Thy-1 promotes bone formation and decreased adipogenic potential of marrow MSCs [44,132] it is conceivable that such alterations could create a pro-inflammatory setting in the marrow microenvironment that is conducive to MDS outgrowth. This has not been examined extensively in the human marrow microenvironment.

2.18.2. Parallels to aging—In aging, MSCs become less regenerative and may undergo senescence, which promotes inflammation and cancer progression [133]. In murine models, aged hematopoietic stem cells drive aging-associated immune remodeling [134]. This may contribute to skewing of stem cell differentiation toward a myeloid pathway with implications for adaptive and innate immunity. Senescent MSCs can regulate inflammatory responses of macrophages and inhibit lymphocytes, but they have a reduced migratory capacity in response to pro-inflammatory signals [135]. These signals include leptin, TNF- α , eotaxin, interferon- γ , vascular cell adhesion molecule-1 (VCAM-1), IFN- β , IL-4, and monocyte chemotactic protein (MCP-1). This may lead to reactive oxidative stress, NF κ B activation, and a positive feedback loop that promotes inflammation and possibly malignancy in the aged mammal [71,136,137]. Just as MDS HSPCs may be subject to pyroptosis, HSPCs from elderly humans have similar tendencies with overexpression of the purinergic receptor P2X7 which activates and cleaves caspase-1 [11]. P2X7 can be inhibited by PPAD (pyridoxal-phosphate-6-azophenyl-2',4'-disulfonate), effectively inhibiting pyroptosis of CD34+ cells [11].

In humans, MDSCs expand with age, and levels of inflammatory cytokines such as TNF- α , IL-6, and IL-1 β also increase. These cytokines are likely activated and expand before MDS emergence. This state may also contribute to a compensatory increase in myeloid progenitor proliferation and the eventual myeloid skewing that occurs with age. Therapeutic interventions that would promote MDSC maturation might have a remitting potential when applied early in the course of MDS. Anti-CD33 antibodies have been proposed as a potential way to accomplish this [138].

3. Summary

MDS is a heterogeneous disease with World Health Organization classification [4] and International Prognostic Scoring System (IPSS) prognostication spanning low to high risk categories with median survival for these groups ranging from 0.4 to 5.7 years [139]. Immunologic aberrations span the spectrum of MDS subtypes. In low-risk MDS, there are increased levels of pro-apoptotic cytokines, low levels of regulatory T-cells, increased Th17 helper T-cells, and T cell mediated inhibition of hematopoiesis. Later in the course of the disease, the hematopoietic clone appears to escape immune surveillance manifested by increased Tregs, low apoptotic rates, and selective growth advantage to the malignant clone which can eventually result in transformation to AML.

Multiple components of the marrow environment and of the MDS cell hierarchy itself can contribute to an inflammatory state which can influence MDS emergence, propagation, and evolution. This state is manifested by enrichment of the NLRP3 inflammasome in concurrent MDS and autoimmune disorders [16], the ability of the NLRP3 inflammasome to drive clonal expansion and pyroptosis independent of genotype [34], and increased blood levels of S100A8 and S100A9 in MDS [36]. Fig. 2 summarizes some of the main inflammatory pathways involved in MDS.

4. Future considerations

Attempts to modulate the marrow microenvironment to suppress inflammation and thus eliminate any advantages conferred to the clonal MDS population may be worthwhile as this would have potential not only to suppress the MDS clone but also to enhance residual normal hematopoiesis. This will require identification of the important molecules and key pathway mediators in these processes as well as development of clinically effective targeting agents. Future studies will be needed to understand if inflammatory changes precede the presence of cell autonomous clonal mutation(s) or are triggered later by cells carrying clonal mutations. How these changes can be targeted for therapeutic benefit will require further extensive laboratory and clinical investigative efforts.

Early in MDS, inflammatory changes in the microenvironment might also contribute to genetic instability and mutational selection processes, so MDS progression could potentially be halted if this inflammatory milieu could be abrogated. It will be necessary to understand when immune modulation should be introduced since in low-grade MDS, inhibiting the inflammatory immune response may decrease apoptosis and pyroptosis and restore normal hematopoiesis. Whether these interventions by themselves would be sufficient to prevent progression or transformation is uncertain as higher risk disease may actually require immune stimulation to suppress the mutated clones(s). Future understanding of the change in microenvironmental components which occurs with disease progression and clonal evolution will therefore be important. It will also be important to understand how immune modulation might best be incorporated with epigenetic or cytotoxic therapies to target the mutated clone as these types of therapies may have concurrent effects on the marrow stroma and inflammatory milieu as well as on the MDS clonal population itself. Table 2 lists

examples of activated trials in MDS which are examining agents with potential effect on inflammatory components of the syndrome.

Practice points

- Immunologic or rheumatic diseases occur commonly in myelodysplastic syndromes.
- In the evaluation of certain immunologic conditions, it is important to observe for dysplasia either concurrently or later in the course of the disease. Diseases where this is important include eosinophilic fasciitis and relapsing polychondritis, amongst others.
- In some cases, elimination of the hematopoietic clone may allow the inflammatory microenvironmental state to revert to normal, but abnormalities in the microenvironment inflammatory milieu may persist for a time even after stem cell transplantation.
- It is important to bear in mind that therapies modulating the immune system may have very different effects in high risk vs. low risk MDS. In low-risk MDS, anti-PD-1 or anti-PD-L1 therapies could exacerbate associated autoimmune diseases. Whether this could be attenuated by concurrent use of hypomethylating agents will need to be examined.
- Therapies used in the treatment of myelodysplastic syndromes to date (lenalidomide and hypomethylating agents) have potential to alter not only the MDS clone but also the marrow microenvironment.

Research agenda

- Much that has been learned about immune modulation in MDS has come from murine and in vitro human studies. Studies directed to human marrow need to be conducted to understand which cells of the marrow environment facilitate the development of niche changes and subsequent nurture of the MDS clone(s).
- Since TLR-mediated pro-inflammatory signaling and constitutive NF κ B signaling may contribute to clonal propagation in MDS, understanding how their inhibition might improve the balance of normal and aberrant hematopoiesis will be of importance [30,115,140].
- Understanding how other environmental components influence cytokines and the immunologic milieu will also be required. For example, extracellular galectins are known to alter cytokine presentation in hematologic disorders and other malignancies. How they are altered in MDS and how they might influence the immunologic milieu is not well understood [141].
- Better understanding of how age-related inflammatory micro-environmental changes relate to MDS initiation is required. [142]

- Studies directed to how or whether the microenvironmental changes in MDS contribute to progression to higher grades or to AML are needed.
- Better preclinical modeling of MDS will aid in understanding the role of inflammation and of the microenvironment as a whole as related to its pathogenesis, maintenance, and evolution. [143]
- Clinical trials will be required to understand how inflammasome inhibitors, TLR inhibitors, NF κ B inhibitors, and other anti-inflammatory agents can be combined with existing and emerging MDS therapies to optimize patient outcomes [144].
- Development of biomarkers such as one described for ASC specks to measure inflammation and pyroptosis in MDS [145] or to measure levels of S100A8 and S100A9 [36] might have diagnostic or prognostic utility in clinical settings.

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Fig. 1.

A Closer Look at Pyroptosis. Cell lysis as well as abnormal proliferation in MDS can be attributed to pyroptosis. Pyroptosis, as described in Section 1.2.3, occurs with NLRP3 inflammasome activation. It has been shown that ROS generated either from mutations or DAMP signaling begins NLRP3 activation. The NFκB pathway is crucial for the production of inflammasome component assembly. Concurrently, ROS also activates the wnt/β-catenin pathway leading to abnormal proliferation. Abbreviations are found in the text. CASP1 = caspase 1.

Fig. 2.

Broad Pathways to Cell Proliferation and Destruction in MDS. Though the specific pathways and intermediates are still being studied, this image shows the generalized understanding of the pathways leading to MDS. Many of the changes can be explained by well-documented changes seen in the cytokine environment. Changes in the inflammatory environment mediated by cytokines lead to changes in the bone marrow microenvironment, increased Myeloid-Derived Suppressor Cells, as well as pyroptosis. Each of these pathways lead to the phenotypic changes characteristic of MDS and also further contribute to an abnormal cytokine environment.

Table 1

Important Cytokines Overexpressed in MDS (based on reference 19).

Cytokine	Change in levels	Cell(s) of origin	Connection to MDS
TNF α	High	macrophages, monocytes, endothelial cells, neutrophils, smooth muscle cells, activated lymphocytes, astrocytes, and adipocytes [22]	Involved in NF κ B pathway which may mediate apoptosis in MDS Some evidence to suggest it may be a prognostic indicator in high-risk MDS
IFN γ	High	T cells, NK cells [23]	Known to have immunomodulatory properties Also modulates NF κ B activity, which may mediate apoptosis in MDS
TGF β	High	Many but not all parenchymal cell types, lymphocytes, monocytes/macrophages, platelets [24]	Modulates cell proliferation and differentiation. Can induce apoptosis.
IL6	High	T cells, B cells, macrophages [22]	Pro-inflammatory cytokine Involved in NF κ B pathway which may mediate apoptosis in MDS
IL8	High	T cells, Macrophages [22]	Important for chemotaxis and phagocytosis Involved in NF κ B pathway which may mediate apoptosis in MDS

Of the many cytokine abnormalities seen in MDS, the most consistent findings have been the increase in the ones listed above. Most of these cytokines have a significant role in the NF κ B pathway, which may mediate apoptosis in MDS and can contribute to inflammasome assembly.

Examples of Currently Accruing or Activated Trials in MDS Incorporating Immunologic Mediators Extracted from www.clinicaltrials.gov; last accessed 2/3/19.

NCT number	Study title	Diseases covered	Study intervention	Comments/references
	A phase I study of AMV564 in patients with intermediate or high-risk MDS	MDS	AMV564	CD33XCD33 Bispecific antibody; could inhibit MDSCs [146]
	Follow up protocol to the phase I/II study of OPN-305 in second-line and first-line lower risk myelodysplastic syndrome	MDS	OPN-305	Toll-like receptor 2 (TLR-2) antibody [130]
	Nivolumab with chemotherapy in refractory MDS	MDS	Nivolumab, azacitidine, fludarabine	Anti-PD-1 agent combined with HMA and chemotherapeutic agent
	Azacitidine and pembrolizumab for patients with MDS	MDS	Azacitidine/pembrolizumab	Pembrolizumab targets PD-1
	A study of azacitidine in myelodysplastic syndrome (MDS) associated to systemic autoimmune and inflammatory disorders	MDS	Azacitidine	Will address effects of azacitidine on concurrent MDS and systemic inflammatory diseases in terms of overall response
	Infliximab in treating patients with myelodysplastic syndrome	MDS	2 doses of infliximab to be compared	Open in low to intermediate risk disease only
	Ibrutinib and Azacitidine for treatment of Higher Risk MDS	MDS	Phase Ib with dose escalation of ibrutinib	Allows prior therapy; [128]
	Ipilimumab and decitabine in treating patients with relapsed or refractory myelodysplastic syndrome or acute myeloid leukemia	MDS AML	Ipilimumab Decitabine	Prior HMA therapy allowed
	Deferasirox in treating patients with very low, low, or intermediate-risk red blood cell transfusion dependent anemia or myelodysplastic syndrome	MDS	Deferasirox	Appropriately open in lower grade MDS where iron overload would be expected to have the greatest impact
	A phase I study of linituzumab combined with lenalidomide in patients with myelodysplastic syndromes (MDS)	MDS	Linituzumab Lenalidomide	Linituzumab is a humanized anti-CD33 monoclonal antibody; effects on MDSCs unknown [147]
	ONC201 in relapsed/refractory acute leukemias and high-risk myelodysplastic syndromes (HR-MDS)	Leukemia/MDS	ONC201 Cytarabine	AKT and ERK inhibitor; induces tumor cell apoptosis via TRAIL (TNF-related apoptosis inducing ligand) [148]
	CD16/IL-15/CD33 Tri-specific killer engagers (TriKES) for high risk heme malignancies	High-risk MDS AML	161,533 Trike	
	Phase I study to evaluate MED14736 in subjects with myelodysplastic syndrome	MDS	MED14736 (durvalumab)	Monoclonal antibody against PD-L1 [149]
	Nivolumab and ipilimumab with 5-azacitidine in patients with myelodysplastic syndromes(MDS)	Leukemia MDS	Nivolumab Ipilimumab 5-azacitidine	
	Phase I/II trial to investigate BI-836858 in MDS	MDS	BI 836858 or best supportive care	Fc engineered anti-CD33 [138]

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NCT number	Study title	Diseases covered	Study intervention	Comments/references
	Phase II INCB024360 study for patients with myelodysplastic syndromes	MDS	INCB024360 (epacadostat)	Inhibitor of indoleamine 2,3-dioxygenase-1 which promotes tumor escape from immune surveillance [150,151]