NEOPLASIA
www.neoplasia.com

Volume 23 Number xxx Month 2021 pp. 1101–1109

Review Article

Myelodysplastic syndromes: Biological and therapeutic consequences of the evolving molecular aberrations landscape ☆,☆☆

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Abstract

Myelodysplastic syndromes (MDS) are clonal hematopoietic disorders with heterogeneous presentation, ranging from indolent disease courses to aggressive diseases similar to acute myeloid leukemia (AML). Approximately 90% of MDS patients harbor recurrent mutations , which – with the exception of mutated *SF3B1* –have not (yet) been included into the diagnostic criteria or risk stratification for MDS. Accumulating evidence suggests their utility for diagnostic workup, treatment indication and prognosis. Subsequently, in patients with unexplained cytopenia or dysplasia identification of these mutations may lead to earlier diagnosis. The acquisition and expansion of additional driver mutations usually antecedes further disease progression to higher risk MDS or secondary AML and thus, can be clinically helpful to detect individuals that may benefit from aggressive treatment approaches. Here, we review our current understanding of somatic gene mutations, gene expression patterns and flow cytometry regarding their relevance for disease evolution from pre-neoplastic states to MDS and potentially AML.

Neoplasia (2021) 23, 1101–1109

Keywords: Myelodysplastic syndrome, Clonal architecture, Clonal evolution, Residual disease, Molecular targets, Measurable residual disease

. SEARCH STRATEGY AND SELECTION CRITERIA

References for this Review were identified through searches of PubMed with the search terms “measurable/minimal residual disease”, “myelodysplastic syndrome”, “clonal evolution”, “disease burden”, and “molecular” from 2005 until December 2020. Articles were also identified through searches of the authors’ own files. Only papers published in English were reviewed. The final reference list was generated on the basis of originality and relevance to the broad scope of this Review.

Introduction

Myelodysplastic syndromes (MDS) are clonal disorders characterized by dysplasia of at least one myeloid cell line, peripheral blood cytopenia, and a propensity to eventually progress into acute myeloid leukemia (AML) [1]. Clinically, MDS presents highly heterogeneous and may range from indolent disease courses with nearly normal life expectancies to aggressive neoplasm similar to that of AML. This clinical diversity underlines the demand for appropriate risk stratification at diagnosis and during disease course as well as personalized treatment approaches to improve outcomes and avoid evitable adverse effects of toxic treatment regimens. A growing understanding of the complex biology and sequence of biological changes in MDS yield not only the hope to improve prognostic and predictive tools for MDS patients but also to enlarge therapeutic options with novel molecular targets [2,3]. For example high teleomerase activity and telomere length have been associated with MDS, and consequently, Imetelstat - an inhibitor of telomerase activity - entered clinical studies to evaluate its potential to reduce transfusion dependence in lower risk MDS [4,5]. Several epigenetic changes have also been shown to play a role in MDS pathogenesis and to impact prognosis, such as microRNA expression patterns [6], or distinct DNA methylation profiles which may identify clusters containing outcome information independent of current MDS prognosis systems [7].

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☆ Funding: None
☆☆ Conflict of Interest: The authors declare no conflict of interest.
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Received 15 July 2021; accepted 2 September 2021

https://doi.org/10.1016/j.neo.2021.09.002

However, today not at last due to practical need in the clinical routine, methods of gene mutation assessment have developed a higher impact on clinical decision making and personalized therapy approaches. Especially the introduction of next generation sequencing (NGS) methods impacts the clinical routine and increasingly allows the identification of recurrent molecular aberrations with several related consequences [8]. Despite the fact that – with the exception of mutated *SF3B1* - these mutations have not (yet) been included into the diagnostic criteria or risk stratification for MDS [1], accumulating evidence suggests their utility for diagnostic workup, treatment indication and prognostic considerations. Studies adapting high throughput sequencing of myeloid genes showed that approximately 90% of MDS patients carry at least one oncogenic mutation [3,8,9], that initiate the disease but may also change with cytotoxic treatments or during natural disease course. Hence, in patients with unexplained cytopenia, the absence of a molecular or cytogenetic alterations constitutes a negative predictive factor for the development of myeloid neoplasm [10]. However, when identifying a molecular alteration, the distinct affected gene impact the probability of developing a myeloid neoplasm. While mutations in genes encoding splicing factors, as well as *JAK2* or *RUNX1* show a high predictive value for the diagnosis of a myeloid neoplasm, mutations in *TET2, DNMT3A, ASXL1* also frequently occur in otherwise healthy individuals [11,12] and are predictive mainly when occurring in combination with other genetic lesions. Subsequently, in patients with unexplained cytopenia and/or dysplasia identification of these mutations may lead to earlier diagnosis and – if clinically necessary – treatment initiation of MDS [10]. During time, the acquisition and expansion of additional driver mutations usually antecedes further disease progression to higher risk MDS or secondary AML and thus, can be clinically helpful to detect individuals that may benefit from aggressive treatment approaches, including allogeneic hematopoietic stem cell transplantation (HSCT) [8].

In MDS patients achieving a morphologic complete remission during treatment the longitudinal evaluation of genetic aberrations may help to estimate the depth of remission and planning of further treatment interventions. Hence, known molecular responses could aid in clinical decision-making, such as dose reductions in older patients under life-long palliative therapy or the optimal timing of an allogeneic HSCT in individuals with curative treatment approaches. Finally, after allogeneic HSCT, repetitive analyses of known molecular alteration may even allow measurable residual disease (MRD) evaluation in MDS patients, quite similar to that already established in AML [13]. However, such longitudinal studies have not been systematically performed so far. Furthermore, thresholds of evolving molecular patterns identifying "danger signals" with regard to disease evolution are not well defined. Lastly, therapeutic consequences in case of clonal evolution or increase of MRD are scarce given the paucity of approved treatment options in MDS [14].

Here, we review our current understanding of somatic gene mutations, and gene expressions in MDS and their relevance for disease evolution from pre-neoplastic states to MDS and potentially secondary AML as well as their applicability for MRD detection when applying therapy to MDS patients, and the respective treatment associations. While this approach by nature cannot be a comprehensive review of every biological aspect, we believe it represents an overview of the data with a stringent up to date clinical value.

The molecular landscape in MDS

There are no molecular changes that are specific for MDS and many recurrent mutations are shared by other myeloid neoplasm and can sometimes even be detected in healthy individuals. Mutations involved in DNA methylation such as *TET2, DNMT3A*, as well as in histone modifiers *ASXL1* and *EZH2* are common in MDS [8], but also frequently occur in aging healthy individuals [11,12]. However, the observed composition and frequencies of mutations differ, as e.g. somatic mutations in spliceosome genes (especially *SF3B1, SRSF2, U2AF1*, and *ZRSR2*), *TP53, NF1, EZH2,* and *BCOR* have been observed more commonly in MDS, while mutations in *FLT3, NPM1, DNMT3A, IDH1*, and *IDH2* are more common in AML [9]. Chronic myelomonocytic leukemia (CMML) is enriched with mutations in *SRSF2* and *TET2*, atypical chronic myeloid leukemia (aCML) with mutations in *ASXL1* and *SETBP1,* and the coexistence of *JAK2* with *SF3B1* is typical for MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN RS-T) [15]. Additional to the composition of mutated genes, also the genomic burden of each mutation differs and allows conclusions about the chronology in which aberrations occurred during disease evolution. Typically early events in MDS are usually found with high variant allele frequencies (VAFs) and include mutations in spliceosome genes, *DNMT3A* mutations, *ASXL1,* or *TET2* [3,8,9,16,17]. Frequent subclonal events that are also known to drive disease progression to higher risk MDS or secondary AML involve mutations in transcription regulators such as *RUNX1* or *CUX1,* as well as signal transducers (i.e. *NRAS, KRAS*, or *CBL*) or cohesin complex components (i.e. *STAG2*, or *RAD21*) [8,9,17]. Table 1 gives a broad overview of recurrently affected genes in MDS and their clinical and prognostic relevance.

Besides gene mutations, also aberrant microRNA [6] and gene expression in peripheral blood or bone marrow, including *BAALC* (brain and acute leukemia, cytoplasmic), *MN1* (meningioma-1), and *WT1* (Wilm's tumor gene) have been shown to impact disease evolution and may refine prognostic information provided by the IPSS-R [18,19]. Following the description of the prognostic significance of high expressed genes correlated with a leukemic stem cell (LCS) signature in AML, Wang et al identified a LCS associated scoring system based on the expression of the 4 genes *LAPTM4B, NGFRAP1, EMP1*, and *CPXM1* [20]. While higher LSC4 scores associated with disease risk as higher IPSS-R scores, complex cytogenetics, and mutations in *RUNX1, ASXL1*, and *TP53*, the LSC4 score also independently predicted prognosis in MDS patients irrespective of IPSS-R risks [20]. However, despite their undoubtful relevance in disease evolution and risk stratification, methodological obstacles in absolute gene quantification and generating comparable results between analyses, PCR quantification methods and laboratories so far prevented their introduction into the clinical practice.

Clonal evolution – en route *to MDS*

Over the past years the introduction of NGS approaches increased the possibility to identify molecular aberrations also in patients in the absence of cytogenetic aberrations and in those with only mild or absent cytopenia. In fact, age-related clonal hematopoiesis (ARCH) is driven by mutations also commonly found in MDS and AML (e.g. especially in the genes *DNMT3A, TET2, ASXL1, JAK2*, or *TP53*) [11,12,21–23]. The condition is associated with an increased risk to develop a myeloid malignancy including MDS, as well as with an increased all-cause mortality, especially by cardio-vascular events [11,12]. This observation in combination with increased detection (intentional or unintentional) of such mutations led to an increasing need to counsel and monitor individuals with detected mutations prospectively, and has prompted first centers to develop specialized clinics [24]. These findings also led to a still continuing debate on minimal diagnostic criteria for MDS and, especially in individuals with an otherwise unexplained cytopenia, the boundaries to MDS may be fluent [10].

However, to bring some clarification into the growing complexity of how to interpret somatic mutations and their prognostic relevance robust definitions were developed. All lack dysplasia in more or equal than 10% of cells, aberrant cytogenetics, or elevated blast counts and, subsequently, do not fulfill the WHO MDS disease criteria [1]. Clonal hematopoiesis of indeterminate potential (CHIP) or ARCH denotes the presence of at least one somatic mutation that is frequently found in myeloid neoplasia at a VAF $\geq$ 2% but without persistent cytopenia or history of an underlying hematologic disorder [25]. CHIP has been shown to associate with a higher risk to develop

Table 1

Relevant mutations and noteworthy clinical consequences / targets in MDS.

Mutated Gene	Clinical Associations	Prognostic and Therapeutic Relevance
DNMT3A	- present in approximately 15% of MDS patients [8,16] - occur very early in disease evolution, most frequently as hotspot mutations in the R882 codon [8,16]	- linked to a higher risk of disease evolution to secondary AML, shorter survival in *de novo* MDS patients [67] and independently from MDS origin after HSCT [56]
TET2	- among the most frequently mutated genes in MDS (20-30%) [3,8]	- associated with increased response to hypomethylating agents, especially when present at high VAFs [42] - associated with shorter overall survival after HSCT [56]
ASXL1	- present in 10% – 20% of MDS patients [3,8] - associated with thrombocytopenia, increased bone marrow blasts, trisomy 8, intermediate-risk karyotype and mutations of *RUNX1, EZH2, IDH1, IDH2, NRAS, JAK2, SETBP1,* and *SRSF2* [16,68]	- predict inferior outcome in MDS and CMML patients [16,68,69], including a shorter overall survival following HSCT [2]
Spliceosome gene mutations	- present in approximately 50% of MDS patients [8] - often represent early events with high VAFs at presentation [7,8]	
SF3B1	- among the most frequently mutated genes in MDS (25% – 35% of patients) [3,8] - strong correlation with ring sideroblast phenotype and ineffective erythropoiesis - may be seen as a distinct nosologic entity [28]	- favorable prognosis [28] - high response rates to luspatercept [39] - K666N mutation may be associated with increased progression of MDS and distinct RNA splicing [70]
IDH1 and *IDH2*	- less common in MDS as compared to AML, affect <5% of MDS patients [42]	- prognostic impact in MDS are still up to debate, *IDH1* mutations may have an adverse prognostic effect [71] - promising data from early clinical trials for IDH inhibitors enasidenib and ivosidenib [72]
RUNX1	- present in approximately 10% of patients [3,8] - linked to a higher incidence of thrombocytopenia [3]	- implied in disease progression and frequently found in secondary AML evolving from MDS [73] - associated with shorter survival following HSCT[2] - may lead to unresponsiveness to lenalidomide in del(5q) MDS [38]
NRAS, KRAS	- present in approximately 10% of MDS patients [3,8] - late, mostly subclonal events [32,33] - a cooperation between genes involved in the cohesin and RAS pathways was observed in 15% – 20% of MDS patients who evolved to secondary AML [32]	- adverse prognostic impact in lower risk MDS patients through a high transformation rate to secondary AML [32,33]

TP53	- present in 5% – 10% of MDS patients [3,8]
	- associate with reduced hemoglobin and platelet counts at MDS presentation [9]
	- closely linked to complex karyotypes
		- provide a survival advantage during radio-chemotherapy and are enriched in individuals with therapy related MDS [13,14]
		- associate with dismal outcomes regardless of the applied therapy (HMA, allogeneic HSCT)[42,56]
		- adverse prognosis may depend on the mutation burden, allelic state and genomic context [74]
		- response to decitabine comparable to that of intermediate risk MDS patients [45]
		- loss of a mutation associates with improved outcome during disease course [65]
		- promising data from early clinical trials for
		  - TP53 reactivator APR-246 in combination with azacitidine [75]
		  - inhibitor of mitochondrial metabolism CPI-613 (Devimistat)
		  - CD47 antibody Magrolimab

(*continued on next page*)

Table 1 (*continued*)

Mutated Gene	Clinical Associations	Prognostic and Therapeutic Relevance
PPM1D	- present in <5% of MDS patients [3,8] - provide a survival advantage during radio-chemotherapy and are enriched in individuals with therapy related MDS [29,55]	- no significant impact on outcomes [55]
NPM1	- among the most frequently mutated genes in AML, but only found in around 2% of MDS cases [8,46]	- associated with an aggressive MDS phenotype and a high progression risk to AML [8,46], should be treated as AML whenever possible - similar to AML, appear to be stable during disease course [8,46] - intensive chemotherapy and HSCT seem to improve outcomes [46] - high activity of combination therapies of venetoclax and HMA in *NPM1*-mutated AML as well as in higher risk MDS [47] hint to a similarly high potential in MDS harboring *NPM1* mutations

hematological cancers at a frequency of approximately 0.5% to 1% per year [26] as well a higher incidence of cardio-vascular events [11,12,27]. A somatic mutation at a VAF > 2% together with a cytopenia but not (yet) fulfilling diagnostic criteria for MDS constitutes a clonal cytopenia of undetermined significance (CCUS) [25]. CCUS associates with a much higher risk of progression to MDS of up to 50% to 90% in 5 years [10], especially when spliceosome gene mutations or co-mutated epigenetic regulators are affected, and with higher number and VAFs of the affected genes [10]. In fact, *SF3B1*-mutated CCUS patients almost invariably develop overt MDS with ring sideroblasts [28]. Still, so far close monitoring to early detect a myeloid neoplasm – if it occurs – should be the only clinical consequence, as not every precursor state will progress into a malignant disease [26].

Already of clinical importance is the presence of mutations in *TP53* or *PPM1D* which associates with or pre-dispose to therapy-related MDS following chemo- or radiotherapy. Likely, their presence will have consequences regarding a more stringent indication for adjuvant chemotherapy in solid tumor patients with low relapse risk in the future [24,29]. However, the consequences of the presence of such mutations, while lacking signs of dysplasia, cytogenetic changes, or blood count abnormalities yet widely remain an area of very active research and great uncertainties regarding boundaries to MDS that overlap in some areas.

Clonal evolution – transforming from MDS to secondary AML

Frequency differences in the mutated genes in low risk MDS, compared to high risk MDS and also to secondary AML indicate that the order of mutation acquisition is not random during progression (Figure 1). Several studies with paired MDS and secondary AML samples have shown that signaling gene mutations (*NRAS, KRAS, FLT3, PTPN11*) and myeloid transcription factors (i.e. *CEBPA, RUNX1*), as well as *TP53*, and cohesin complex components (*STAG2, RAD21*), along with new cytogenetic abnormalities, expand or emerge at the time of disease progression [9,16,17,26,30,31]. In many cases, disease progression is associated with clonal evolution, typically defined by the expansion of a subclone with a unique set of mutation patterns that drive disease progression to high risk MDS or secondary AML. Especially the combination of *STAG2* mutations and *NRAS* mutations may play an important role in progress to secondary AML in a subgroup of patients [32]. Furthermore, mutations in the genes *FLT3, PTPN11, NRAS,* and *NPM1* have been associated with a fast progression from MDS to AML [30,33], and mutations in *ASXL1* with progression from CMML to secondary AML [34]. Thus, monitoring tumor burden and clonal evolution using serial sequencing may provide advantages over using e.g. the blast count - which often underestimates the tumor burden - and may allow for early detection of disease progression prior to clinical deterioration [35].

Molecular markers predicting treatment response

Molecular aberrations underlie the pathogenic mechanism driving MDS, and subsequently, can be utilized to estimate treatment responses (Figure 2). A long-known example in MDS is the 5q-syndrome with its particular phenotype through haploinsufficiency of a variety of genes, subsequent high response rates to lenalidomide [36], and resistance mechanism through development of *TP53* mutations with their underlying pathogenesis [37]. Also the presence of *RUNX1* and *GATA2* mutations may render del(5q) MDS unresponsive to lenalidomide induced megakaryocytic differentiation and apoptosis [38]. Accordingly, *SF3B1*-mutated MDS not only shows a relatively favorable prognosis in general, but also constitutes a condition with a high likelihood to respond to luspatercept with abolishment of transfusion requirement, a recently approved treatment option for low risk MDS patients [28,39].

In low risk MDS treatment with erythropoiesis stimulating agents (ESA) or luspatercept aims at improving anemia. However, the molecular changes that impact responses is only poorly investigated. While Kosmider et al did not identify a specific typical MDS mutation, they showed that the presence of more than 2 mutations associated with a lower likelihood of responses [40], while a recent American society of Hematology (ASH) abstract with darbepoetin suggested that in this context, *ASXL1* mutations might be linked to lower response rates [41].

Also in patients with high risk MDS, in whom hypomethylating agents (HMA) remain the current standard of care with clinical responses of approximately 40% to 50% and complete remission rates of 10% to 15%, molecular alterations may allow response prediction. HMA inhibit DNA methyltransferases which leads to a decreased methylation of DNA cytosine residues. Of the genes frequently mutated in MDS that encode proteins involved in epigenetic regulations, *TET2* mutations at VAFs >10% have been repeatedly shown to predict response to HMA [42,43], especially in the context of unmutated *ASXL1* [42], but did not result in longer survival in 2 analyses. In contrast, a third study suggested similar response rates but significantly longer survival in patients with either *TET2* or *EZH2* mutations under treatment with azacitidine in two independent cohorts [44]. Additionally, in a murine model Tet2 loss sensitized cells to treatment with azacitidine *in vivo* which supports these clinical observations [42]. On the

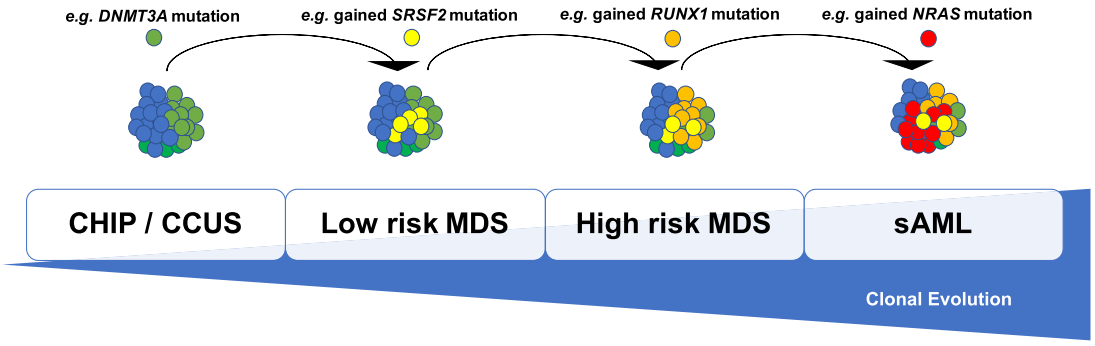

Fig. 1. Schematic display of a hierarchical, branching model of the natural MDS disease course. Acquisition of additional genetic abnormalities over time which gradually lead to a more aggressive disease phenotype.

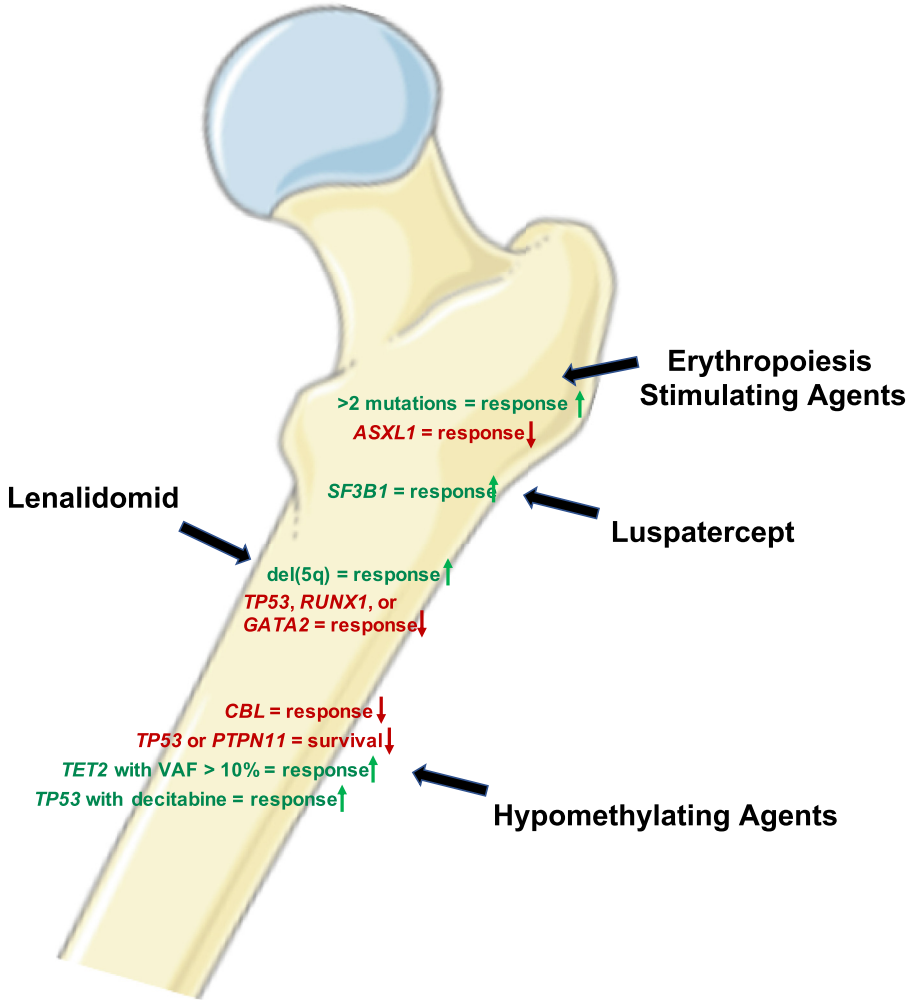

Fig. 2. Genetic factors able to predict responses for different MDS treatment options (Graphic was constructed using Servier Medical Art).

other hand, *CBL* mutations were linked to a lower response rate to HMA while *TP53* and *PTPN11* mutations resulted in shorter survival, but did not affect initially response to treatment [42,44]. In fact, Bejar et al especially pointed out that they were not able to identify a mutation profile predicting treatment failure of HMA [42]. Another study suggested that the use of decitabine may improve response rates in *TP53* mutated MDS patients compared to standard chemotherapy and may provide a bridge to allogeneic HSCT as curative treatment option for some patients [45]. For the rare but aggressive MDS subgroup with mutated *NPM1* a potential benefit of intensive chemotherapy and HSCT has been described, indicating that these patients should be treated similar to patients diagnosed with AML [46]. However, combination therapies of venetoclax and HMA have been shown to have high activity in *NPM1*-mutated AML as well as in higher risk MDS [47]. Subsequently, it will be interesting to investigate this combination also

in this rare MDS subgroup harboring *NPM1* mutations. Certainly, many of the described clinical observations would benefit from prospective trials further confirming the observed results.

Molecular markers in transplanted MDS patients

As of today, allogeneic HSCT remains the only treatment option capable to fully eradicate and cure MDS. Generally, allogeneic HSCT is mainly applied in high risk MDS, where it has demonstrated improved long term survival compared to other treatment options, especially HMA [48]. In contrast, in low risk MDS patients the expected longer survival and a substantial transplant-related toxicity has led to refrain from allogeneic HSCT for the majority of patients in this group. However, clinical factors, as well as the presence of *TP53, RUNX1*, or *ASXL1* mutations may modify the approach and the center specific non-relapse mortality (NRM) is a key parameter when considering allogeneic HSCT in low risk MDS [49]. Higher intensity conditioning may be preferable for disease control, but the preferred regimen especially in an older and comorbid patient population is still under debate [50–52]. Another important aspect is the tumor burden of the patients going into transplantation. Many centers use HMA or AML-induction-like therapies, depending on the pre-transplant tumor burden and patient age to reduce blast levels prior to allogeneic HSCT. However, here the best approach is also up to debate and the existing data is not uncontroversial [53,54]. Given all these uncertainties the growing knowledge of MDS biology may aid in refining and individualizing the therapeutic approaches.

Due to the descripted therapeutic approach in general, the mutational landscape in transplanted MDS patients is skewed towards aberrations more frequently found in high risk MDS [55]. In a pivotal study by Lindsley et al *TP53* mutations – present in 19% of the patients in the analyzed cohort - associated with shorter overall survival and higher relapse rates following allogeneic HSCT, independently of the applied conditioning regimen [55]. RAS-pathway mutations (defined as *NRAS, KRAS, PTPN11, CBL, NF1, RIT1, FLT3*, and *KIT*) on the other hand associated with shorter overall survival following allogeneic HSCT in younger patients (<40 yr) only after reduced intensity conditioning (RIC). Thus, patients with RAS-pathway mutations may benefit from myeloablative conditioning (MAC) in this group. The presence of *JAK2* mutations were associated with higher rates of death without relapse, regardless of the conditioning regimen – although the reason remains unknown. In an Italian study including 274 MDS patients somatic mutation in *ASXL1, RUNX1*, or *TP53* were independently associated with unfavorable outcomes and shorter survival after allogeneic HSCT [2]. Furthermore, in another smaller study of 87 MDS patients who underwent allogeneic HSCT for MDS, mutations in the genes *TP53, TET2*, and *DNMT3A* associated with shorter overall survival. Noteworthy, in this study all 18 *TP53*-mutant patients died within five years after HSCT [56]. Intriguingly, the microenvironment of *TP53* mutant MDS shows an immune privileged, evasive phenotype with a PDL1 overexpression which may contribute also to an inferior graft-versus-disease response after allogeneic HSCT [57]. These findings provide a possible target for an immune-checkpoint-based approach to improve outcomes in *TP53*-mutated MDS patients destined for allogeneic HSCT.

MRD detection in MDS

Until today, only a few published studies fill the relative void of MRD assessment in MDS patients. Most of them have been conducted in the context of an allogeneic HSCT, which currently remains the only treatment capable to fully eradicate the disease. Still, post-transplant relapse or progression remains a major cause of transplant failure, and longitudinal MRD testing following allogeneic HSCT will help to identify patients at risk of disease progression. So far, known potential MRD markers in MDS include (CD34-lineage specific) chimerism analysis [58,59], flow cytometry [60], and *WT1* expression levels [60–62], but all only included small patient numbers. In terms of traceable driver mutations, a recent study of 53 patients including 14 with MDS analyzed circulating tumor DNA by sensitive digital droplet PCR assays for individual driver mutations after allogeneic HSCT. Increasing levels of the individual mutational burden between one month and three months was a sensitive predictor of relapse following allogeneic HSCT [63]. In another study that searched bone marrow for known gene mutations adapting NGS 30 days after performing HSCT, the risk of disease progression was higher among patients in whom mutations were detected compared to those in whom these mutations were not detected [64]. In a recent paper that analyzed the impact of NGS-based mutation negativity during disease course in a heterogenous patient population that included 95 MDS patients with different treatments, including allogeneic HSCT, it was shown that achieving NGS-based mutation negativity associated with improved outcomes [65], which was especially true for the loss of *TP53* mutations [65]. First prospective analyses already utilized MRD to pre-emptively treat impeding relapse and showed feasibility to potentially prevent or delay hematological relapse. The RELAZA2 trial tested an azacitidine-based MRD-guided therapy in 53 AML and MDS patients that achieved complete remission following either intensive chemotherapy or allogeneic HSCT with a traceable molecular marker (i.e. mainly *NPM1* mutations) or CD34+ peripheral blood chimerism [66]. Rautenberg et al monitored 35 MDS and AML patients for peripheral blood *WT1* expression following allogeneic HSCT [62], also initiating treatment with azacitidine in patients with elevated levels. After 6 cycles of azacitidine treatment, 37% of patients achieved a *WT1* level normalization which correlated with improved outcomes. Noteworthy, these studies mostly used peripheral blood for MRD monitoring avoiding unpleasant bone marrow biopsies for the patients. However, so far large studies analyzing MRD in MDS following remission achievement including in the context of an allogeneic HSCT are missing and, thus, it remains an open question, which methods, targets, material (peripheral blood vs bone marrow) or time-point would be most informative.

For the detection of MDS-associated mutations for MRD analysis in the context of cytoreductive therapy without HSCT, data remains limited – likely also due to the difficulty to discriminate between CHIP and malignant populations. However, often VAF levels of MDS-associated mutations clearly exceed the bone marrow blast count and also lower-risk MDS patients with a normal blast count can have an molecular or cytogenetic aberrations in nearly all bone marrow cells [9]. This indicates that the blast count in MDS patients may frequently underestimate the actual disease burden. Subsequently, close monitoring of known mutations may provide additional prognostic information, albeit not comparable to the extent known from acute leukemias.

Conclusion and future perspectives

Our knowledge on MDS biology is getting increasingly complex and continuously growing at an unprecedented pace. Zealously running NGS panels for MDS-related mutations leading to CHIP or CCUS diagnosis in (still) apparently healthy individuals result in challenging discussions and decisions regarding further actions [24]. Especially the detection of recurrent mutations, gene expression patterns, and flow cytometric analyses have resulted in continuously improved risk stratification and the development of personalized therapies. With the growing knowledge of the complex MDS and pre-MDS biology also comes the opportunity for therapeutic trials which will help to clinically address actionable genetic changes in MDS.

Author contributions

MJ and SSch wrote the first draft of the manuscript. ASK, KHM, and UP contributed to the writing and editing and all authors finalized the manuscript.

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