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Perspective
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Old Dogs, New Tricks: Revisiting Immune Modulatory Approaches for Myelodysplastic Syndromes

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Profound immune dysregulation is an increasingly recognized feature of myelodysplastic syndromes (MDS), contributing to ineffective hematopoiesis and driving disease progression. Immune dysregulation in MDS is highly complex and composed of many interdependent factors, including clonal hematopoietic cells with somatic mutations providing faulty signals to the immune system and altered cells of the bone marrow microenvironment contributing to inflammation and immunosuppression. Cellular components of disturbed immune regulation include T and natural killer cells, myeloid-derived suppressor cells as well as MSC. In this perspective, we highlight the role of the various players contributing to immune dysregulation in MDS and discuss novel therapeutic approaches currently being designed to improve treatment options.

MDS: A heterogeneous disease with distinct elements of immune dysregulation

MDS are clonal hematopoietic stem/progenitor cell (HSPC) disorders characterized by ineffective hematopoiesis, peripheral cytopenias, and a risk for transformation to acute myeloid leukemia (AML). Distinct acquired epigenetic and genetic mutations have been detected in MDS HSPC and are considered disease-initiating events.[1] Recent work has also highlighted the role of the immune system as well as of the bone marrow mesenchymal stromal cells (MSC) compartment (ie, stem cell niche) in the development and progression of MDS.[2,3] The interplay between clonal hematopoietic cells, cells of immune system, and the specific bone marrow microenvironment is central to how MDS manifests itself.

Dysregulation of the immune system has long been considered a defining feature of MDS. Autoimmune disorders can be commonly observed in MDS patients with varying frequency reported between 10% and 30%.[4–6] Clinical presentation is quite diverse and encompasses disorders such as vasculitis (often presenting as Sweet syndrome), seronegative polyarthritis, neutrophilic dermatosis polychondritis, systemic lupus erythematodes (SLE), and Sjogren syndrome.[6,7] Rarer manifestations include glomerulonephritis and polyneuropathy. Epidemiological studies have linked autoimmune disorders and MDS, and certain autoimmune diseases have a higher risk for development of MDS, most notably rheumatoid arthritis and SLE but also immune thrombocytopenia, autoimmune hemolytic anemia, myasthenia gravis, and giant cell arteritis.[8,9] Furthermore, patients receiving azathioprine therapy for autoimmune disease (most commonly SLE) have a 7-fold higher risk of developing MDS.[10]

The immune system in MDS shows an inflammatory response (Fig. 1) with an increased release of inflammatory cytokines and immune mediators such as tumor-necrosis factor alpha (TNF-alpha), interferon-gamma (IFN-gamma), transforming-growth factor-ß, indoleamine-2,3-dioxygenase (IDO), nitric oxide (NO), and various interleukins such as interleukin-6 (IL-6) and interleukin-10 (IL-10).[11] Cytokines are expressed by clonal hematopoietic cells as well as by MSC in the bone marrow microenvironment. In addition, activated T cells locally secrete IFN-gamma, which stimulates MSC and contributes to the inflammatory phenotype. Immune cell function is also impaired in MDS. Levels of regulatory T cells (T-regs) in the peripheral blood are decreased in low-risk MDS and counts of cytotoxic CD8+ T cells as well as natural killer (NK) cells are higher compared to healthy age-matched controls. By contrast, expansion of T-regs, especially memory T-regs, can be observed in higher-risk MDS, indicating an increasingly immunosuppressive state in advanced disease.[12,13] MDS patients have increased

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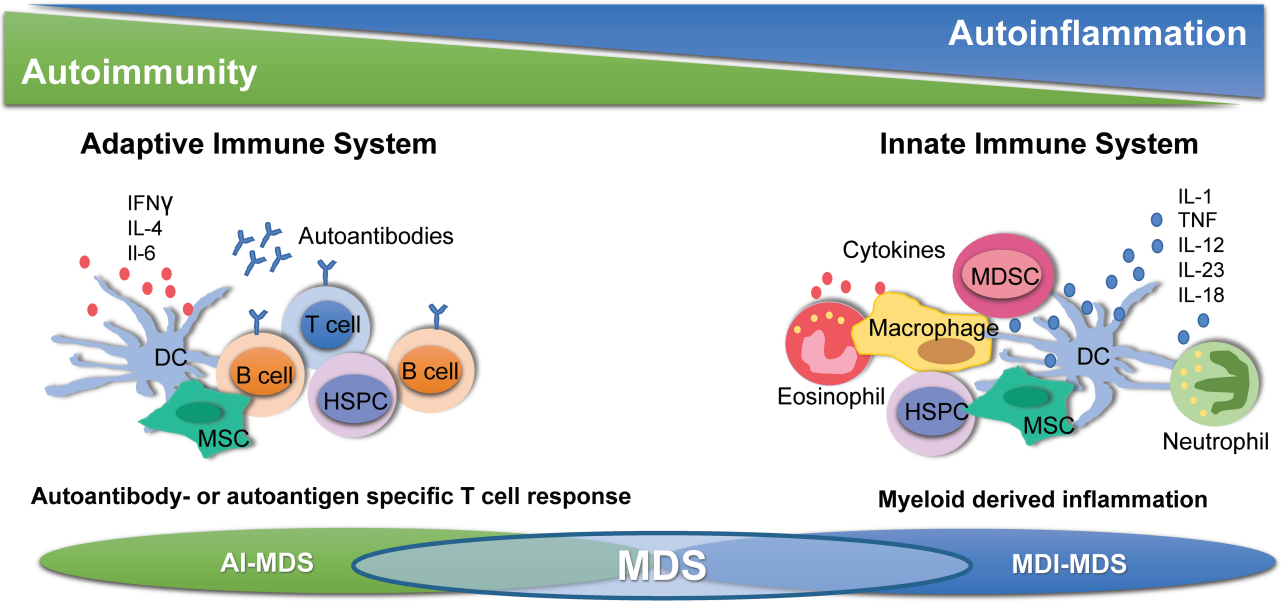

Figure 1. Immune dysregulation in MDS. MDS is characterized by dysregulation of the adaptive as well as the innate immune system. Autoimmune mechanisms (AI-MDS, left panel) and myeloid-derived inflammation (MDI-MDS, right panel) contribute to specific phenotypes in low- and high-risk MDS, which can be targeted by immune modulatory therapies. DC = dendritic cell, HSPC = hematopoietic stem and progenitor cell, MDI = myelodysplastic syndrome, MDSC = myeloid-derived suppressor cell, MSC = mesenchymal stromal cell.

numbers of myeloid-derived suppressor cells (MDSC), which are nonclonal immunosuppressive effector cells that also mediate a pro-inflammatory response.[14,15] The main feature of MDSC is a potent suppression of T cell function.[16] MDSC become activated by toll-like receptor (TLR) signaling as part of innate immune activation, specifically by calcium-binding proteins S100A9 and S100A8, which are TLR4 and CD33 ligands.[14] TLRs have been found to be upregulated in the bone marrow of MDS patients and expression can be correlated with increased apoptosis in low-risk MDS.[17] Recently, it has additionally been shown that S100A9 and reactive oxygen species produced by MDSC as well as clonal HSPC themselves activate the NLRP3 pattern recognition receptor, leading to inflammasome assembly and activation of inflammatory cell death.[18] Induction of S100A9/S100A8 also leads to a p53-dependent differentiation defect in erythroblasts in MDS with (del5q).[19]

Finally, MSC as crucial components of the stem cell niche in the bone marrow are increasingly being recognized as important players in MDS pathogenesis and progression (Fig. 1). MSC are critical for regulating self-renewal, survival, and differentiation of HSPC.[20] MSC communicate with HSPC directly by cell-cell contact and indirectly through secreted factors and production of extracellular matrix. In addition, MSC replenish osteoblasts as well as adipocytes in the bone marrow niche and have diverse immunoregulatory functions. In MDS, MSC and clonal HSPC exist in a close and codependent relationship. MDS MSC are essential for propagation of human MDS HSPC in vivo in xenograft models and cross-talk between MDS MSC and clonal HSPC has been shown to reinforce clonal dominance of MDS cells in the niche.[21] MDS MSC are phenotypically and functionally altered and display a prominent inflammatory program, which contributes to negative regulation of normal hematopoiesis.[2,22–24] MSC have profound immunosuppressive properties, which are mediated in part by production of IDO, which suppresses T cell proliferation and activation.[25] While healthy MSC exhibit immunosuppressive effects and are thus used to treat graft-versus-host disease after allogeneic transplant, there is some evidence that MSC-mediated immunosuppression is decreased in low-risk MDS and may actually increase with disease progression to higher-risk MDS and secondary AML.[26]

In sum, the bulk of accumulated data show that the immune system is profoundly dysregulated in MDS. Activation of the innate immune system is considered a hallmark of MDS, and early stages of the disease are characterized by immune activation and inflammation. It is still unknown whether this is a cause or a consequence of (genetic) alterations within the HSPC pool. Progression to higher risk MDS and transformation to secondary AML is accompanied by dysregulation of the adaptive immune system and is defined by increased immunosuppression and progressive immune evasion mechanisms allowing unchecked proliferation of myeloid blasts.

Rationale for immune modulatory therapies in MDS

Immune modulatory therapies have long been employed for MDS, most commonly in the form of immunosuppressive therapy (IST) for (hypoplastic) low-risk MDS. More recently, the availability of checkpoint modulating agents and other T cell therapies and their clinical success in solid tumors have generated interest in these agents as a novel therapeutic approach for higher-risk MDS and secondary AML. The rationale for immune modulation as a treatment for MDS is based on 2 premises. First, in low-risk MDS the observed cytopenias are in part a result of immune activation and thus may be amenable to IST. Second, progression to higher-risk MDS or secondary AML is associated with immune evasion mechanisms enabling expansion of the leukemic clone, particularly myeloid blasts, which may be

amenable to T cell therapies. Based on these 2 premises, immunosuppressive agents are primarily employed in low-risk MDS whereas more recently, immune modulatory drugs, including checkpoint modulators and other T cell activating therapies, have entered the scene as a novel approach to treat high-risk MDS and secondary AML.

Strategies for low-risk MDS

Immunosuppressive agents

IST with antithymocyte globulin (ATG, either horse or rabbit), with or without addition of cyclosporine (CSA), has been evaluated for treatment of low-risk MDS in a number of phase II clinical trials with small numbers of patients and response rates ranging from 16% to 67%.[27] Various predictors of response have been described in these trials, most notably MDS-SLD (formerly refractory anemia, RA) with absence of ring sideroblasts, a hypoplastic bone marrow, DR15 HLA type, younger age (<60 years), female sex, trisomy 8, and short duration of transfusion dependence.[28] Presence of a coexisting PNH clone had no influence on response to IST. However, a recent large retrospective analysis of 367 MDS patients treated with IST failed to confirm the predictive value of these previously described biomarkers of response.[29] Interestingly, while the overall response rate to IST was 45%, the presence of *SF3B1* mutations negatively affected response, which correlates with previous observations noting a decreased response in MDS with ring sideroblasts (MDS-RS, formerly RARS). To date, there is only 1 prospective randomized trial comparing ATG + CSA to best supportive care performed by the HOVON-SAKK cooperative group.[30] In this trial of 74 low-risk transfusion-dependent MDS patients, hematologic response (CR, PR, and HI) at 6 months was 31% compared to 9% with a response duration of 16.4 months in the ATG + CSA arm. Response rates were highest for patients with a hypocellular bone marrow. However, transformation-free survival and overall survival did not differ between patients receiving ATG + CSA or best supportive care. Currently, ATG is still routinely used in the clinic in rare cases with hypoplastic MDS and a normal karyotype.

Very high response rates of 72% were also reported using the anti-CD52 monoclonal antibody alemtuzumab in a cohort of low-risk MDS patients with a high likelihood of response to IST based on HLA-DR15 expression, age, and duration of transfusion dependence.[31] Interestingly, even cytogenetic responses including in patients with monosomy 7 have been observed. As CD52 is expressed on all lymphocyte subsets, with a higher density on T cells than on B cells, alemtuzumab induces widespread T cell depletion. However, due to licensing issues the drug is no longer available for the treatment of hematologic diseases.

Lenalidomide

Lenalidomide, an immune modulatory drug (ImiD), has a high rate of activity in low-risk MDS with del(5q), the most prevalent cytogenetic abnormality, with achievement of transfusion independence in 56% to 67% and cytogenetic complete remissions in 45%.[32,33] Lenalidomide is thus considered the treatment of choice for transfusion-dependent low-risk MDS with del(5q), although in some countries first-line therapy with erythropoietin is also used in these patients.[34] A recently completed randomized double-blind phase III European trial is investigating the efficacy of lenalidomide versus placebo in MDS del(5q) patients with a hemoglobin value <12 g/dL who have not yet become transfusion-dependent (SINTRA-Rev trial, EudraCT 2009-013619-36). The trial is designed to determine if lenalidomide can extend the period of transfusion independency of MDS del(5q) patients.

As an immunomodulatory agent with pleiotropic effects on the T cell repertoire as well as on the bone marrow microenvironment,[35–37] lenalidomide has also been investigated in low-risk MDS without del(5q) with response rates around 25% and in higher-risk MDS and AML with del(5q). The results of these trials suggest that lenalidomide could be effective in a subset of patients with low-risk MDS without del(5q), but so far it has not been possible to identify prognostic markers that could be associated with response.[38] Lenalidomide improves the function of MDS-derived MSC to support HSPC in in vitro culture models and thus may contribute to improved hematopoiesis by also affecting the stem cell niche.

Suppression of innate immune activation

Given the activation of the innate immune system with sustained inflammation in the bone marrow microenvironment mediated by MDSC and through TLR signaling, some clinical trials have been initiated to target these components. Because MDSC express high levels of CD33, there has been some interest in employing anti-CD33 directed therapies to deplete MDSC numbers in the bone marrow. Both a monoclonal antibody against CD33 (BI 836858) as well as a novel CD3/CD33 bispecific tetravalent antibody that recognizes both CD33 and CD3 (AMLV564) have been evaluated in preclinical models.[39] The anti-CD33 antibody was shown to prevent CD33-mediated cytokine secretion in the bone marrow, correlating with a significant increase in hematopoietic colony formation in vitro as well as directly reducing MDSC through antibody-dependent cellular toxicity.[40] In vitro treatment of bone marrow mononuclear cells from MDS patients with the bispecific antibody BI 836858 eliminated CD33 + MDSC and led to expansion of CD4+ and CD8+ T cells as well as improved hematopoietic colony formation in vitro.[41] Both antibodies have now entered Phase I/II trials (NCT02240706 and NCT03516591). Interestingly, these preclinical studies also provided some first evidence that targeting CD33 may increase sensitivity to checkpoint inhibitors, thus augmenting immune response against the MDS clone.[41]

Targeting TLR signaling pathways has also been explored as means to modulate innate immune activation, for instance, by blocking TLR2 receptor. In vitro inhibition of TLR2 in cultured BM CD34 cells from patients with lower-risk MDS resulted in increased formation of erythroid colonies.[42] However, for higher-risk MDS and secondary AML, more recent data suggest that targeting the innate immune system alone will be insufficient, as compensatory immune escape pathways become activated as the disease progresses, similar to what has been shown for solid tumors.[43] This insight has prompted the concept of combining targets of innate immunity with checkpoint modulators.

Strategies for high-risk MDS

Countering immune evasion mechanisms

As outlined above, evasion of adaptive immune response is a feature of advanced MDS. Accordingly, recent efforts have focused on reactivating T cell responses through use of checkpoint modulators or novel T cell therapies.

Programmed cell death-1 (PD-1) is a negative regulatory receptor expressed on the surface of activated T cells as well as B

cells and NK cells. It binds to PD-L1, which is a negative costimulatory ligand expressed on malignant cells, including MDS stem cells and leukemic blasts.[43] IFN-gamma, produced by T cells as well as the bone marrow microenvironment, induces up-regulation of PD-L1 in leukemia cells. In a mouse model of AML, inhibition of the PD-1/PD-L1 pathway led to a significant reduction of AML burden in vivo and a prolonged murine survival, providing a preclinical rationale for use of checkpoint modulators in myeloid leukemia.[39] Clinical trials of checkpoint modulation using PD-1 as well as PD-L1 inhibitors are currently underway in MDS and AML. The cytotoxic T-lymphocyte associated antigen 4 expressed on T cells is also part of an inhibitory checkpoint pathway and can be targeted by the monoclonal antibody ipilimumab to reverse T cell inhibition. So far, PD-1/PD-L1 inhibitors as well as CTLA4 inhibitors seem to have limited efficacy as single agents in advanced disease. In a recently reported Phase I trial of ipilimumab in MDS patients failing treatment with hypomethylating agents (HMA), responses consisted mainly of disease stabilization.[44] By contrast, a second trial of single-agent ipilimumab in HMA failure patients reported an overall response rate of 30% and accrual is currently continuing.[45] The Keynote-013 Phase Ib trial (NCT01953692) evaluated the PD-1 inhibitor pembrolizumab for patients with various advanced hematologic malignancies, including 28 MDS patients after treatment with HMA. The overall response rate was 4%.[46] First-line pembrolizumab therapy, however, was shown to induce a clinical and molecular response in a patient with secondary AML receiving the drug for melanoma.[47] Similar to pembrolizumab, no responses were seen in advanced MDS patients treated with the PD-1 inhibitor nivolumab after HMA failure.[45] Interestingly, in MDS and secondary AML, PD-L1 expression levels are higher in hematopoietic cells than in those of healthy volunteers and resistance to the HMA azacitidine and decitabine is additionally associated with up-regulation of PD-1 and PD-L1 on leukemic cells, representing an immune evasion mechanism against HMA.[43] However, this may be exploited therapeutically, as blockade of PD-L1 in combination with HMA therapy may actually increase the antitumor response. This combinatory approach is being tested in several ongoing clinical trials for higher-risk MDS and AML as a first-line therapy (pembrolizumab + azacitidine, NCT03094637). Nivolumab + azacitidine has shown impressive overall response rates of 80% in a small exploratory Phase II trial.[45] Anti-PDL-1 antibodies, such as durvalumab and atezolizumab, are also being evaluated as first-line therapies in combination with HMA (NCT02775903 and NCT02508870), as is the combination of CTLA4 and PD-1 blockade with ipilimumab and nivolumab (NCT02530463).

Bispecific T cell engager antibody therapies

Finally, bispecific antibodies aimed at redirecting T cell killing by engagement of a specific target on the cancer cell are also being explored as novel therapeutic agents in MDS. Flotetuzumab is a CD3 × CD123 bispecific T cell engager antibody that has shown promising results in a recently presented Phase I trial of relapsed or refractory MDS and AML patients with a complete remission rate of 26% and an overall response rate of 42%.[48] CD123 is expressed at high levels on leukemic stem cells and is differentially overexpressed in 93% of AML and 50% of MDS patients and previous work has shown a correlation between CD123+ cell frequency and prognosis.[44,49] However, the anti-CD123 monoclonal antibody talacotuzumab recently failed to show any meaningful activity in advanced MDS and the trial was halted due to excess toxicity (NCT03011034). Thus, the early flotetuzumab results attest to the potency of harnessing a specific T cell response in myeloid leukemia. Interestingly and similar to observations with HMA, PD-L1 expression on leukemic blasts increased in patients no longer responding to flotetuzumab, suggesting the combination of flotetuzumab and checkpoint modulators such as PD-1/PD-L1 inhibitors may be beneficial. This may also be true for bispecific antibodies targeting CD3 and CD33, a molecule highly expressed on myeloid blasts in AML and MDS.[50–52] (AMG330, NCT02520427; GEM33, EudraCT 2017-001707-77). Lysis of AML cells by T cells through engagement of AMG330 in vitro was shown to be augmented by inhibition of PD-L1.[53] To this end, novel bifunctional checkpoint inhibitory T cell engaging antibodies combining T cell redirection to CD33 with locally restricted checkpoint blockade are also being developed.[54] As these results are preclinical and bispecific antibodies are only yet in Phase I trials, whether combining bispecific antibodies with PD-1/PD-L1 inhibitors is feasible in terms of toxicity and efficacious in terms of clinical responses remains to be seen.

Summary

Immune modulatory approaches show high promise in the treatment of MDS. Given the heterogeneity of the disease, both in terms of risk stratification as well as highly variable genetic traits and the type of immune dysregulation present, it will be of utmost importance to correctly identify which patients will most likely benefit from which approach. For a small group of carefully selected low-risk MDS patients, IST with ATG shows high response rates with durable remissions and an acceptable toxicity profile. However, more recent efforts are focused on targeting the inflammatory phenotype (in particular the expanding MDSC compartment) in low-risk MDS induced through modulation of the innate immune system by targeting TLR signaling or MDSC directly through inhibition of CD33. By contrast, higher-risk MDS and AML is characterized by an immunosuppressive microenvironment and increased immune escape mechanisms allowing unchecked proliferation of immature progenitor cells. Thus, T cell directed therapies such as checkpoint modulation in combination with HMA or bispecific T cell engager antibody therapies to reverse immunosuppression and activate T cell responses are novel treatment options currently being investigated for this group of patients. The ideal target for bispecific T cell engager antibodies in myeloid disease has yet to be determined, but CD33 as well as CD123 seem to hold promise in patients with AML as well as MDS with high-risk features such as elevated blast counts.

As most of the clinical trials are still in their early stages, experience with these approaches for MDS is currently limited. Thus, critical issues such as optimal combination, dosing and scheduling of agents as well as identification of patient populations most likely to benefit from immune modulatory therapies remain to be answered by the currently ongoing clinical trials.

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