review articles

Integrating the "Immunome" in the Stratificatio of Myelodysplastic Syndromes and Future Clinical Trial Design

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abstract

Myelodysplastic syndromes (MDS) are characterized by ineffective hematopoiesis and often include a dysregulation and dysfunction of the immune system. In the context of population aging, MDS incidence is set to increase substantially, with exponential increases in health care costs, given the limited and expensive treatment options for these patients. Treatment selection is mainly based on calculated risk categories according to a Revised International Prognostic Scoring System (IPSS-R). However, although IPSS-R is an excellent predictor of disease progression, it is an ineffective predictor of response to disease-modifying therapies. Redressing these unmet needs, the "immunome" is a key, multifaceted component in the initiation and overall response against malignant cells in MDS, and the current omission of immune status monitoring may in part explain the insufficiencies of current prognostic stratification methods. Nevertheless, integrating these and other recent molecular advances into clinical practice proves difficult. This review highlights the complexity of immune dysregulation in MDS pathophysiology and the fine balance between smoldering inflammation, adaptive immunity, and somatic mutations in promoting or suppressing malignant clones. We review the existing knowledge and discuss how state-of-the-art immune monitoring strategies could potentially permit novel patient substratification, thereby empowering practical predictions of response to treatment in MDS. We propose novel multicenter studies, which are needed to achieve this goal.

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INTRODUCTION

Myelodysplastic syndromes (MDS) represent a group of acquired clonal disorders of hematopoietic stem and progenitor cells (HSPCs), characterized by ineffective hematopoiesis, peripheral cytopenias, genetic instability, and an increased risk of progression to acute myeloid leukemia (AML).[1] Considering the higher prevalence in elderly patients, the population aging in developed countries, as well as higher diagnostic awareness, the incidence of MDS is set to increase substantially in coming decades.[2]

Clinical outcomes can vary greatly, even between patients considered to have the same MDS subtype. Thus, MDS display marked heterogeneity regarding prognosis and the risk of disease progression. To overcome this heterogeneity, the International Prognostic Scoring System (IPSS) was introduced, and then later revised (IPSS-R), with the aim to provide discriminatory prognostic risk assessment regarding overall survival and risk of progression to AML.[3] Although the IPSS-R reliably predicts the risk of disease progression, it is not an effective tool to predict response to disease-modifying therapies.[4] This is not surprising, because the IPSS-R, like the original IPSS, was developed based on clinical data from patients with untreated MDS. Recent advances in targeted and large-scale next-generation sequencing (NGS) have helped to illuminate the dynamic genomic landscape in MDS.[5-7] Although none of the most common recurrent somatic mutations is disease defining, some have an independent impact on overall survival, such as in *TP53*.[8] Thus, addition of molecular data to the IPSS-R can improve its predictive power.[5,8,9]

Recent advances have also highlighted the role of immune dysregulation in MDS pathogenesis but are currently omitted from IPSS-R. This includes both abnormal activation of innate immune pathways and associated inflammation as well as aberrant cellular immune responses of independent prognostic value, which dynamically evolve during disease progression.[10-13] The addition of comprehensive immunologic data to prognostic models could, similar to mutational data, further help to refine risk stratification across the boundary of lower- and higher-risk MDS. We envisage that continued clarification of the immune pathways that are dysregulated in selected MDS subtypes will improve patient stratification, improve the use and outcomes of existing treatments and

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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novel immunotherapies, and drive the development of new targeted drugs. In this review, we highlight recent advances in the understanding of immune dysregulation in MDS, discuss their clinical implications and potential therapeutic applications, and outline how immune profiling could be implemented in future clinical trials.

PREDISPOSING AND POTENTIAL DRIVING IMMUNE FACTORS

Smoldering Inflammation and Immunosenescence

Chronic inflammation as a result of long-lasting exposure to persistent infection or sterile inflammation is a well-established predisposing factor for cancer,[14,15] and increasing evidence implicates the activation of innate immune signaling in age-related hematopoietic senescence,[16] bone loss,[17] and MDS.[18] In fact, normal human aging represents a state of chronic low-grade sterile inflammation, similar to that originally described as "para-inflammation" by Medzhitov[19] and commonly referred to as "inflammaging."[20] Stressed, damaged or otherwise malfunctioning, and/or dead cells release endogenous inducers of sterile inflammation, including damage-associated molecular patterns (DAMPs) like high-mobility-group-protein B1 and alarmin S100 proteins, which can be sensed through different receptors, such as Toll-like receptors (TLRs) and cytosolic nucleotide-binding domain and leucine-rich repeat pattern recognition receptors (NLRs).[19,20] The physiologic purpose of the ensuing inflammatory response early in life and adulthood is to restore functionality and homeostasis in the tissue. However, in old age, a period in life largely not foreseen by evolution, the continuous exposure to inflammatory stimuli/stressors (the "immune biography") becomes detrimental, setting the biologic background favoring the susceptibility to age-related inflammatory disorders, autoimmunity, and deterioration of hematopoiesis. A reduced capacity to defend against pathogens and to initiate adaptive immunity is observed in aging humans, together with enhanced proinflammatory reactions fueled by endogenous/self-molecular garbage.[20,21] The presence of "smoldering" inflammation in the elderly may aid the proliferation and survival of malignant MDS clones driven by genetic alterations (including a recently described condition known as clonal hematopoiesis of indeterminate potential [CHIP][22]), subvert adaptive immunity, and alter cellular responses to therapeutic intervention.

NLRP3 Inflammasome: A Driver of Chronic Inflammation in MDS

Increased levels of DAMPs (eg, S100A8/9) and activated NLR family, pyrin domain-containing protein 3 (NLRP3) inflammasomes are evident in MDS, particularly lower-risk disease.[18,23-25] Notably, MDS HSPCs are specifically susceptible to DAMPs because they overexpress TLRs[26,27] along with signal transducers, such as IRAK1[28] and TRAF6.[29] Ligation of S100A8/9 to TLR4 induces NF-κB–mediated transcription of proinflammatory cytokines, including pro–interleukin (IL)-1β and IL-18, and transcriptional priming of inflammasome components.[30] Once activated, the NLRP3 inflammasome directs caspase-1–dependent conversion of pro–IL-1β/IL-18 to their active forms and inflammatory pyroptotic cell death.[18] The consecutive release of proinflammatory cytokines, reactive oxygen species, and other intracellular contents into the extracellular milieu further activates the NLRP3 inflammasome, driving pyroptosis of HSPCs, consequent cytopenias, and an inflammatory circuit (Fig 1). This milieu may support the propagation of the MDS clone through various pathways, including Wnt/β-catenin signaling[31] or aberrant activation of the IL-1/p38MAPK pathway.[32] NLRP3 inflammasome activation seems to be licensed by S100A8/9 and MDS-related gene mutations and is also evident in patients with del(5q) MDS, featuring activation of the p53-S100A8/9-TLR4 axis.[10,18,24] However, whether inflammasome activation is a general feature of lower-risk MDS or particular subgroups needs to be evaluated in larger cohorts in the future.

TLR signaling pathway activation in MDS HSPCs makes the TLR axis a promising therapeutic target (Table 1). In addition, novel NLRP3 inflammasome inhibitors or approved IL-1β inhibitors are in clinical development and may offer therapeutic promise in MDS,[10] which highlights the importance of refined patient stratification to identify patients with prominent "autoinflammatory" features, who are therefore most likely to benefit from inflammasome pathway inhibition.

Somatic Mutations and Inflammatory Status

A complex and dynamic landscape of genetic mutations and cytogenetic lesions is evident in MDS.[5,33] Acquisition of serial mutations and clonal diversification not only reflect on disease progression but also give an indication of the (in-)efficacy of the immune system to control outgrowth of malignant clones, as suggested in other malignancies.[34,35] Underlying smoldering inflammation could contribute to the genomic instability and acquisition of additional mutations, as shown in gastrointestinal malignancies.[36,37] In MDS, mutations affecting epigenetic modifiers (eg, *TET2*, *ASXL1*) and RNA splicing factors (eg, *SF3B1*, *SRSF2*) seem to represent predominantly "founder" events.[33] Mutations in several of these genes have been linked to activated NLRP3 inflammasomes and enhanced innate immune signaling.[18,38-40] Such mutant gene licensing of innate signaling pathways in myeloid progenitors may provide the selective immune pressure conducive to malignant progression in MDS/AML. On the other hand, the observation of founder mutations in the lymphoid lineage raises questions about the potential effect of intrinsically

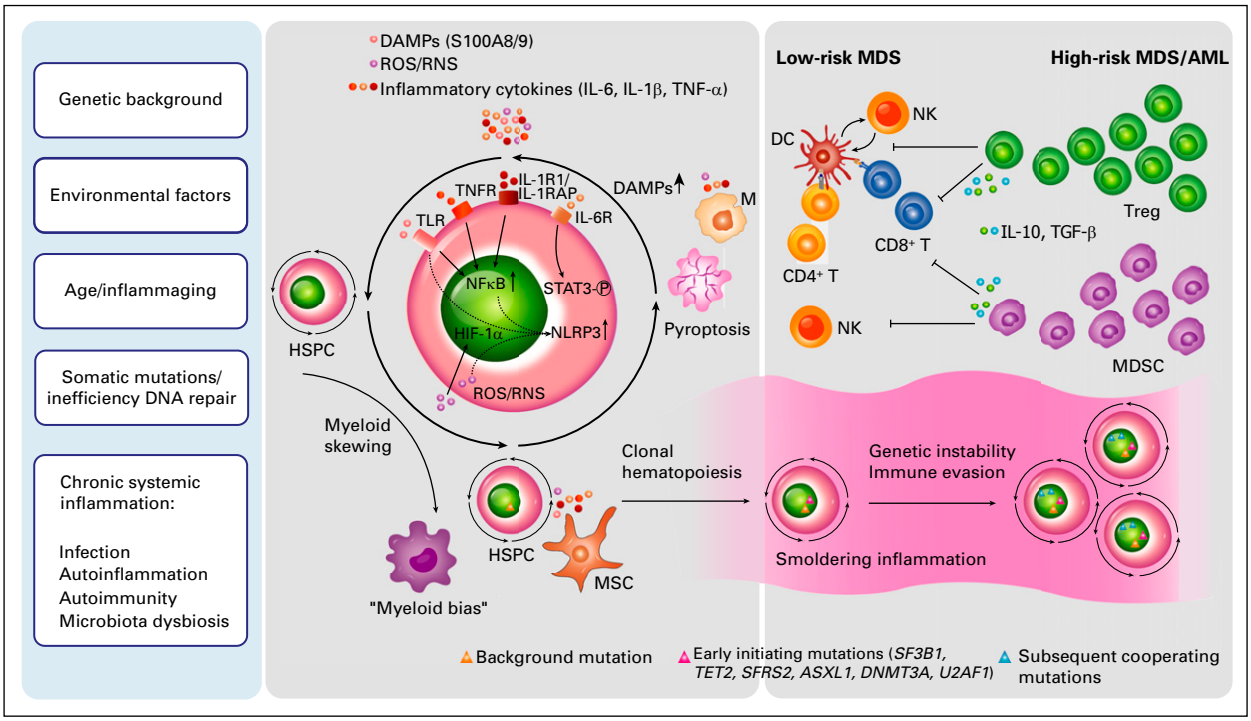

FIG 1. The immune contexture in myelodysplastic syndrome (MDS). Certain conditions associated with chronic immune stimulation, such as aging, chronic infection, and autoimmune disease, may contribute to set the biologic background for MDS development (left). Chronic immune stimulation leads to sustained TLR activation that may drive hematopoietic skewing and loss of stem cell quiescence. Initial events may induce a "myeloid bias" of hematopoietic stem cells and multipotent progenitors, and such a bias could skew the accumulation of somatic mutations conferring clonal advantage and/or differentiation defects toward the myeloid lineage. Elevated levels of proinflammatory cytokines, reactive oxygen species (ROS)/reactive nitrogen species (RNS), and damage-associated molecular patterns (DAMPs) induce activation of the NLRP3 inflammasome, resulting in pyroptosis of hematopoietic stem and precursor cells (HSPCs), consequent cytopenias, an inflammasome-driven inflammatory circuit, and an increasing dysfunction of the hematopoietic stem cell niche, including mesenchymal alterations (middle). Subsequently, the presence of smoldering inflammation may support the propagation of premalignant clones (eg, via ROS-dependent Wnt/β-catenin pathway) and subvert adaptive immunity (right). The immune contexture dynamically changes with disease progression. In higher-risk MDS, an expansion of myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) contributes to the suppression of antitumor responses and immune evasion of malignant clones. Regarding $CD4^+$ T-cell subsets, which display significant plasticity in response to changing environmental cues, different $CD4^+$ T-cell signatures are to be expected in MDS subtypes, with predictive value for disease progression and response to therapy, as shown in other diseases like aplastic anemia.[148] AML, acute myeloid leukemia; *ASXL1*, additional sex combs-like 1, transcriptional regulator; DC, dendritic cell; *DNMT3A*, DNA methyltransferase 3 alpha; HIF-1α, hypoxia-inducible factor 1, alpha subunit; IL-1R1, interleukin-1 receptor, type 1; IL-1RAP, interleukin-1 receptor accessory protein; M, macrophage; MSC, mesenchymal stromal cell; NK, natural killer cell; NLRP3, nucleotide-binding domain and leucine-rich repeat pattern recognition receptor (NLR) family, pyrin domain-containing protein 3; *SF3B1*, RNA splicing factor 3B, subunit 1; *SRSF2*, serine/arginine-rich splicing factor 2; STAT3-P, signal transducer and activator of transcription 3, phosphorylated; *TET2*, tet methylcytosine dioxygenase 2; TLR, Toll-like receptor; TNFR, tumor necrosis factor receptor; *U2AF1*, U2 small nuclear RNA auxiliary factor 1.

aberrant lymphocytes on the adaptive immune response and MDS/AML pathogenesis.[33,41]

The intricate relationship between mutagenesis and inflammatory processes is not limited to established MDS. Patients with CHIP,[22] a condition that likely precedes MDS and is characterized by the presence of MDS-related mutations in *DNMT3A*, *TET2*, *ASXL1*, or *JAK2*, were found to have an increased risk of inflammatory-related diseases, such as coronary heart disease.[42,43] Recent studies point to the existence of shared autoinflammatory NLRP3-related pathways in CHIP/MDS and associated comorbidities,[44] and suggest *NLRP3* as a shared genetic risk factor for MDS and paraneoplastic Sweet syndrome.[45]

The other important and yet poorly investigated aspect of MDS pathophysiology is the reciprocal effect of the (cellular) immune response on frequency and type of somatic mutations and whether these mutations induce immunogenic neoantigens, as shown in other malignancies.[34] Because of the overall lower somatic mutation burden in both AML and MDS compared with other types of tumors,[46] the potential immunogenicity of these mutations is largely

TABLE 1. Novel Therapeutic Agents Evaluating Immune Targets in MDS

Class	Drug	Target	Patient Group	Clinical Trial	Reference
TLR inhibition	OPN-305 (mAb)	TLR2	HMA failure lower-risk MDS	NCT02363491: phase I-II, completed	155
	IRAK-1/4 inhibitor	IRAK-1/4	N/A	Preclinical	28
	Bortezomib	NF-κB pathway, TRAF6 inhibition	Lower-risk MDS with p65 activation	NCT01891968: phase II, completed	156
NLRP3 inhibition	Ibrutinib	BTK inhibitor, regulator of NLRP3 inflammasome	Higher-risk MDS	Phase I, recruiting; in combination with Len (NCT03359460) or Aza (NCT02553941)	
Cytokine inhibition	Luspatercept (ACE-536)	TGF-β superfamily ligands	Lower-risk MDS	NCT02631070: phase III, active, not recruiting and NCT03682536: phase III, recruiting; luspatercept *v* epoetin alpha	157,158
Checkpoint inhibitors	Ipilimumab/ nivolumab	CTLA-4/PD-1	Untreated MDS, post–HMA failure	NCT02530463: phase II, recruiting; alone or in combination with Aza	93
	Durvalumab	PD-L1	Untreated higher-risk MDS, AML	NCT02775903: phase II, active, not recruiting; in combination with Aza	
	Atezolizumab	PD-L1	HMA R/R MDS, HMA-naïve MDS	NCT02508870: phase I, suspended; alone or in combination with Aza	
	Pembrolizumab	PD-1	IPSS int-1 or higher (HMA-naïve and HMA failure)	NCT03094637: phase II, recruiting; in combination with Aza	159
	Hu5F9-G4 (5F9)	CD47	R/R AML or MDS, treatment-naïve unfit AML or higher-risk MDS	NCT03248479: phase IB, recruiting; alone or in combination with Aza	100
NK therapies	CD16/IL-15/CD33 TRIKE	CD16/CD33	High-risk MDS, R/R AML, CD33 hematologic malignancies	NCT03214666: phase I/II, not yet recruiting	69
	Lirilumab	KIR2DL1/2L3	Lower-risk and higher-risk MDS without prior HMA therapy	NCT02599649: phase II, completed; alone or in combination with Aza/nivolumab	
MDSC elimination	BI 836858	CD33	R/R lower-risk MDS (HMA-naïve and HMA failure)	NCT02240706: phase I-II, recruiting	91
Vaccine therapies	DEC-205/NY-ESO-1 fusion protein CDX-1401	—	IPSS (int-1, int-2, high), AML	NCT03358719: phase I, recruiting	90
	Potential neoantigen-based vaccine approach	MDS cells expressing defined neoantigens	MDS or CCUS	NCT03072498: sample collection, recruiting	
CAR T cells	CM-CS1 T-cell infusion	NKG2D	MDS-EB, AML, MM	NCT02203825: phase I, completed	

Abbreviations: AML, acute myeloid leukemia; Aza, 5-azacytidine; BTK, Bruton's tyrosine kinase; CAR, chimeric antigen receptor; CCUS, clonal cytopenia of undetermined significance; HMA, hypomethylating agent; int-1, intermediate-1; int-2, intermediate-2; IPSS, International Prognostic Scoring System; Len, lenalidomide; mAb, monoclonal antibody; MDS, myelodysplastic syndrome; MDSC, myeloid-derived suppressor cell; MDS-EB, MDS with excess blasts; MM, multiple myeloma; N/A, not applicable; R/R, refractory/relapsed; TRIKE, trispecific killer engager.

unexplored. We previously adopted an algorithm to predict neoantigens and combined this with mass cytometry to identify neoantigen-related immune signatures.[47] This initial investigation suggested that the presence of predicted neoantigens has a protective effect in patients with lower-risk disease.

The Microbiome and Its Impact on Inflammation and Immunome

Profound changes in the microbiota and its interaction with the immune system are increasingly recognized to contribute to chronic inflammatory diseases, including hematologic disorders.[48,49] Various factors can reduce microbial diversity and commensalism, including treatment with broad-spectrum antibiotics, poor dietary patterns, drugs, chemotherapy, and environmental factors. For example, depletion of intestinal microbial flora by broad-spectrum antibiotic treatment of mice has been shown to cause a decrease in HSPC numbers and concomitant anemia, highlighting the intricate relationship between host-microbiome and hematopoiesis.[50]

Although no detailed study exists concerning the microbiome composition in MDS, the role of microbial-dependent inflammation in the development of preleukemic myeloproliferation has been demonstrated recently in *Tet2*-deficient mice, in which intrinsic (*Tet2* deficiency–induced IL-6Rα overexpression) and extrinsic (microbial-induced IL-6) inflammatory cues cooperate and trigger proliferation of highly sensitive *Tet2*-deficient hematopoietic progenitor cells.[39] Clinically, overuse of antibiotics and/or a poor dietary pattern/nutritional reserve is also common in MDS/AML and could lead to decreases of microbial diversity and commensalism in the gut, resulting in compromised immune responses and increased risk of inflammation. One study concerning relapse after allogeneic hematopoietic stem-cell transplantation (HSCT) demonstrated that higher abundance of a bacterial group composed mostly of *Eubacterium limosum* could decrease the risk of relapse and disease progression.[51] Lack of commensal microbes like *E. limosum* or their immunomodulatory metabolites (eg, short-chain fatty acids) can increase the risk of gut permeability and result in translocation of pathobionts and overexpression of inflammatory cytokines.[52] Thus, identifying microbiome signatures that contribute to immune system deterioration in MDS may lead to novel therapeutic strategies to control inflammation and potentially prevent disease progression.

Immune Dysregulation in MDS: Autoimmunity or Autoinflammation?

Although there is evidence for the presence of both innate immune-related autoinflammation as well as adaptive autoimmune responses in MDS,[10,53,54] these two terms are sometimes used interchangeably, which may cause some confusion. The term autoimmunity names a condition associated with the presence of autoreactive T cells and high autoantibody titers, whereas autoinflammation generally refers to a condition with dysregulated myeloid-driven innate immune responses only. This view clearly separated autoinflammation and autoimmunity as distinct immunologic diseases. However, and this may be true for MDS, some chronic inflammatory diseases may lie on a spectrum from autoinflammatory to autoimmune, sharing genetic associations and common inflammatory pathways (TLR, PI3K-Akt, and NF-κB signaling), and connecting by variable degrees of interaction between innate and adaptive immune responses[55,56] (Fig 2).

Autoimmune features were long considered as a coincidence rather than a predisposing factor for MDS. Spurred from case reports and smaller studies, a large population-based study was designed, which demonstrated an increased risk of MDS among patients with antecedent autoimmune disease (AID; odds ratio [OR], 2.1; 95% CI, 1.7 to 2.6) or infectious disease (OR, 1.3; 95% CI, 1.1 to 1.5), indicating that chronic immune stimulation (the immune biography) might act as a trigger for MDS development.[57] On the other hand, AID can be a favorable prognostic factor in patients with established MDS,[54] but additional large prospective studies are necessary to confirm these results.

IMMUNE SURVEILLANCE, MICROENVIRONMENT, AND MDS PROGRESSION

Immune Surveillance and MDS Progression

The immune response to cancer requires a series of carefully regulated events that in principle should amplify and broaden cellular immune responses.[58] Chronic inflammation affects immune surveillance and has two overlapping effects in MDS. On the one hand, DAMPs and/or founder gene mutations license the NLRP3 inflammasome to generate an inflammatory feed-forward process characterized by excess proinflammatory cytokines, such as IL-1β, TNF-α, and IFN-γ (Fig 1). Proinflammatory cytokines may facilitate the selection of neoplastic clones by simultaneously enhancing their growth and exhausting non-neoplastic clones, as demonstrated by the paradoxical effects of IL-1β on AML versus normal progenitors.[32] On the other hand, cytokine-mediated induction of immune-inhibitory molecules like programmed cell death-ligand 1 (PD-L1) may contribute to T-cell suppression and reduced immune surveillance.[59] Furthermore, excess DAMPs may expand myeloid-derived suppressor cells (MDSCs),[60] which overproduce suppressive cytokines, such as IL-10 and transforming growth factor-β, contributing to the subsequent immunosuppression and ineffective hematopoiesis.[60,61]

In general, low-risk disease is related to a more proinflammatory immune response and higher numbers of effector-type cells, such as IL-17$^+$ CD4$^+$ cells,[11] and higher-risk disease is characterized by a predominantly suppressive milieu with significant expansion of immunosuppressive cells, such as Tregs[62,63] and MDSCs,[12,60] accompanied by a reduction in the number and function of bone marrow (BM) dendritic cells,[64] peripheral CD8$^+$ T cells,[65] and natural killer (NK) cells[66] (Fig 1). The proliferative capacity of Tregs appears compromised during earlier disease stages but is restored during disease progression.[67] A positive correlation between the numbers of circulating MDSCs and Tregs has been observed, suggesting a role of MDSCs in the expansion of Tregs and subsequent disease progression.[12] Moreover, an independent prognostic value of peripheral Treg and BM progenitor B-cell frequencies in lower-risk MDS has been suggested.[13,62] Reduced NK function in higher-risk MDS likely supports immune evasion and disease progression.[66,68] Hence, a novel strategy to restore NK cell function and overcome MDSC-mediated suppression in patients with MDS has been proposed (Table 1).[69] In addition, the presence of KIR haplotype A on NK cells may represent an independent risk factor for the progression of MDS to AML.[70]

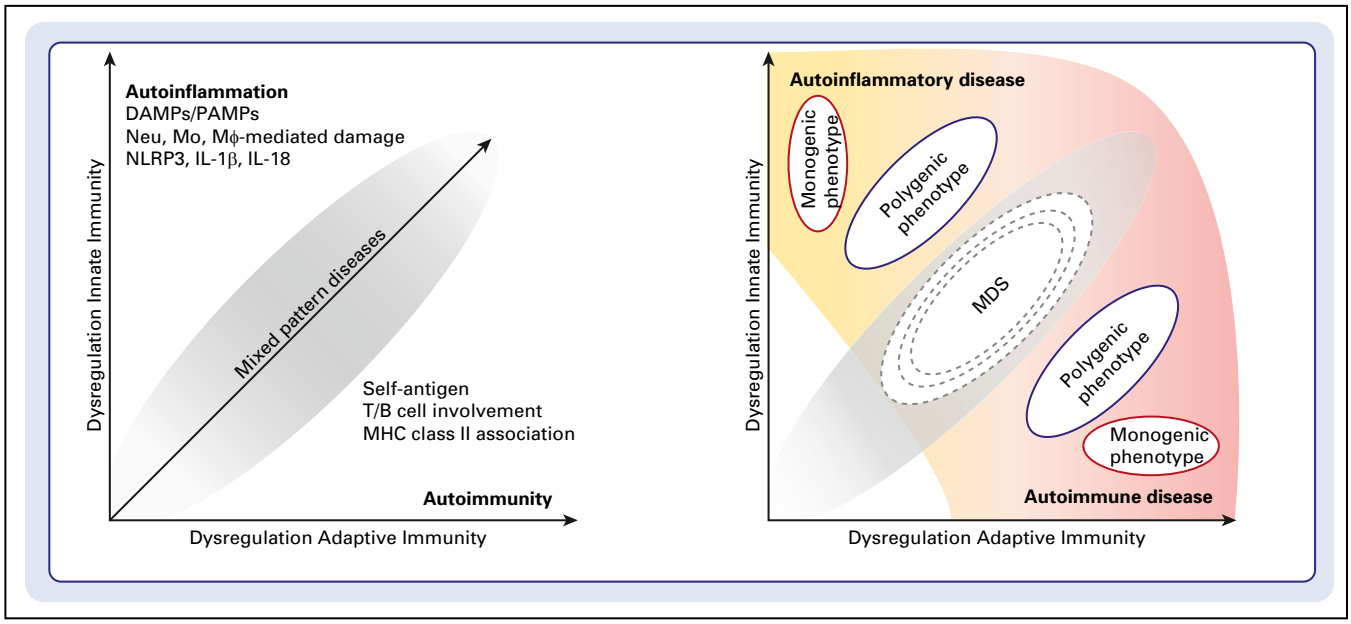

FIG 2. Myelodysplastic syndrome (MDS) across the autoinflammatory/autoimmune disease continuum. The clinical heterogeneity of MDS may reflect the variable contribution of autoinflammatory and autoimmune processes to disease pathogenesis. The classic autoinflammatory syndromes are usually related to monogenic (eg, cryopyrin-associated periodic syndromes [CAPS], TNF receptor–associated periodic syndrome) or polygenic mutations (eg, Crohn's disease) in genes important in the regulation of the innate immune response. Several autoinflammatory disorders, including CAPS[149] and Crohn's disease,[150] have been linked to mutations/genetic variants in nucleotide-binding domain and leucine-rich repeat pattern recognition receptor (NLR) family, pyrin domain-containing protein 3 (*NLRP3*) and overproduction of IL-1β. The adaptive immune response plays the predominant role in the clinical expression of monogenic (eg, immune dysregulation polyendocrinopathy enteropathy X-linked syndrome) and polygenic (eg, rheumatoid arthritis, systemic lupus erythematosus [SLE]) autoimmune diseases. However, innate immune mechanisms, in particular the NLRP3 inflammasome, are also emerging as important players in various autoimmune diseases, including SLE.[151] Some diseases, referred to as mixed-pattern diseases, are on the borderline between autoimmune and autoinflammatory diseases and may share genetic associations, treatment responses, and clinical manifestations.[152] DAMPs, danger-associated molecular patterns; Mϕ, macrophage; MHC, major histocompatibility complex; Mo, monocyte; Neu, neutrophil; PAMPs, pathogen-associated molecular patterns.

Overall, similar to the role of inflammation in the initiation of MDS, the cellular immune response in established MDS is multifactorial and follows a stepwise transformation from an activated protective to a more immunosuppressive response as the disease progresses. Discrete patterns of cytokine expression may be evident throughout MDS progression, and an integrative approach is required to study specific components of MDS pathogenesis in relation to cytokine network dynamics and immune cell states.

Microenvironment and MDS Progression

Inflammatory cues from the surrounding microenvironment may actively contribute to the formation and/or maintenance of a mutagenic environment in MDS and may also suppress immune effector responses.[71-74] Mesenchymal stromal cells (MSCs) and their progeny are important components of the HSPC niche and regulate hematopoiesis by cell-to-cell contact or through paracrine signals.[75] MSCs undergo functional decline with systemic aging.[76] This is further aggravated in MDS/AML MSCs, which have accumulated structural, epigenetic, and functional alterations, have chromosomal aberrations different from those found in HSPCs, and display activation of key inflammatory pathways.[77-81] Interestingly, MDS hematopoietic cells can instruct healthy MSCs to acquire MDS-like features.[78] In turn, MDS MSCs produce a variety of cytokines and other factors (eg, S100A8/9[25,81]) and exert immunomodulatory/-suppressive functions that could further promote propagation of malignant HSCs.[25,82] Mesenchymal S100A8/9 expression has been shown to be predictive of leukemic evolution and progression-free survival in a cohort of homogeneously treated patients with low-risk MDS, suggesting molecular characteristics of the mesenchymal niche as an important determinant of disease outcome.[25]

CLINICAL EXPERIENCE WITH IMMUNE INTERVENTIONS

Immunomodulatory therapies have long been used for MDS, with benefits for selected patient subgroups. Immunosuppressive therapy (IST) with antithymocyte globulin (ATG), and in combination with prednisone or cyclosporine, provides a therapeutic option for selected lower-risk patients, particularly those with hypoplastic MDS, a still poorly defined subgroup.[83-86] The immunomodulatory drug lenalidomide has shown a high rate of activity in lower-risk del(5q) MDS[87] but also yields sustained responses in 26.9% lower-risk non-del(5q) MDS, while predictive

immunologic biomarkers associated with this response are lacking.[88] Allogeneic HSCT is another type of immunotherapy that has long been used in MDS and could lead to a beneficial graft-versus-leukemia effect. The success of this therapeutic approach may also be based on its capacity to reprogram the niche-driven immune dysregulation in MDS.

Although recent progress in cancer immunology and the emergence of novel cancer immunotherapies brought new hope for many patients with cancer, including those with MDS and AML[69,89-92] (Table 1), the overall response rates to these therapies are variable and $< 50\%$ in the majority of malignancies, including MDS. So far, single-agent application of PD-1/PD-L1 as well as cytotoxic T-lymphocyte–associated antigen 4 checkpoint inhibitors has shown limited efficacy in advanced disease after hypomethylating agent (HMA) failure, with variable overall response rates as low as 0% for nivolumab (0/15),[93] 4% for pembrolizumab (1/27),[94] and 3.4% (1/29) to 22% (2/9) for ipilimumab.[93,95] Hence, combination strategies with checkpoint inhibitors both in the upfront as well as HMA-refractory setting to counteract HMA-induced checkpoint upregulation are currently under intensive investigation.[89,92,96] Nonetheless, single-agent therapy might display disease-modifying activity in selected patients, including elderly patients with AML.[97] Recent studies have also indicated the potential of targeting the innate immune checkpoint CD47-SIRPα in cancer, including hematologic cancers.[98,99] So far, blocking the interaction between the "don't-eat me" signal CD47 and the phagocyte inhibitory immunoreceptor SIRPα has shown low activity in a small AML/MDS cohort (1/10), but initial results from the combination therapy with 5-Aza are promising.[100] Altogether, there is growing evidence that the combination of drugs with different mechanisms of action might offer clinical benefit in MDS/AML, while the search for reliable biomarkers for response continues. This will require innovative and multicenter clinical trial designs to obtain meaningful results in larger patient cohorts.[101] It is worth mentioning that reliable predictors are also lacking for routine monotherapies. For instance, recent studies have evaluated how mutations correlate with clinical benefit from HMA therapy. Although earlier studies reported a favorable effect of *TET2* mutations on response rates,[102,103] this association was not confirmed in a different cohort.[104]

Finding predictive biomarker(s) for response to therapy is of particular relevance for the elderly population, which often displays lower response and higher toxicity rates. However, finding a magic "fits all" predictive biomarker in MDS is an unlikely scenario, considering the complexity of the disease and the role of several genetic, immunologic, and environmental factors in its pathophysiology. Technological advances in recent years, thanks to affordable omics experiments, led to a so-called "big data revolution." The challenge, however, is to integrate the massive amount of data and create computational models to build knowledge and identify signatures that are important in patients' stratification for immunotherapy.[105] To overcome this challenge, a more comprehensive and combinatorial approach is necessary, which uses individual biomarkers as part of the bigger picture rather than the whole story.

SYSTEMS IMMUNOLOGY: A WAY FORWARD

Framework for Comprehensive Immune Monitoring in Clinical Trials

Overall, sufficient evidence exists to support the role of the immunome as an important and independent factor in the stratification of patients with MDS/AML. Nonetheless, immune responses against malignant clones require coordination between cell types and across tissues, and a systems immunity screening approach is necessary to evaluate the overall "immune fitness" in cancer, as previously shown.[106] Data from recent cancer studies highlighting the power of integrative approaches are encouraging.[105,107] Nevertheless, there is still no standard or widely accepted method for monitoring the overall immune response in hemato-oncology in general or MDS in particular. Data from state-of-the-art immune monitoring strategies need to be merged with clinical data and other omics data for multiomics-driven analysis to identify robust and predictive immune signatures and map the interaction between disease-associated inflammation and potentially host-beneficial cellular immune responses (Fig 3). Multiomics-driven analysis has shown the power to identify key molecular pathways in cancer progression and could identify pathway-enriched cancer driver modules on the basis of DNA, RNA, and protein data.[108] For instance, web tools like LinkedOmics provide a user-friendly platform to explore, analyze, and compare cancer multiomics data within and across tumor types.[109] The widespread use of NGS technologies and the maturation of cutting-edge technologies, such as single-cell RNA-seq,[110] CITE-seq[111]/Ab-seq,[112] and mass cytometry by time of flight (CyTOF),[113] generate large datasets that can be mined for immunologically relevant parameters and serve as input for integrative data analysis.

Over the last years, NGS technologies are becoming increasingly important in the clinical setting for mutational profiling in MDS, using comprehensive myeloid NGS panels.[114] In many clinics, multiparameter flow cytometry (MFC) is increasingly used to reinforce MDS diagnosis.[115,116] MFC has also been extensively applied to characterize the immune landscape in MDS[11,12,60,62-67,117] and has demonstrated utility for monitoring immune-modifying agents in high-risk MDS/AML[118] or minimal residual disease monitoring, as has been shown in multiple myeloma.[119] CyTOF, which achieves an even higher resolution of the single-cell proteome, has been broadly

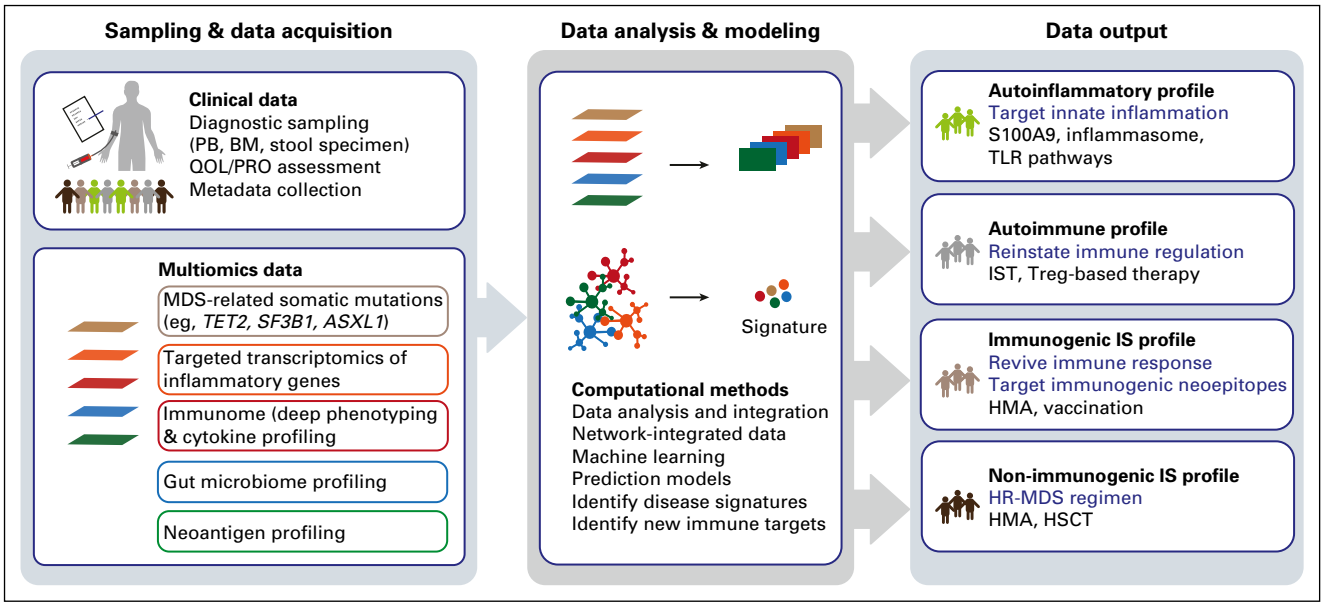

FIG 3. Multiomics pipeline for myelodysplastic syndrome (MDS). Implementing systems biology approaches in MDS is an unmet and urgent clinical need not only to understand the pathophysiology of this complex disease but also to create a more personalized approach to therapy. Multiple types of highly complex and rich omics data are being generated in large scale and are particularly helpful in risk stratification for patients with MDS and for identifying novel therapeutic targets. Different data types, including clinical, genomic (multigene next-generation sequencing–based panels), transcriptomic (single-cell RNA-seq), targeted transcriptomic (NanoString[153]), proteomic/immunophenotypic (CyTOF, flow cytometry), and metagenomic (16S ribosomal RNA sequencing, high-throughput shotgun sequencing) datasets, will be combined with the development of a bioinformatics pipeline, allowing an integrative view of the immunome in patients with MDS. The advent of new technologies like TARGET-seq,[154] which combines high-sensitivity single-cell mutational analysis and parallel RNA-seq, will further help to resolve inflammatory signatures of MDS genetic subclones and nonmutant cells. The analytical pipeline will use customized computational methods to incorporate single-cell and bulk multiomics data, leveraging on mathematical models to provide a holistic view of all components and modeling of biologic networks to identify disease signatures. This provides an unprecedented opportunity to identify immune profiles, examine the association between common driver mutations and immune subtype, and better understand how somatic mutations and immune cell activation states affect the disease course, response to treatment, and outcome. *ASXL1*, additional sex combs-like 1, transcriptional regulator; BM, bone marrow; HMA, hypomethylating agent; HR, higher-risk; HSCT, hematopoietic stem cell transplantation; IS, immunosuppressive; IST, immunosuppressive therapy; PB, peripheral blood; QOL/PRO, quality of life/patient-reported outcome; *SF3B1*, RNA splicing factor 3B, subunit 1; *TET2*, tet methylcytosine dioxygenase 2; TLR, Toll-like receptor; Treg, regulatory T cell.

applied in the solid cancer field to profile the tumor immune landscape,[120,121] to monitor checkpoint-blockade–induced immune responses, and to predict response to PD-1 immunotherapy.[122,123] CyTOF has also been already successfully adopted for immunophenotypic analysis of clinical samples in MDS,[124] for prospective immune monitoring of patients with chronic myeloid leukemia (CML),[125] and to further characterize the immune signature in a wider range of T-cell subsets in MDS.[126]

There are, however, two important questions to be addressed: (1) Which immunologic markers to use? and (2) How will we define an immunoscore? We are still in the early days, but resources are already available that could be used and customized for MDS/AML. In an attempt to identify and characterize all major human immune cell lineages in a single assay, Hartmann et al[127] have designed and validated a CyTOF panel that can be incorporated into cancer immunotherapy trials. This framework provides a set of markers also relevant for future clinical trials in MDS and may be extended by markers relevant for additional immunophenotyping of immune cell subsets and HSPCs (Appendix Table A1, online only).[128-137]

In solid tumors, infiltrating T cells have been generally associated with a positive prognosis, which led to the development of the immunoscore, a scoring system based on the quantification of cytotoxic and memory T cells in the tumor center and invasive margin.[138,139] Although this immunohistochemical tool has demonstrated prognostic value for solid tumors,[140] it cannot be directly applied to the MDS/AML BM microenvironment, which lacks a clear invasive margin and a tumor core. However, automated image analysis of BM tissues in combination with flow cytometry and clinical parameters has been shown useful for predicting treatment responses in CML.[141] A comprehensive immunoscore for MDS will likely be based on multivariate features derived from genomic, transcriptomic, and proteomic data (Fig 3; Appendix Fig A1, online only). The solid tumor field provides examples of how such immune profiling can be used to train predictive models and generate

immunoscores.[142-144] Overall, this will require an expanding computational toolbox to process, analyze, and visualize the highly complex and heterogeneous datasets being generated on bulk tissue and at single-cell level (reviewed by Finotello et al[145]) as well as validation of predictive biomarkers in independent cohorts and across MDS subtypes.

Moreover, comprehensive interrogation of cancer immunity in MDS requires longitudinal as well as paired sampling to evaluate the impact of a given therapy on peripheral blood immune cells and the BM immune microenvironment. Combinatorial agents, such as 5-Aza and lenalidomide, can exert direct immunomodulatory effects on immune cells and BM MSCs.[79,146,147] Thus, careful dissection of the net immunomodulatory effects of combination therapy through serial assessment can provide adequate information regarding activation of alternative pathways and inform subsequent clinical trials.

Dissecting Good and Not so Good Immune Responses

Although it is, for instance, possible that autoinflammatory and autoimmune features are present in a single patient, a dominant clinical representation of one of these conditions is more likely. An important aspect of immune profiling in MDS would therefore be to identify patients with MDS with an underlying autoimmune response that could benefit from IST or potentially Treg-based therapies to reinstate immune regulation (Fig 3). Immune profiling may also help to identify patients with lower-risk MDS who harbor a signature characteristic of smoldering innate inflammation in the absence of autoimmune disease. These patients may benefit from novel therapies targeting S100A8/9-related inflammasome activation or TLR pathways. Patients with potentially immunogenic somatic mutations may benefit from novel vaccination therapies with or without immune checkpoint inhibitors to reinstate the beneficial immune response against dysplastic clones. On the other hand, it is equally important to identify patients without dominant inflammatory/autoimmune features or immunogenic somatic mutations who are less likely to respond to novel immunotherapies and may benefit from other forms of therapies, such as early HSCT.

CONCLUSION

In conclusion, collection of comprehensive omics datasets will leverage the development of a computational pipeline specific to MDS that will help to identify key features at various biologic levels and their interconnectivity, and to better predict patient outcomes. To achieve this, well-coordinated studies on large cohorts of patients are crucial to combine known as well as potentially relevant predictive immunologic biomarkers with clinical data. We expect that applying validated immune signatures to routine clinical investigations will improve patients' stratification for therapeutic intervention and ultimately improve patient outcomes.

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EQUAL CONTRIBUTION

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Integrating the "Immunome" in the Stratification of Myelodysplastic Syndromes and Future Clinical Trial Design

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APPENDIX

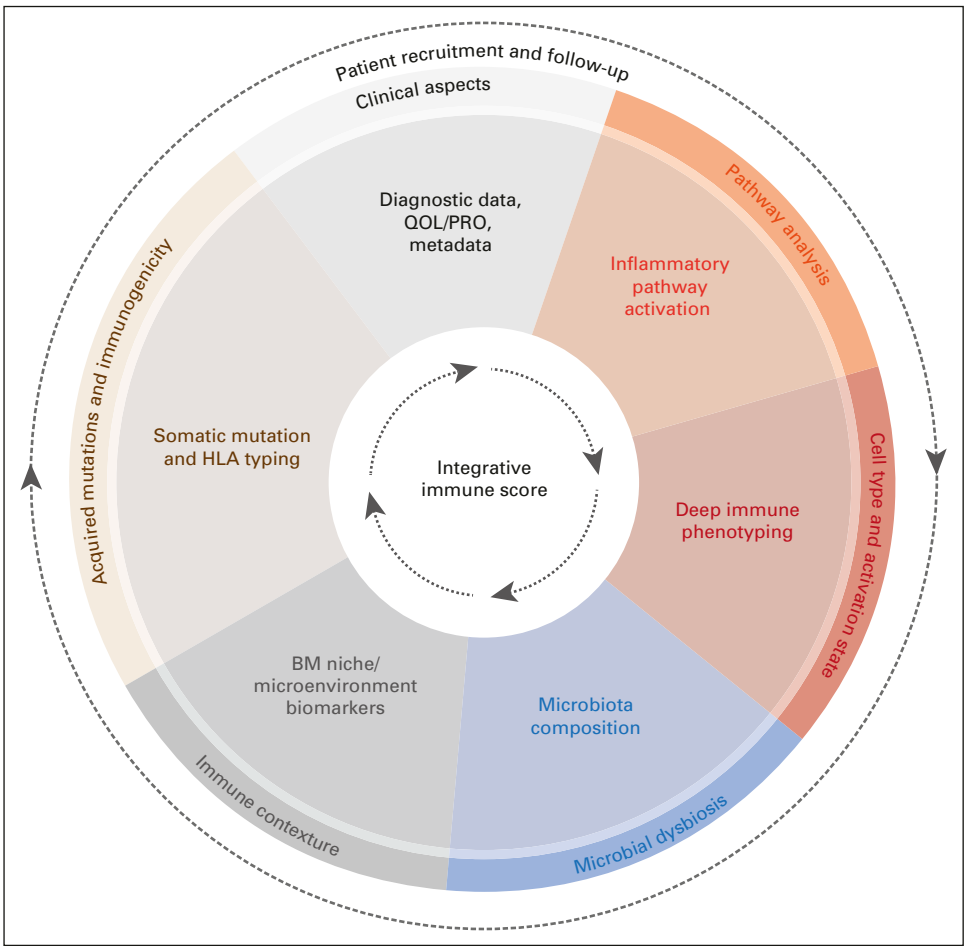

FIG A1. Integrative immunoscore for myelodysplastic syndrome (MDS). Integration of data from different omic platforms with clinical data could identify a biomarker panel to improve stratification of patients with MDS. BM, bone marrow; HLA, human leukocyte antigen; QOL/PRO, quality of life/patient-reported outcome.

TABLE A1. Immune Cell Markers of Relevance for Future Clinical Trials in MDS

Cell Type	Antigen/Marker	Reference
T-cell subsets and checkpoints (Th, CTLs: naïve, effector, effector memory, central memory, regulatory T cells; NKT; γδ T cells)	CD3, CD4, CD8a, CD25, CD27, CD28*, CD38, CD45RA, CD45RO, CD56, CD69*, CD95*, CD127 (IL-7Rα), CD152 (CTLA-4), CD161*, CD197 (CCR7), CD223 (LAG-3)*, CD279 (PD-1), FoxP3, IL-17*, HLA-DR, T-bet, γδTCR, TIM-3	11,13,47,59,63,65,67,126,128,129,138,148
B-cell subsets (naïve, memory, plasmablasts/plasma cells)	CD10*, CD19, CD24*, CD27, CD38, CD40*, HLA-DR, IgD*	130
NK-cell subsets (cytokine-producing, cytolytic)	CD8a, CD16, CD38, CD56, CD57*, CD69*, CD161*, T-bet, NK receptors*	66,131,132
Myeloid-cell types/subsets (classic/intermediate/nonclassical monocytes, macrophages, cDCs/pDCs, MDSCs, neutrophils, basophils, eosinophils, mast cells)	CD11b, CD11c, CD13*, CD14, CD15*, CD16, CD33, CD64*, CD66b*, CD117, CD123, CD203c*, CD273 (PD-L2)*, CD274 (PD-L1), FcεR1α, HLA-DR	64,115,117,122,133,134
HSPCs and checkpoints (stem cells, progenitor populations)	CD10*, CD34*, CD38, CD45RA, CD71*, CD90*, CD117, CD123, CD133*, CD135 (FLT-3)*, CD273 (PD-L2)*, CD274 (PD-L1), CD279 (PD-1), IL1RAP*	13,59,115,133,135-137
General markers	CD45, CD235ab, CD61, DNA, live/dead, lineage markers (for exclusion)	

NOTE. Adapted and modified from Hartmann et al.[127]

Abbreviations: cDCs, conventional dendritic cells; CTLs, cytotoxic T cells; HSPCs, hematopoietic stem and precursor cells; MDSCs, myeloid-derived suppressor cells; NK, natural killer; NKT, natural killer T cells; pDCs, plasmacytoid dendritic cells; Th, T helper cells.

*Additional markers are indicated.