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Review Article

Integrating the 'Immunome' in the stratification of myelodysplastic syndromes and future clinical trial design

Running title: Systems immunology; a way forward in MDS

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Abstract

Myelodysplastic syndromes (MDS) are characterised by ineffective haematopoiesis and often include a dysregulation and dysfunction of the immune system. In the context of population ageing, MDS incidence is set to rise 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 smouldering 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 sub-stratification, thereby empowering practical predictions of response to treatment in MDS. We propose novel multicentre studies, which are needed to achieve this goal.

Keywords: MDS, immune dysregulation, immune profile, patient stratification

Introduction

Myelodysplastic syndromes (MDS) represent a group of acquired clonal disorders of haematopoietic stem and progenitor cells (HSPCs), characterised by ineffective haematopoiesis, peripheral cytopenias, genetic instability, and an increased risk of progression to acute myeloid leukaemia (AML)[1]. Considering the higher prevalence in elderly patients, the population ageing in developed countries as well as higher diagnostic awareness, the incidence of MDS is set to rise 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 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]. Whilst 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 since the IPSS-R, like the original IPSS, was developed based on clinical data from untreated MDS patients. 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, the use and outcomes of existing treatments and 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 as well as potential therapeutic applications, and outline how immune profiling could be implemented in future clinical trials.

Predisposing and potential driving immune factors

a) Smouldering inflammation and immunosenescence

Chronic inflammation due to 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 signalling in age-related haematopoietic senescence[16], bone loss[17], and MDS[18]. In fact, normal human ageing 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 (HMGB1) 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 physiological 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 favouring the susceptibility to age-related inflammatory disorders, autoimmunity, and deterioration of haematopoiesis. A reduced capacity to defend against pathogens and to initiate adaptive immunity is observed in ageing humans, together with enhanced pro-inflammatory reactions fuelled by 'endogenous/self-molecular garbage'[20, 21]. The presence of 'smouldering' 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 haematopoiesis of indeterminate potential [CHIP][22]), subvert adaptive immunity, and alter cellular responses to therapeutic intervention.

b) NLRP3 inflammasome: a driver of chronic inflammation in MDS

Increased levels of DAMPs (e.g. 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 since 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 pro-inflammatory 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 pro-inflammatory cytokines, reactive oxygen species (ROS), 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 signalling[31] or aberrant activation of the IL-1/p38MAPK pathway[32]. NLRP3 inflammasome activation appears to be licensed by S100A8/9 and MDS-related gene mutations and is also evident in del(5q) MDS patients, 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 signalling 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, therefore most likely to benefit from inflammasome pathway inhibition.

c) 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 smouldering 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 (e.g. *TET2*, *ASXL1*) and RNA splicing factors (e.g. *SF3B1*, *SRSF2*) appear to represent predominantly 'founder' events[33]. Mutations in several of these genes have been linked to activated NLRP3 inflammasomes and enhanced innate immune signalling[18, 38–40]. Such mutant gene licensing of innate signalling 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 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 characterised 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 co-morbidities[44], and suggest *NLRP3* as a shared genetic risk factor for MDS and para-neoplastic 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]. Due to the overall lower somatic mutation burden in both AML and MDS compared to other types of tumours[46], the potential immunogenicity of these mutations is largely 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.

d) The microbiome and its impact on inflammation and immunome

Profound changes in the microbiota and its interaction with the immune system are increasingly recognised to contribute to chronic inflammatory diseases, including

haematologic 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 anaemia, highlighting the intricate relationship between host-microbiome and haematopoiesis[50].

Although no detailed study exists concerning the microbiome composition in MDS, the role of microbial-dependent inflammation in the development of pre-leukaemic 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 haematopoietic 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 haematopoietic 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 (e.g. 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.

e) 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' coins a condition associated with the presence of autoreactive T cells and high autoantibody titres, whereas 'autoinflammation' generally refers to a condition with dysregulated myeloid-driven innate immune responses only. This view clearly separated autoinflammation and autoimmunity as distinct immunological 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, common inflammatory pathways (TLR, PI3K-Akt, and NF-κB signalling), 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) (OR 2.1; 95% CI 1.7-2.6) or infectious disease (OR 1.3; 95% CI 1.1-1.5), 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 favourable 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

a) 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 characterised by excess pro-inflammatory cytokines, such as IL-1β, TNF-α, and IFN-γ (FIG. 1). Pro-inflammatory 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]. Moreover, cytokine-mediated induction of immunoinhibitory molecules like programmed cell death-ligand 1 (PD-L1) may contribute to T cell suppression and reduced immune surveillance[59]. On the other hand, excess DAMPs may expand myeloid-derived suppressor cells (MDSCs)[60], which overproduce suppressive cytokines, such as IL-10 and transforming growth factor-β (TGF-β), contributing to immunosuppression and ineffective haematopoiesis[60, 61].

In general, low-risk disease is related to a more pro-inflammatory immune response and higher numbers of effector-type cells, such as IL-17$^+$ CD4$^+$ cells[11], while higher-risk disease is characterised 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[65], and 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 MDS patients 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].

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.

b) 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 might suppress immune effector responses[71–74]. Mesenchymal stromal cells (MSCs) and their progeny are important components of the HSPC niche and regulate haematopoiesis by cell-to-cell contact or through paracrine signals[75]. MSCs undergo functional decline with systemic ageing [76]. This is further aggravated in MDS/AML MSCs, which have accumulated structural, epigenetic and functional alterations, chromosomal aberrations different from those found in HSPCs, and display activation of key inflammatory pathways[77–81]. Interestingly, MDS haematopoietic cells

can instruct healthy MSCs to acquire MDS-like features[78]. In turn, MDS MSCs produce a variety of cytokines and other factors (e.g. 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 leukaemic evolution and progression-free survival in a cohort of homogeneously treated low-risk MDS patients, 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 employed 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 immunological biomarkers associated with this response are lacking[88]. Allogeneic HSCT is another type of immunotherapy which has long been used in MDS and could lead to a beneficial graft-versus-leukaemia (GvL) effect. The success of this therapeutic approach may also be based on its capacity to reprogram the niche-driven immune dysregulation in MDS.

While recent progress in cancer immunology and the emergence of novel cancer immunotherapies brought new hope for many cancer patients, including those with MDS and AML[69, 89–92] (TABLE 1), the overall response rates to these therapies are variable and less than 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 (CTLA-4) checkpoint inhibitors

(CIs) 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)-22% (2/9) for ipilimumab[93, 95]. Hence, combination strategies with CIs 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 AML patients[97]. Recent studies have also indicated the potential of targeting the innate immune checkpoint CD47-SIRPα in cancer, including haematologic 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 mechanism of action might offer clinical benefit in MDS/AML, while the search for reliable biomarkers for response continues. This will require innovative and multicentre 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. While earlier studies reported a favourable 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, immunological, 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 utilises individual biomarkers as part of the bigger picture rather than the whole story.

Systems Immunology; a way forward

a) 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 MDS/AML patients` stratification. 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 haemato-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 based on DNA, RNA, and protein data[108]. For instance, web tools like LinkedOmics provide a user-friendly platform to explore, analyse, and compare cancer multiomics data within and across tumour 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 increasingly used in the clinical setting for mutational profiling in MDS, utilising 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 characterise 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 applied in the solid cancer field to profile the tumour immune landscape[120, 121], to monitor checkpoint-blockade-induced immune responses, and predict response to PD-1 immunotherapy[122, 123]. CyTOF has also been already successfully adopted for immunophenotypic analysis of clinical samples in MDS[124], prospective immune monitoring of patients with chronic myeloid leukaemia (CML)[125], and to further characterise 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 immunological markers to use? 2) How will we define an immunoscore? We are still in the early days but resources are already available, which could be used and customised for MDS/AML. In an attempt to identify and characterise all major human immune cell lineages in a single assay, Hartmann et al. have designed and validated a CyTOF panel that can be incorporated into cancer immunotherapy trials[127]. This framework provides a set of markers also relevant for future clinical trials in MDS and may be extended by markers relevant for further immunophenotyping of immune cell subsets and HSPCs (supplementary TABLE S1).

In solid tumours, 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 tumour centre and invasive margin[128, 129]. While this immunohistochemical tool has demonstrated prognostic value for solid tumours[130], it cannot be directly applied to the MDS/AML BM microenvironment, which lacks a clear invasive margin and a tumour 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[131]. A comprehensive immunoscore for MDS will likely be based on multivariate features derived from genomic, transcriptomic, and proteomic data (FIG. 3 and supplementary FIG. S1). The solid tumour field provides examples of how such immune profiling can be used to train predictive models and generate immunoscores[132–134]. Overall, this will require an expanding computational toolbox to process, analyse and visualise the highly complex and heterogeneous datasets being generated on bulk tissue and at single-cell level (reviewed by Finotello et al.[135]) 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, 136, 137]. 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.

b) Dissecting good and not so good immune responses

While 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 MDS patients with an underlying autoimmune response that could benefit from immunosuppressive therapy (IST) or potentially Treg-based therapies to reinstate immune regulation (FIG. 3). Immune profiling may also help to identify lower-risk MDS patients who harbour a signature characteristic of smouldering 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 CIs 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 biological levels, 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 immunological 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.

Figure legends

Fig. 1: The immune contexture in MDS. Certain conditions associated with chronic immune stimulation, such as ageing, 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 haematopoietic skewing and loss of stem cell quiescence. Initial events may induce a 'myeloid bias' of HSCs and multipotent progenitors, and such a bias could skew the accumulation of somatic mutations conferring clonal advantage and/or differentiation defects towards the myeloid lineage. Elevated levels of pro-inflammatory cytokines, reactive oxygen/nitrogen species, and DAMPs induce activation of the NLRP3 inflammasome, resulting in pyroptosis of HSPCs, consequent cytopenias, an inflammasome-driven inflammatory circuit, and an increasing dysfunction of the haematopoietic stem cell niche including mesenchymal alterations (middle). Subsequently, the presence of smouldering inflammation may support the propagation of pre-malignant clones (e.g. 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 MDSCs and Tregs contributes to the suppression of antitumour 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 anaemia[138]. Abbreviations: *ASXL1*, additional sex combs-like 1, transcriptional regulator; DAMP, damage-associated molecular pattern; DC, dendritic cell; *DNMT3A*, DNA methyltransferase 3 alpha; HIF-1α, hypoxia-inducible factor 1, alpha subunit; HSPC, haematopoietic stem and precursor cell; IL-1R1, interleukin-1 receptor, type 1; IL-1RAP, interleukin-1 receptor accessory protein; M,

410 macrophage; MDSC, myeloid-derived suppressor cell; MSC; mesenchymal stromal cell; NK,
411 natural killer cell; NLRP3; nucleotide-binding domain and leucine-rich repeat pattern
412 recognition receptor (NLR) family, pyrin domain-containing protein 3; ROS, reactive oxygen
413 species; RNS, reactive nitrogen species; *SF3B1*, RNA splicing factor 3B, subunit 1; *SRSF2*,
414 serine/arginine-rich splicing factor 2; STAT3-P, signal transducer and activator of transcription
415 3, phosphorylated; *TET2*, tet methylcytosine dioxygenase 2; TLR, Toll-like receptor; TNFR,
416 tumour necrosis factor receptor; Treg, regulatory T cell; *U2AF1*, U2 small nuclear RNA auxiliary
417 factor 1.

418 **Fig. 2: MDS across the autoinflammatory/autoimmune disease continuum.** The clinical
419 heterogeneity of MDS may reflect the variable contribution of autoinflammatory and
420 autoimmune processes to disease pathogenesis. The classic autoinflammatory syndromes are
421 usually related to monogenic (e.g. cryopyrin-associated periodic syndromes [CAPS], TNF
422 receptor-associated periodic syndrome [TRAPS]) or polygenic mutations (e.g. Crohn's disease)
423 in genes important in the regulation of the innate immune response. Several
424 autoinflammatory disorders, including CAPS[139] and Crohn's disease[140], have been linked to
425 mutations/genetic variants in NLRP3 and overproduction of IL-1β. The adaptive immune
426 response plays the predominant role in the clinical expression of monogenic (e.g. immune
427 dysregulation polyendocrinopathy enteropathy X-linked syndrome [IPEX]) and polygenic (e.g.
428 rheumatoid arthritis, systemic lupus erythematosus [SLE]) autoimmune diseases. However,
429 innate immune mechanisms, in particular the NLRP3 inflammasome, are also emerging as
430 important players in various autoimmune diseases, including SLE[141]. Some diseases, referred
431 to as mixed-pattern diseases, are on the borderline between autoimmune and
432 autoinflammatory diseases, and may share genetic associations, treatment responses and
433 clinical manifestations[142]. Abbreviations: DAMPs, danger-associated molecular patterns; Mϕ,

macrophage; MHC class II, major histocompatibility complex class II; Mo, monocyte; Neu, neutrophil; NLRP3; nucleotide-binding domain and leucine-rich repeat pattern recognition receptor (NLR) family, pyrin domain-containing protein 3; PAMPs, pathogen-associated molecular patterns.

Fig. 3: Multiomics pipeline for MDS. Implementing systems biology approaches in MDS is an unmet and urgent clinical need to not only understand the pathophysiology of this complex disease but also to create a more personalised approach to therapy. Multiple types of highly complex and rich omics data are being generated in large scale and are particularly helpful in MDS patients' risk stratification and for identifying novel therapeutic targets. Different data types, including clinical, genomic (multigene NGS-based sequencing panels), transcriptomic (single-cell RNA-seq), targeted transcriptomic (NanoString[143]), proteomic/immunophenotypic (CyTOF, flow cytometry), and metagenomic (16S ribosomal rRNA sequencing, high-throughput shotgun sequencing) datasets, will be combined with the development of a bioinformatics pipeline, allowing an integrative view of the immunome in MDS patients. The advent of new technologies like TARGET-seq[144], which combines high-sensitivity single-cell mutational analysis and parallel RNA-seq, will further help to resolve inflammatory signatures of MDS genetic subclones and non-mutant cells. The analytical pipeline will employ customized computational methods to incorporate single-cell and bulk multiomics data, leveraging on mathematical models to provide a holistic view of all components and modelling of biological 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 to better understand how somatic mutations and immune cell activation states impact the disease course, response to treatment, and outcome. Abbreviations: *ASXL1*, additional sex combs-like 1, transcriptional regulator; BM, bone

458 marrow; HMA, hypomethylating agent; HR, higher-risk; HSCT, haematopoietic stem cell
459 transplantation; IS, immunosuppressive; IST, immunosuppressive therapy; PB, peripheral
460 blood; QOL/PRO, quality of life/patient reported outcome; *SF3B1*, RNA splicing factor 3B,
461 subunit 1; *TET2*, tet methylcytosine dioxygenase 2; TLR, Toll-like receptor.

462 Table legends

463 **Table 1: Novel therapeutic agents evaluating immune targets in MDS**

464 Aza, 5-azacytidine; BTK, Bruton's tyrosine kinase; CAR, chimeric antigen receptor; CCUS, clonal
465 cytopenia of undetermined significance; HMA, hypomethylating agent; int-1, intermediate-1;
466 int-2, intermediate-2; Len, lenalidomide; MM, multiple myeloma; RAEB, refractory anemia
467 with excess blasts; R/R, refractory/relapsed; TRIKE; trispecific killer engager.

468

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Author contributions

S.W., S.K., and U.P. designed the review. All authors contributed to the writing and editing of the manuscript.

References

1. Tefferi A, Vardiman JW: Myelodysplastic Syndromes. N Engl J Med 361:1872–1885, 2009

2. Sekeres MA: The epidemiology of myelodysplastic syndromes. Hematol Oncol Clin North Am 24:287–294, 2010

3. Greenberg PL, Tuechler H, Schanz J, et al: Revised international prognostic scoring system for myelodysplastic syndromes. Blood 120:2454–2465, 2012

4. Blum S, Gattermann N, Martins F, et al: Is the IPSS-R Useful for Patients with MDS Receiving Disease-Modifying Treatment? Blood 128:5526 LP – 5526, 2016

5. Haferlach T, Nagata Y, Grossmann V, et al: Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia 28:241–247, 2014

6. Makishima H, Yoshizato T, Yoshida K, et al: Dynamics of clonal evolution in myelodysplastic syndromes. Nat Genet 49:204–212, 2017

7. Pellagatti A, Armstrong RN, Steeples V, et al: Impact of spliceosome mutations on RNA splicing in myelodysplasia: Dysregulated genes/pathways and clinical associations. Blood 132:1225–1240, 2018

8. Nazha A, Al-Issa K, Hamilton BK, et al: Adding molecular data to prognostic models can improve predictive power in treated patients with myelodysplastic syndromes. Leukemia 31:2848–2850, 2017

9. Nazha A, Narkhede M, Radivoyevitch T, et al: Incorporation of molecular data into the Revised International Prognostic Scoring System in treated patients with myelodysplastic syndromes. Leukemia 30:2214–2220, 2016

505 **10**. Sallman DA, List A: The central role of inflammatory signaling in the pathogenesis of
506 myelodysplastic syndromes. Blood 133:1039–1048, 2019

507 **11**. Kordasti SY, Afzali B, Lim Z, et al: IL-17-producing CD4+T cells, pro-inflammatory cytokines
508 and apoptosis are increased in low risk myelodysplastic syndrome. Br J Haematol 145:64–72,
509 2009

510 **12**. Kittang AO, Kordasti S, Sand KE, et al: Expansion of myeloid derived suppressor cells
511 correlates with number of T regulatory cells and disease progression in myelodysplastic
512 syndrome. Oncoimmunology 5:e1062208-1–9, 2016

513 **13**. Kahn JD, Chamuleau MED, Westers TM, et al: Regulatory T cells and progenitor B cells are
514 independent prognostic predictors in lower risk myelodysplastic syndromes. Haematologica
515 100:e220–e222, 2015

516 **14**. Elinav E, Nowarski R, Thaiss CAA, et al: Inflammation-induced cancer: Crosstalk between
517 tumours, immune cells and microorganisms. Nat Rev Cancer 13:759–771, 2013

518 **15**. Diakos CI, Charles KA, McMillan DC, et al: Cancer-related inflammation and treatment
519 effectiveness. Lancet Oncol 15:e493–e503, 2014

520 **16**. Esplin BL, Shimazu T, Welner RS, et al: Chronic Exposure to a TLR Ligand Injures
521 Hematopoietic Stem Cells. J Immunol 186:5367–5375, 2011

522 **17**. Youm YH, Grant RW, McCabe LR, et al: Canonical Nlrp3 inflammasome links systemic low-
523 grade inflammation to functional decline in aging. Cell Metab 18:519–532, 2013

524 **18**. Basiorka AA, McGraw KL, Eksioglu EA, et al: The NLRP3 inflammasome functions as a driver
525 of the myelodysplastic syndrome phenotype. Blood 128:2960–2975, 2016

526 **19**. Medzhitov R: Origin and physiological roles of inflammation. Nature 454:428–435, 2008

527 **20**. Franceschi C, Garagnani P, Vitale G, et al: Inflammaging and 'Garb-aging.' Trends
528 Endocrinol Metab 28:199–212, 2017

529 **21**. Boraschi D, Aguado MT, Dutel C, et al: The Gracefully Aging Immune System. Sci Transl
530 Med 5:185ps8 LP-185ps8, 2013

531 **22**. Steensma DP, Bejar R, Jaiswal S, et al: Perspective : Clonal Hematopoiesis of Indeterminate
532 Potential and Its Distinction from Myelodysplastic Syndromes. Blood 126:9–16, 2015

533 **23**. Velegraki M, Papakonstanti E, Mavroudi I, et al: Impaired clearance of apoptotic cells leads
534 to HMGB1 release in the bone marrow of patients with myelodysplastic syndromes and
535 induces TLR4-mediated cytokine production. Haematologica 98:1206–1215, 2013

536 **24**. Schneider RK, Schenone M, Ferreira MV, et al: Rps14 haploinsufficiency causes a block in
537 erythroid differentiation mediated by S100A8 and S100A9. Nat Med 22:288–297, 2016

538 **25**. Zambetti NA, Ping Z, Chen S, et al: Mesenchymal Inflammation Drives Genotoxic Stress in
539 Hematopoietic Stem Cells and Predicts Disease Evolution in Human Pre-leukemia. Cell Stem
540 Cell 19:613–627, 2016

541 **26**. Maratheftis CI, Andreakos E, Moutsopoulos HM, et al: Toll-like receptor-4 is up-regulated
542 in hematopoietic progenitor cells and contributes to increased apoptosis in myelodysplastic
543 syndromes. Clin Cancer Res 13:1154–1160, 2007

544 **27**. Wei Y, Dimicoli S, Bueso-Ramos C, et al: Toll-like receptor alterations in myelodysplastic
545 syndrome. Leukemia 27:1832–1840, 2013

546 **28**. Rhyasen GW, Bolanos L, Fang J, et al: Targeting IRAK1 as a Therapeutic Approach for

547 Myelodysplastic Syndrome. Cancer Cell 24:90–104, 2013

548 **29**. Fang J, Bolanos LC, Choi K, et al: Ubiquitination of hnRNPA1 by TRAF6 links chronic innate
549 immune signaling with myelodysplasia. Nat Immunol 18:236–245, 2017

550 **30**. Simard JC, Cesaro A, Chapeton-Montes J, et al: S100A8 and S100A9 induce cytokine
551 expression and regulate the NLRP3 inflammasome via ROS-dependent activation of NF-κB(1.).
552 PLoS One 8:e72138, 2013

553 **31**. Bhagat TD, Chen S, Bartenstein M, et al: Epigenetically aberrant stroma in MDS propagates
554 disease via Wnt/β-catenin activation. Cancer Res 77:4846–4857, 2017

555 **32**. Carey A, Edwards DK, Eide CA, et al: Identification of Interleukin-1 by Functional Screening
556 as a Key Mediator of Cellular Expansion and Disease Progression in Acute Myeloid Leukemia.
557 Cell Rep 18:3204–3218, 2017

558 **33**. Mossner M, Jann JC, Wittig J, et al: Mutational hierarchies in myelodysplastic syndromes
559 dynamically adapt and evolve upon therapy response and failure. Blood 128:1246–1259, 2016

560 **34**. McGranahan N, Furness AJS, Rosenthal R, et al: Clonal neoantigens elicit T cell
561 immunoreactivity and sensitivity to immune checkpoint blockade. Science (80-) 351:1463–
562 1469, 2016

563 **35**. Wolf Y, Bartok O, Patkar S, et al: UVB-Induced Tumor Heterogeneity Diminishes Immune
564 Response in Melanoma. Cell 179:219-235.e21, 2019

565 **36**. Hollstein PE, Shaw RJ: Inflamed T cells and stroma drive gut tumors. Science (80-) 361:332
566 LP – 333, 2018

567 **37**. Hirsch D, Wangsa D, Zhu YJ, et al: Dynamics of genome alterations in Crohn's disease-

associated colorectal carcinogenesis. Clin Cancer Res 24:4997–5011, 2018

38. Pollyea DA, Harris C, Rabe JL, et al: Myelodysplastic syndrome-associated spliceosome gene mutations enhance innate immune signaling. Haematologica 104:e388–e392, 2019

39. Meisel M, Hinterleitner R, Pacis A, et al: Microbial signals drive pre-leukaemic myeloproliferation in a Tet2-deficient host. Nature 557:580–584, 2018

40. Smith MA, Choudhary GS, Pellagatti A, et al: U2AF1 mutations induce oncogenic IRAK4 isoforms and activate innate immune pathways in myeloid malignancies. Nat Cell Biol 21:640–650, 2019

41. Thol F, Klesse S, Köhler L, et al: Acute myeloid leukemia derived from lympho-myeloid clonal hematopoiesis. Leukemia 31:1286–1295, 2017

42. Jaiswal S, Natarajan P, Silver AJ, et al: Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease. N Engl J Med 377:111–121, 2017

43. Cook EK, Izukawa T, Young S, et al: Feeding the Fire: The Comorbid and Inflammatory Backdrop of Clonal Hematopoiesis of Indeterminate Potential (CHIP) By Mutation Subtype. Blood 130:426 LP – 426, 2017

44. Fuster JJ, MacLauchlan S, Zuriaga MA, et al: Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. Science (80-) 355:842–847, 2017

45. Mian SA, Kulasekararaj AG, Kizilors A, et al: NLRP3 Inflammosome Polymorphisms Are Enriched in Myelodysplastic Syndrome Patients with Autoimmune Disorders. Blood 126:1659–1659, 2015

46. Martincorena I, Campbell PJ: Somatic mutation in cancer and normal cells. Science (80-)

349:1483–1489, 2015

47. Coats T, Smith AE, Mourikis A, et al: Neoantigens in MDS Are Associated with Two Novel CD4+ T Cell Subsets and Improved Overall Survival. Blood 130:2958 LP – 2958, 2017

48. Belkaid Y, Hand TW: Role of the microbiota in immunity and inflammation. Cell 157:121–141, 2014

49. Manzo VE, Bhatt AS: The human microbiome in hematopoiesis and hematologic disorders. Blood 126:311–318, 2015

50. Josefsdottir KS, Baldridge MT, Kadmon CS, et al: Antibiotics impair murine hematopoiesis by depleting the intestinal microbiota. Blood 129:729–739, 2017

51. Peled JU, Devlin SM, Staffas A, et al: Intestinal microbiota and Relapse after hematopoietic-Cell transplantation. J Clin Oncol 35:1650–1659, 2017

52. Kanauchi O, Fukuda M, Matsumoto Y, et al: Eubacterium limosum ameliorates experimental colitis and metabolite of microbe attenuates colonic inflammatory action with increase of mucosal integrity. World J Gastroenterol 12:1071–1077, 2006

53. Kulasekararaj AG, Kordasti S, Basu T, et al: Chronic relapsing remitting Sweet syndrome - a harbinger of myelodysplastic syndrome. Br J Haematol 170:649–656, 2015

54. Komrokji RS, Kulasekararaj A, Al Ali NH, et al: Autoimmune diseases and myelodysplastic syndromes. Am J Hematol 91:E280–E283, 2016

55. Caso F, Costa L, Nucera V, et al: From autoinflammation to autoimmunity: old and recent findings. Clin Rheumatol 37:2305–2321, 2018

56. Arakelyan A, Nersisyan L, Poghosyan D, et al: Autoimmunity and autoinflammation: A

610 systems view on signaling pathway dysregulation profiles. PLoS One 12:e0187572, 2017

611 **57**. Kristinsson SY, Björkholm M, Hultcrantz M, et al: Chronic immune stimulation might act as
612 a trigger for the development of acute myeloid leukemia or myelodysplastic syndromes. J Clin
613 Oncol 29:2897–2903, 2011

614 **58**. Chen DS, Mellman I: Oncology meets immunology: The cancer-immunity cycle. Immunity
615 39:1–10, 2013

616 **59**. Kondo A, Yamashita T, Tamura H, et al: Interferon-γ and tumor necrosis factor-α induce an
617 immunoinhibitory molecule, B7-H1, via nuclear factor-κB activation in blasts in
618 myelodysplastic syndromes. Blood 116:1124–1131, 2010

619 **60**. Chen X, Eksioglu E a, Zhou J, et al: Induction of myelodysplasia by myeloid- derived
620 suppressor cells. J Clin Invest 123:4595–4611, 2013

621 **61**. Zhou L, Nguyen AN, Sohal D, et al: Inhibition of the TGF-beta receptor I kinase promotes
622 hematopoiesis in MDS. Blood 112:3434–3443, 2008

623 **62**. Kordasti SY, Ingram W, Hayden J, et al: CD4 + CD25 high Foxp3 + regulatory T cells in
624 myelodysplastic syndrome (MDS). Blood 110:847–850, 2007

625 **63**. Kotsianidis I, Bouchliou I, Nakou E, et al: Kinetics, function and bone marrow trafficking of
626 CD4+CD25+FOXP3+regulatory T cells in myelodysplastic syndromes (MDS). Leukemia 23:510–
627 518, 2009

628 **64**. Saft L, Björklund E, Berg E, et al: Bone marrow dendritic cells are reduced in patients with
629 high-risk myelodysplastic syndromes. Leuk Res 37:266–273, 2013

630 **65**. Lopes MR, Traina F, Campos P de M, et al: IL10 inversely correlates with the percentage of

631 CD8+ cells in MDS patients. Leuk Res 37:541–546, 2013

632 **66**. Epling-Burnette PK, Bai F, Painter JS, et al: Reduced natural killer (NK) function associated
633 with high-risk myelodysplastic syndrome (MDS) and reduced expression of activating NK
634 receptors. Blood 109:4816–4824, 2007

635 **67**. Bouchliou I, Miltiades P, Nakou E, et al: Th17 and Foxp3+ T regulatory cell dynamics and
636 distribution in myelodysplastic syndromes. Clin Immunol 139:350–359, 2011

637 **68**. Hejazi M, Manser AR, Fröbel J, et al: Impaired cytotoxicity associated with defective natural
638 killer cell differentiation in myelodysplastic syndromes. Haematologica 100:643–652, 2015

639 **69**. Sarhan D, Brandt L, Felices M, et al: 161533 TriKE stimulates NK-cell function to overcome
640 myeloid-derived suppressor cells in MDS. Blood Adv 2:1459–1469, 2018

641 **70**. Stringaris K, Marin D, Barrett AJ, et al: KIR gene haplotype: An independent predictor of
642 clinical outcome in MDS patients. Blood 128:2819–2823, 2016

643 **71**. Raaijmakers MHGP, Mukherjee S, Guo S, et al: Bone progenitor dysfunction induces
644 myelodysplasia and secondary leukaemia. Nature 464:852–857, 2010

645 **72**. Raaijmakers MHGP: Myelodysplastic syndromes: Revisiting the role of the bone marrow
646 microenvironment in disease pathogenesis. Int J Hematol 95:17–25, 2012

647 **73**. Kode A, Manavalan JS, Mosialou I, et al: Leukaemogenesis induced by an activating β-
648 catenin mutation in osteoblasts. Nature 506:240–244, 2014

649 **74**. Bulycheva E, Rauner M, Medyouf H, et al: Myelodysplasia is in the niche: Novel concepts
650 and emerging therapies. Leukemia 29:259–268, 2015

651 **75**. Morrison SJ, Scadden DT: The bone marrow niche for haematopoietic stem cells. Nature

505:327–334, 2014

76. Zhou S, Greenberger JS, Epperly MW, et al: Age-related intrinsic changes in human bone-marrow-derived mesenchymal stem cells and their differentiation to osteoblasts. Aging Cell 7:335–343, 2008

77. Blau O, Baldus CD, Hofmann W, et al: Mesenchymal stromal cells of myelodysplastic syndrome and acute myeloid leukemia patients have distinct genetic abnormalities compared with leukemic blasts. Blood 118:5583–5592, 2011

78. Medyouf H, Mossner M, Jann JC, et al: Myelodysplastic cells in patients reprogram mesenchymal stromal cells to establish a transplantable stem cell niche disease unit. Cell Stem Cell 14:824–837, 2014

79. Ferrer RA, Wobus M, List C, et al: Mesenchymal stromal cells from patients with myelodyplastic syndrome display distinct functional alterations that are modulated by lenalidomide. Haematologica 98:1677–1685, 2013

80. Geyh S, Öz S, Cadeddu RP, et al: Insufficient stromal support in MDS results from molecular and functional deficits of mesenchymal stromal cells. Leukemia 27:1841–1851, 2013

81. Chen S, Zambetti NA, Bindels EMJ, et al: Massive parallel RNA sequencing of highly purified mesenchymal elements in low-risk MDS reveals tissue-context-dependent activation of inflammatory programs. Leukemia 30:1938–1942, 2016

82. Lopes MR, Pereira JKN, De Melo Campos P, et al: De novo AML exhibits greater microenvironment dysregulation compared to AML with myelodysplasia-related changes. Sci Rep 7:40707, 2017

673 **83**. Kochenderfer JN, Kobayashi S, Wieder ED, et al: Loss of T-lymphocyte clonal dominance in
674 patients with myelodysplastic syndrome responsive to immunosuppression. Blood 100:3639–
675 3645, 2002

676 **84**. Lim ZY, Killick S, Germing U, et al: Low IPSS score and bone marrow hypocellularity in MDS
677 patients predict hematological responses to antithymocyte globulin. Leukemia 21:1436–1441,
678 2007

679 **85**. Passweg JR, Giagounidis AAN, Simcock M, et al: Immunosuppressive therapy for patients
680 with myelodysplastic syndrome: A prospective randomized multicenter phase III trial
681 comparing antithymocyte globulin plus cyclosporine with best supportive care - SAKK 33/99.
682 J Clin Oncol 29:303–309, 2011

683 **86**. Stahl M, Deveaux M, De Witte T, et al: The use of immunosuppressive therapy in MDS:
684 clinical outcomes and their predictors in a large international patient cohort. Blood Adv
685 2:1765–1772, 2018

686 **87**. Fenaux P, Giagounidis A, Selleslag D, et al: A randomized phase 3 study of Len vs placebo
687 in transfusion-dependent patients with Low- or Int1-risk MDS with del5q. Blood 118:3765–
688 3777, 2011

689 **88**. Santini V, Almeida A, Giagounidis A, et al: Randomized phase III study of lenalidomide
690 versus placebo in RBC transfusion-dependent patients with lower-risk non-del(5q)
691 myelodysplastic syndromes and ineligible for or refractory to erythropoiesis-stimulating
692 agents. J Clin Oncol 34:2988–2996, 2016

693 **89**. Sallman DA, Davila ML: Is Disease-Specific Immunotherapy a Potential Reality for MDS?
694 Clin Lymphoma Myeloma Leuk 17:S26–S30, 2017

90. Griffiths EA, Srivastava P, Matsuzaki J, et al: NY-ESO-1 vaccination in combination with decitabine induces antigen-specific T-lymphocyte responses in patients with myelodysplastic syndrome. Clin Cancer Res 24:1019–1029, 2018

91. Eksioglu EA, Chen X, Heider KH, et al: Novel therapeutic approach to improve hematopoiesis in low risk MDS by targeting MDSCs with the Fc-engineered CD33 antibody BI 836858. Leukemia 31:2172–2180, 2017

92. Ivy KS, Brent Ferrell P: Disordered Immune Regulation and its Therapeutic Targeting in Myelodysplastic Syndromes. Curr Hematol Malig Rep 13:244–255, 2018

93. Garcia-Manero G, Daver NG, Montalban-Bravo G, et al: A Phase II Study Evaluating the Combination of Nivolumab (Nivo) or Ipilimumab (Ipi) with Azacitidine in Pts with Previously Treated or Untreated Myelodysplastic Syndromes (MDS). Blood 128:344 LP – 344, 2016

94. Garcia-Manero G, Tallman MS, Martinelli G, et al: Pembrolizumab, a PD-1 Inhibitor, in Patients with Myelodysplastic Syndrome (MDS) after Failure of Hypomethylating Agent Treatment. Blood 128:345 LP – 345, 2016

95. Zeidan AM, Knaus HA, Robinson TM, et al: A multi-center Phase 1 trial of ipilimumab in myelodysplastic syndrome patients following hypomethylating agent failure. Clin Cancer Res clincanres.3763.2017, 2018

96. Kubasch AS, Platzbecker U: Beyond the edge of hypomethylating agents: Novel combination strategies for older adults with advanced MDS and AML. Cancers (Basel) 10:158, 2018

97. Kubasch AS, Wehner R, Bazzurri S, et al: Clinical, molecular, and immunological responses to pembrolizumab treatment of synchronous melanoma and acute myeloid leukemia. Blood

Adv 2:1187–1190, 2018

98. Matlung HL, Szilagyi K, Barclay NA, et al: The CD47-SIRPα signaling axis as an innate immune checkpoint in cancer. Immunol Rev 276:145–164, 2017

99. Majeti R, Chao MP, Alizadeh AA, et al: CD47 Is an Adverse Prognostic Factor and Therapeutic Antibody Target on Human Acute Myeloid Leukemia Stem Cells. Cell 138:286–299, 2009

100. Sallman D, Donnellan W, Asch A, et al: THE FIRST-IN-CLASS ANTI-CD47 ANTIBODY HU5F9-G4 IS ACTIVE AND WELL TOLERATED ALONE OR IN COMBINATION WITH AZACITIDINE IN AML AND MDS PATIENTS: INITIAL PHASE 1B RESULTS. HemaSphere 3:394, 2019

101. Kaiser J: Too much of a good thing? Science (80-) 359:1346 LP – 1347, 2018

102. Bejar R, Lord A, Stevenson K, et al: TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients. Blood 124:2705–2712, 2014

103. Lee EJ, Zeidan AM: Genome sequencing in myelodysplastic syndromes: Can molecular mutations predict benefit from hypomethylating agent therapy? Expert Rev Hematol 8:155–158, 2015

104. Idossa D, Lasho TL, Finke CM, et al: Mutations and karyotype predict treatment response in myelodysplastic syndromes. Am J Hematol 93:1420–1426, 2018

105. Szeto GL, Finley SD: Integrative Approaches to Cancer Immunotherapy. Trends in Cancer 5:400–410, 2019

106. Spitzer MH, Carmi Y, Reticker-Flynn NE, et al: Systemic Immunity Is Required for Effective Cancer Immunotherapy. Cell 168:487-502.e15, 2017

738 **107**. McGrail DJ, Federico L, Li Y, et al: Multi-omics analysis reveals neoantigen-independent
739 immune cell infiltration in copy-number driven cancers. Nat Commun 9:1317, 2018

740 **108**. Silverbush D, Cristea S, Yanovich-Arad G, et al: Simultaneous Integration of Multi-omics
741 Data Improves the Identification of Cancer Driver Modules. Cell Syst 8:456-466.e5, 2019

742 **109**. Vasaikar S V., Straub P, Wang J, et al: LinkedOmics: Analyzing multi-omics data within and
743 across 32 cancer types. Nucleic Acids Res 46:D956–D963, 2018

744 **110**. Ziegenhain C, Vieth B, Parekh S, et al: Comparative Analysis of Single-Cell RNA Sequencing
745 Methods. Mol Cell 65:631-643.e4, 2017

746 **111**. Stoeckius M, Hafemeister C, Stephenson W, et al: Simultaneous epitope and
747 transcriptome measurement in single cells. Nat Methods 14:865–868, 2017

748 **112**. Shahi P, Kim SC, Haliburton JR, et al: Abseq: Ultrahigh-throughput single cell protein
749 profiling with droplet microfluidic barcoding. Sci Rep 7:44447, 2017

750 **113**. Baranov VI, Lou X, Bandura DR, et al: Mass Cytometry: Technique for Real Time Single
751 Cell Multitarget Immunoassay Based on Inductively Coupled Plasma Time-of-Flight Mass
752 Spectrometry. Anal Chem 81:6813–6822, 2009

753 **114**. Bacher U, Kohlmann A, Haferlach T: Mutational profiling in patients with MDS: Ready for
754 every-day use in the clinic? Best Pract Res Clin Haematol 28:32–42, 2015

755 **115**. Bento LC, Correia RP, Pitangueiras Mangueira CL, et al: The Use of Flow Cytometry in
756 Myelodysplastic Syndromes: A Review. Front Oncol 7:270, 2017

757 **116**. Oelschlaegel U, Westers TM, Mohr B, et al: Myelodysplastic syndromes with a deletion
758 5q display a characteristic immunophenotypic profile suitable for diagnostics and response

759 monitoring. Haematologica 100:e93 LP-e96, 2015

760 **117**. Schmidt CS, Aranda Lopez P, Dopheide JF, et al: Phenotypic and functional
761 characterization of neutrophils and monocytes from patients with myelodysplastic syndrome
762 by flow cytometry. Cell Immunol 308:19–26, 2016

763 **118**. Jain P, Klotz J, Dunavin N, et al: Cellular immune profiling after sequential clofarabine and
764 lenalidomide for high risk myelodysplastic syndromes and acute myeloid leukemia. Leuk Res
765 Reports 7:40–44, 2017

766 **119**. Paiva B, Cedena MT, Puig N, et al: Minimal residual disease monitoring and immune
767 profiling in multiple myeloma in elderly patients. Blood 127:3165–3174, 2016

768 **120**. Chevrier S, Levine JH, Zanotelli VRT, et al: An Immune Atlas of Clear Cell Renal Cell
769 Carcinoma. Cell 169:736-749.e18, 2017

770 **121**. Lavin Y, Kobayashi S, Leader A, et al: Innate Immune Landscape in Early Lung
771 Adenocarcinoma by Paired Single-Cell Analyses. Cell 169:750-765.e17, 2017

772 **122**. Krieg C, Nowicka M, Guglietta S, et al: Author Correction: High-dimensional single-cell
773 analysis predicts response to anti-PD-1 immunotherapy. Nat Med 24:144–153, 2018

774 **123**. Wei SC, Levine JH, Cogdill AP, et al: Distinct Cellular Mechanisms Underlie Anti-CTLA-4
775 and Anti-PD-1 Checkpoint Blockade. Cell 170:1120-1133.e17, 2017

776 **124**. Behbehani GK, Finck R, Samusik N, et al: Profiling Myelodysplastic Syndromes by Mass
777 Cytometry Demonstrates Abnormal Progenitor Cell Phenotype and Differentiation. bioRxiv
778 604397, 2019

779 **125**. Gullaksen SE, Skavland J, Gavasso S, et al: Single cell immune profiling by mass cytometry

of newly diagnosed chronic phase chronic myeloid leukemia treated with nilotinib. Haematologica 102:1361–1367, 2017

126. Coats T, Smith A e, Mourikis TP, et al: Mass Cytometry Reveals PD1 Upregulation Is an Early Step in MDS Disease Progression. Blood 128:4296 LP – 4296, 2016

127. Hartmann FJ, Babdor J, Gherardini PF, et al: Comprehensive Immune Monitoring of Clinical Trials to Advance Human Immunotherapy. Cell Rep 28:819-831.e4, 2019

128. Pagès F, Mlecnik B, Marliot F, et al: International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. Lancet 391:2128–2139, 2018

129. Galon J, Lugli A, Bifulco C, et al: World-Wide Immunoscore Task Force: Meeting report from the "Melanoma Bridge", Napoli, November 30th-December 3rd, 2016. J Transl Med 15:212, 2017

130. Kümpers C, Jokic M, Haase O, et al: Immune Cell Infiltration of the Primary Tumor, Not PD-L1 Status, Is Associated With Improved Response to Checkpoint Inhibition in Metastatic Melanoma. Front Med 6:27, 2019

131. Brück O, Blom S, Dufva O, et al: Immune cell contexture in the bone marrow tumor microenvironment impacts therapy response in CML. Leukemia 32:1643–1656, 2018

132. Thorsson V, Gibbs DL, Brown SD, et al: The Immune Landscape of Cancer. Immunity 48:812-830.e14, 2018

133. Auslander N, Zhang G, Lee JS, et al: Robust prediction of response to immune checkpoint blockade therapy in metastatic melanoma. Nat Med 24:1545–1549, 2018

134. Jiang P, Gu S, Pan D, et al: Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response. Nat Med 24:1550–1558, 2018

135. Finotello F, Rieder D, Hackl H, et al: Next-generation computational tools for interrogating cancer immunity. Nat Rev Genet 6, 2019

136. Adès L, Fenaux P: Immunomodulating drugs in myelodysplastic syndromes. Hematology Am Soc Hematol Educ Program 2011:556–560, 2011

137. Wenk C, Garz AK, Grath S, et al: Direct modulation of the bone marrow mesenchymal stromal cell compartment by azacitidine enhances healthy hematopoiesis. Blood Adv 2:3447–3461, 2018

138. Kordasti S, Costantini B, Seidl T, et al: Deep phenotyping of Tregs identifies an immune signature for idiopathic aplastic anemia and predicts response to treatment. Blood 128:1193–1205, 2016

139. de Torre-Minguela C, del Castillo PM, Pelegrín P: The NLRP3 and pyrin inflammasomes: Implications in the pathophysiology of autoinflammatory diseases. Front Immunol 8:43, 2017

140. Schoultz I, Verma D, Halfvarsson J, et al: Combined polymorphisms in genes encoding the inflammasome components NALP3 and CARD8 confer susceptibility to crohn's disease in swedish men. Am J Gastroenterol 104:1180–1188, 2009

141. Kahlenberg MJ, Kaplan MJ: The Inflammasome and lupus- another innate immune mechanism contributing to disease pathogenesis? Curr Opin Rheumatol 26:475–481, 2014

142. McGonagle D, McDermott MF: A Proposed Classification of the Immunological Diseases. PLoS Med 3:e297, 2006

143. Geiss GK, Bumgarner RE, Birditt B, et al: Direct multiplexed measurement of gene expression with color-coded probe pairs. Nat Biotechnol 26:317–25, 2008

144. Rodriguez-Meira A, Buck G, Clark SA, et al: Unravelling Intratumoral Heterogeneity through High-Sensitivity Single-Cell Mutational Analysis and Parallel RNA Sequencing. Mol Cell 73:1292-1305.e8, 2019

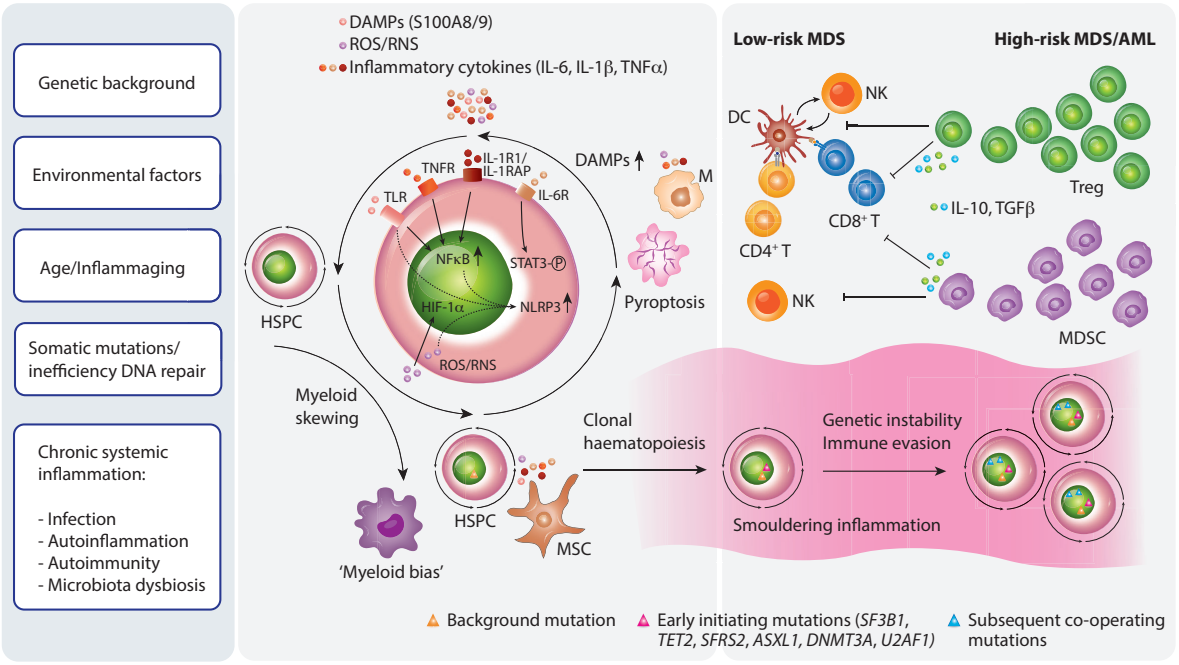
145. Garcia-Manero G, Montalban-Bravo G, Yang H, et al: Un update of a phase II exploratory study of OPN-305, a toll-like receptor 2 antibody, in patients with lower risk myelodysplastic syndromes with prior hypomethylating agent therapy. Haematol Conf 22th Congr Eur Hematol Assoc Spain , 2017

146. Daher M, Hidalgo Lopez JE, Randhawa JK, et al: An exploratory clinical trial of bortezomib in patients with lower risk myelodysplastic syndromes. Am J Hematol 92:674–682, 2017

147. Fenaux P, Platzbecker U, Mufti GJ, et al: The Medalist Trial: Results of a Phase 3, Randomized, Double-Blind, Placebo-Controlled Study of Luspatercept to Treat Anemia in Patients with Very Low-, Low-, or Intermediate-Risk Myelodysplastic Syndromes (MDS) with Ring Sideroblasts (RS) Who Require Red. Blood 132:1 LP – 1, 2018

148. Chien KS, Cortes JE, Borthakur G, et al: Preliminary Results from a Phase II Study of the Combination of Azacitidine and Pembrolizumab in Patients with Higher-Risk Myelodysplastic Syndrome. Blood 132:Abstract 464, 2018

FIG. 1



840

FIG. 2

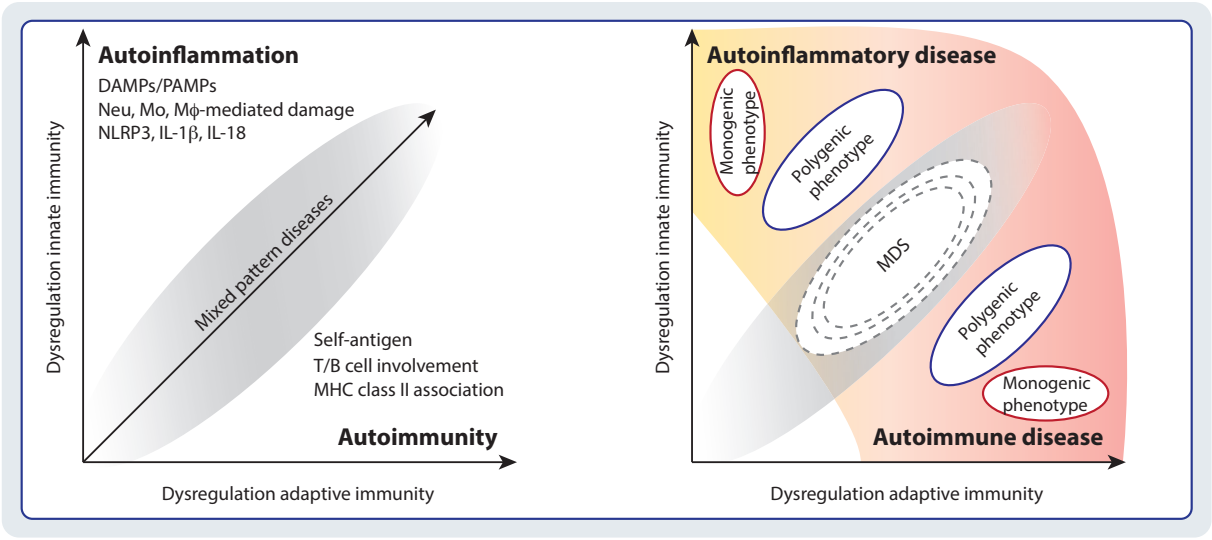


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FIG. 3

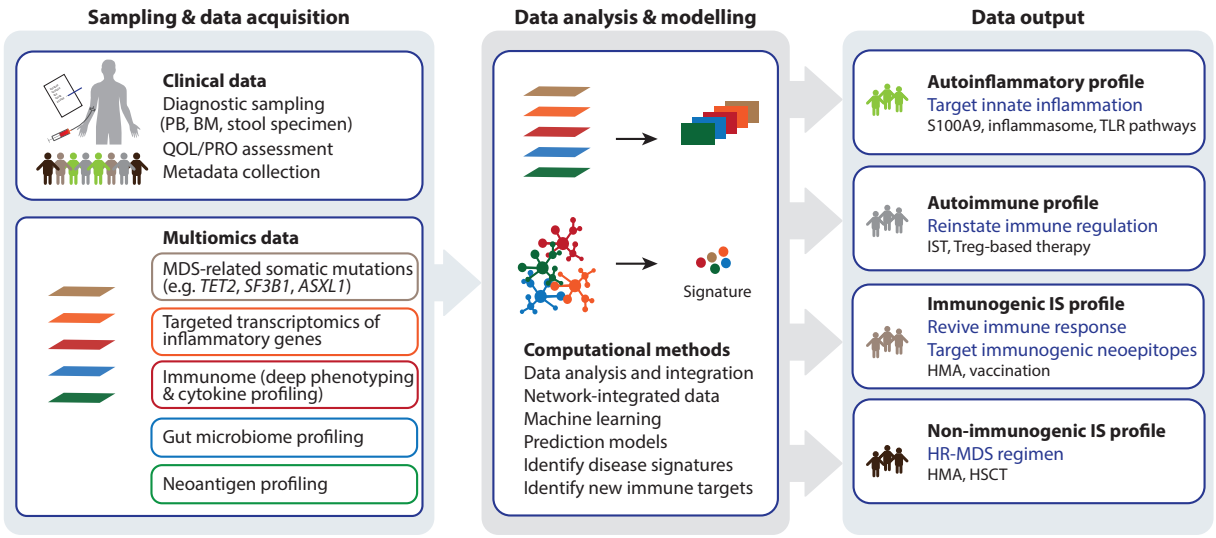
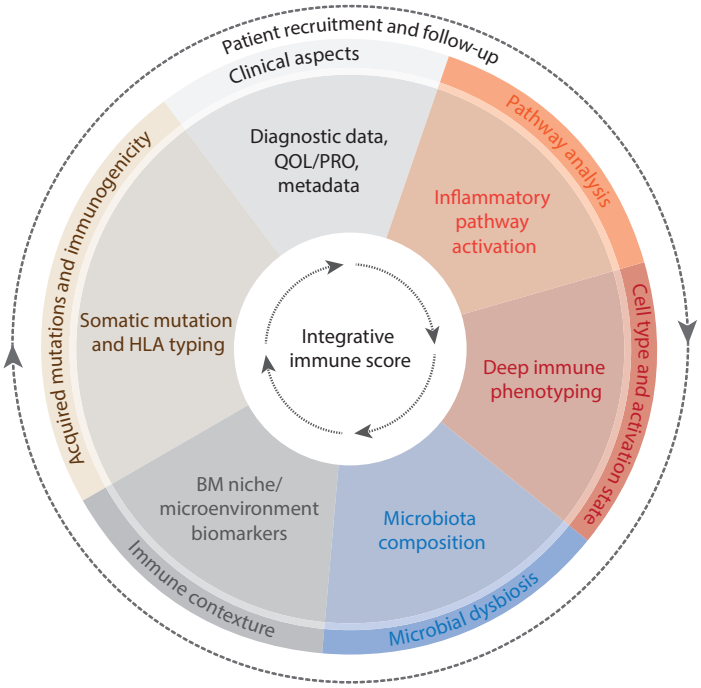


FIG. S1



Supplementary Fig. S1: Integrative immunoscore for MDS. Online version only. Integration of data from different omic platforms with clinical data could identify a biomarker panel to improve stratification of MDS patients.
Abbreviations: BM, bone marrow; HLA, human leukocyte antigen; QOL/PRO, quality of life/patient reported outcome.