Hypoplastic myelodysplastic syndrome and acquired aplastic anemia: Immune-mediated bone marrow failure syndromes (Review)

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Abstract. Hypoplastic myelodysplastic syndrome (hMDS) and aplastic anemia (AA) are rare hematopoietic disorders characterized by pancytopenia with hypoplastic bone marrow (BM). hMDS and idiopathic AA share overlapping clinico-pathological features, making a diagnosis very difficult. The differential diagnosis is mainly based on the presence of dysgranulopoiesis, dysmegakaryocytopoiesis, an increased percentage of blasts, and abnormal karyotype, all favouring the diagnosis of hMDS. An accurate diagnosis has important clinical implications, as the prognosis and treatment can be quite different for these diseases. Patients with hMDS have a greater risk of neoplastic progression, a shorter survival time and a lower response to immunosuppressive therapy compared with patients with AA. There is compelling evidence that these distinct clinical entities share a common pathophysiology based on the damage of hematopoietic stem and progenitor cells (HSPCs) by cytotoxic T cells. Expanded T cells overproduce proinflammatory cytokines (interferon-γ and tumor necrosis factor-α), resulting in decreased proliferation and increased apoptosis of HSPCs. The antigens that trigger this abnormal immune response are not known, but potential candidates have been suggested, including Wilms tumor protein 1 and human leukocyte antigen class I molecules. Our understanding of the molecular pathogenesis of these BM failure syndromes has been improved by next-generation sequencing, which has enabled the identification of a large spectrum of mutations. It has also brought new challenges, such as the interpretation of variants of uncertain significance and clonal hematopoiesis of indeterminate potential. The present review discusses the main clinicopathological differences between hMDS and acquired

AA, focuses on the molecular background and highlights the importance of molecular testing.

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1. Introduction

Myelodysplastic syndrome (MDS) is a clonal hematopoietic stem cell (HSC) disorder characterized by ineffective erythropoiesis, dysplasia involving one or more cell lineages, peripheral cytopenia and an increased risk of transformation to acute myeloid leukemia (AML). In developed countries, the incidence of MDS increases progressively with age and the annual incidence of the disease is estimated to be 4 cases per 100,000 people, rising to 30 cases per 100,000 people in those >70 years old (1,2). Men have a higher incidence rate than women (2). Although the bone marrow (BM) of most patients with MDS is normo- or hypercellular (NH-MDS), 10-20% of patients with MDS have hypocellular BM (cellularity <20-30% in the BM trephine biopsy) (2,3). This subset is referred to as hypoplastic MDS (hMDS) in the World Health Organization (WHO) classification of myeloid neoplasms (4), but it is not currently considered a separate entity. Most cases of hMDS are classified as MDS with single- and multiple-lineage dysplasia in the WHO classification system. Hypocellular BM is predominantly found in low-risk MDS, but can also be observed in high-risk MDS (5,6).

hMDS shares some clinical manifestations with NH-MDS, such as cytopenia, BM dyspoiesis, clonal chromosomal changes and the possibility of transformation to AML. By contrast, it shows distinctive features associated with decreased BM cellularity, including more profound neutropenia and thrombocytopenia, and a lower percentage of blasts (7). Furthermore, patients with hMDS have less frequent abnormal karyotypes, a higher response rate to immunosuppressive therapy (IST)

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and a more favourable prognosis compared with patients with NH-MDS. Notably, patients with hMDS tend to be younger (hMDS is the most common MDS type in pediatric patients) (7). Since marrow cellularity decreases with age, age-adjusted criteria of hypocellularity have been proposed (e.g., <30% cellularity in patients ≤70 years and <20% cellularity in patients >70 years) (8).

hMDS is initially treated as low-risk MDS, but treatment may be tailored according to the degree of similarity to aplastic anemia (AA) or MDS. AA-like treatment is based on IST with anti-thymocyte globulin (ATG) and cyclosporine A, which suppresses the activity of aberrant T cells and helps with BM recovery. Approximately 50% of low-risk MDS patients show an objective IST response, which is associated with hypocellular BM and increased rates of transfusion independence (9). A high overall response rate (ORR) (73%) was reported in a study that focused only on hMDS treated with IST (10). Supportive care in low-risk MDS includes red blood cell (RBC) transfusions, antibiotics and erythropoietin for stimulation of RBC production. Hypomethylating agents (HMAs), such as azacytidine or decitabine, have recently been administered to high-risk patients, but these agents are effective in only ~50% of MDS patients in the short term, and a number of patients develop drug resistance and progress to AML (11). Targeted therapy using BCL2 and immune checkpoint inhibitors is being tested in combination with HMAs. HMA therapy may be a reasonable option for patients with hMDS who have high-risk cytogenetics and unfavourable somatic mutations (12). HSC transplantation (HSCT) is the only curative option for patients with MDS; however, numerous patients are not eligible for HSCT due to comorbidities usually associated with older age (13). Recently, Zhou *et al* (14) evaluated the outcomes of exclusively hMDS patients after allogenic HSCT; the patients had favourable survival rate, and none of them relapsed within a follow-up period of ~3 years.

AA is a rare BM failure (BMF) characterized by hypoplastic or aplastic BM, a paucity of hematopoietic stem and progenitor cells (HSPCs), and pancytopenia of the peripheral blood. In North America and Europe, the incidence of AA is 2-3 cases per million per year, but may be three-fold higher in Asian populations (15). AA is a disease that affects the young, typically within the first three decades of life, with a median age of onset of ~20 years old. The second peak occurs at ~60 years old (16). In some cases, inherited conditions, such as Fanconi anemia, Shwachman-Diamond syndrome and dyskeratosis congenital, can damage stem cells and lead to AA (17). Acquired AA is more frequent, and it may be caused by toxic chemicals, radiation or idiosyncratic reactions to medications or infections (18). However, in >50% of cases, there is no identifiable cause and the condition is then referred to as idiopathic AA (iAA). In iAA, a dysregulated immune system destroys HSCs either directly by activation of apoptosis or indirectly by overproduction of inflammatory cytokines. Evolution to MDS or AML occurs in up to 20% of AA patients, especially in those with an incomplete response to IST (19).

Patients with mild or moderate AA generally do not require immediate treatment, but patients with severe AA should be treated as soon as possible after diagnosis. A crucial part of patient care is supportive treatment that is focused on the prevention of infections (antibiotics) and bleeding (RBC/platelet/granulocyte transfusions). Immunosuppression with ATG and cyclosporine A is frontline treatment in older patients with AA and in patients for who matched BM donors are not available. A total of 60-70% of patients with AA show long-term durable ORR after IST (20) and may show higher response rates for IST compared with those with hMDS (21). Paroxysmal nocturnal hemoglobinuria (PNH) clones have recently been shown to be a good predictor of IST response in AA as well as MDS (22). Some patients with AA treated with IST develop clonal hematopoiesis or somatic mutations and progress to MDS or AML (23). Corticosteroids, such as methylprednisolone, are often used with immunosuppressants. Furthermore, AA therapy includes BM stimulants, such as granulocyte monocyte colony-stimulating factor or platelet growth factor (eltrombopag). Generally, HSCT is reserved for young patients and those with severe AA (<50 years old) who are more likely to have potentially fatal complications. Recently, Zhu *et al* (24) performed a meta-analysis of studies on HSCT and IST in AA, and observed longer survival times in patients after first-line allo-HSCT compared with times in those treated with first-line IST (24). However, the potential risks and benefits of HSCT should be considered for each individual patient.

Patients with hMDS and AA share overlapping clinical and pathological features; thus, distinguishing between these patients can be very difficult. An accurate diagnosis has important clinical implications, as prognosis and treatment can be quite different for these diseases. The differential diagnosis is mainly based on the presence of dysgranulopoiesis, dysmegakaryocytopoiesis, any ring sideroblasts, an increased percentage of blasts and abnormal karyotype, all favouring the diagnosis of hMDS (7). hMDS has a greater risk of neoplastic progression and a shorter survival time compared with AA (Table I) (3,7). Clonal cytogenetic abnormalities are considered typical of MDS, but they are usually found in only half of all MDS patients, and cytogenetic analyses may be less reliable when the BM is hypocellular (3). An increased percentage of CD34$^+$ cells and a tendency of positive cells to form aggregates may be useful in distinguishing hypoplastic myeloid neoplasms (hMDS and hypocellular AML) from AA (25). Furthermore, elevated levels of serum thrombopoietin have recently been reported in AA compared with those in hMDS and may also help to discriminate between these disorders (26).

2. Mutational landscape

MDS. MDS develops through a multistep process encompassing an initial deleterious genetic event within a HSC and successive genetic abnormalities, leading to clonal expansion and malignant transformation (27). In recent years, the understanding of the molecular pathogenesis of MDS has been markedly improved by next-generation sequencing (NGS), which has enabled the identification of a large spectrum of new mutations across all MDS subtypes. There are >40 significantly mutated genes in MDS, and these mutations account for nearly 90% of patients with MDS (28). Functionally, the mutations are grouped into several categories based on their prevalence: RNA splicing factors [splicing factor 3B subunit 1 (*SF3B1*), serine and arginine rich splicing factor 2 (*SRSF2*), zinc finger CCCH-type, RNA binding motif and serine/arginine rich 2 (*ZRSR2*) and U2

Table I. Clinicopathological features of AA, hMDS and NH-MDS.

Features	AA	hMDS	NH-MDS	(Refs.)
Clinical				
Age	Bimodal	Younger/older	Older	(3,84)
Transfusion dependence	++	+	+/-	(84)
Transformation to MDS/AML	~10%	~20%	~40%	(3,30,34)
Response to IST	++	+	-	(3,9,20,21)
Survival	+/-	+/-	-	30,84
Laboratory				
Bone marrow cellularity	Decreased	Decreased	Normal/increased	(3)
Dysplasia	Erythroid only	Bilineage or trilineage	Single or multilineage	(3,5)
Macrocytosis	With PNH ++	Prevalent ++	+	(3,84)
Blasts	Absent	Normal/increased	Often increased	(3)
Genetic				
Abnormal cytogenetics	4-11%	50%	30-70%	(3,119,120)
Frequent chromosomal aberrations	UPD in 6p, -7/del (7q), +6, +8, +15, del (13q)	-5/del (5q), -7/del (7q), +8, 17pLOH, del (20q), UPDs in 4q, 11q, 13q, 14q	-5/del(5q), -7/del (7q), +8, 17pLOH, del (20q), UPDs in 4q, 11q, 13q, 14q	(119,120)
Mutations	5-20%	~35%	>60%	(5,29,30,119,121)
Commonly mutated genes	*PIGA, BCOR/BCORL1, ASXL1, DNMT3A*	*TET2, DNMT3A, RUNX1, NPM1, AXL1, PIGA, STAG2*	*SF3B1, TET2, ASXL1, RUNX1, DNMT3A, IDH1/2, STAG2, TP53*	(29,30,119,121)
Variant allele frequency	<10%	~35%	>45%	(29,34,85,119)
Telomere shortening	++	+	+/++	(104,109)
Immunological				
T cell activation	+	+	+/-	(3,85,127)
T cell repertoire	Highly increased oligoclonal and polyclonal CTLs; highly increased Th cells and polarized toward Th1; decreased Tregs	Increased clonal and oligoclonal CTLs; increased Th cells and polarized toward Th1; decreased Tregs	Increased and oligoclonal CTLs; increased Th17 cells; increased Tregs	(84,85,126-128)
PNH clone	Up to 60%	Up to 40%	Up to 20%	(5,33,84,91)

AA, aplastic anemia; hMDS, hypoplastic myelodysplastic syndrome; NH-MDS, normo-/hypercellular MDS; AML, acute myeloid leukemia; IST, immunosuppressive therapy; PNH, paroxysmal nocturnal hemoglobinuria; del, deletion; UPD, uniparental disomy; LOH, loss of heterozygosity; CTL, cytotoxic T cell; Th, T helper cell; Tregs, regulatory T cell; +, presence or response or favourable; -, absence or no response or poor.

small nuclear RNA auxiliary factor 1/2 (*U2AF1/2*)], epigenetic regulators [Tet methylcytosine dioxygenase 2 (*TET2*), DNA methyltransferase 3α (*DNMT3A*) and isocitrate dehydrogenase (NADP(+)) 1/2 (*IDH1/2*)], components of the cohesion complex (stromal antigen 2, CCCTC-binding factor, structural maintenance of chromosomes 1A and RAD21 cohesin complex component), chromatin modifiers [ASXL transcriptional regulator 1 (*ASXL1*) and enhancer of zeste 2 polycomb repressive complex 2 subunit (*EZH2*)], transcription factors [tumor protein p53 (*TP53*), RUNX family transcription factor 1 (*RUNX1*), ETS variant transcription factor 1 (*ETV1*) and GATA binding protein 2 (*GATA2*)], signal transduction molecules [Fms related receptor tyrosine kinase 3 (*FLT3*), Janus kinase 2 (*JAK2*), MPL proto-oncogene thrombopoietin receptor (*MPL*), GNAS complex locus and KIT proto-oncogene receptor tyrosine kinase], RAS pathway [KRAS proto-oncogene GTPase, NRAS proto-oncogene GTPase (*NRAS*), Cbl proto-oncogene, neurofibromin 1 and protein tyrosine phosphatase non-receptor type 11 (*PTPN11*)] and DNA repair [ATM serine/threonine kinase, BRCA1/BRCA2-containing complex subunit 3, DNA cross-link repair 1C and FA complementation group L]. Mutations in RNA splicing and DNA methylation genes seem to occur early and are considered founder mutations in >50% of patients with MDS (28). Mutations provide a wide range of prognostic information, from benign to malignant and from good to poor overall survival (OS) time. For example, *TP53*, *EZH2*, ETS variant transcription factor 6 (*ETV6*), *RUNX1*, *ASXL1* and *SRSF2* mutations predict shorter survival time. The *SF3B1* mutation is strongly associated with ring sideroblasts and thus has been included as a diagnostic criterion in MDS with ring sideroblasts (4).

There are several reports concerning differences in the mutational landscapes between hMDS and NH-MDS (Fig. 1). Nazha *et al* (29) compared the mutational profiles of 62 genes between patients with hMDS and NH-MDS. Patients with hMDS acquired fewer somatic mutations and had smaller driver clones compared with patients with NH-MDS. Splicing somatic mutations were determined predominantly in patients with NH-MDS, as driver clones were found exclusively in these patients. The study hypothesized that the immune system in patients with hMDS may suppress the driver clone by inhibiting its growth and genetic evolution, thus limiting the acquisition of downstream somatic lesions. Notably, some driver clones, such as *SF3B1*, *SRSF2*, *TET2*, *ASXL1* and BCL-6 coreceptor (*BCOR*), may overcome this inhibitory effect (29). Yao *et al* (30) detected at least one gene mutation (17 genes) in 35% of patients with hMDS, and the most common mutation was an SF3B1 mutation. Patients with hMDS exhibited significantly lower incidence rates of *RUNX1*, *ASXL1*, *DNMT3A*, *EZH2* and *TP53* mutations, and a lower number of mutations per subject compared with patients with NH-MDS; however, the number was significantly higher in comparison with the number in patients with AA. Schwartz *et al* (31) used a whole exome sequencing approach to describe somatic and germline changes in pediatric MDS and found prevalent Ras/MAPK pathway mutations compared with that in adult MDS. Huang *et al* (6) did not find any difference in the incidence of *RAS*, acute myeloid leukemia 1 protein, *JAK2*, *PTPN11* or *FLT3*/internal tandem duplication mutations between hMDS and non-hMDS. Bono *et al* (5) reported mutational data from

a 24-gene panel on a large cohort of hMDS patients (n=93) and detected one or more somatic mutations in 38% of patients with hMDS. In comparison to non-hMDS patients (n=239), the patients with hMDS had a lower number of mutations per subject, but this number was significantly higher than that found in the patients with AA. The prevalence of splicing mutations (*SF3B1* and *SRSF2*) and co-mutation patterns of *TET2*, *DNMT3A* and *ASXL1* was lower in hMDS compared with that in non-hMDS. The integration of mutational data into a scoring formula enabled the separation hMDS patients with myeloid neoplasm-like profiles from those with non-malignant profiles. It was suggested that hMDS more likely represents a mixture of entities along a spectrum rather than a homogeneous in-between category (5).

Taken together, these results suggest that the mutational profile of hMDS overlaps with the profile of NH-MDS, except for the lower incidence of mutations in splicing factors and in *ASXL1* and *IDH1/2* genes. Patients with hMDS have fewer somatic mutations, and overall, smaller driver clones.

AA. In AA, the most frequently mutated genes are phosphatidylinositol glycan anchor biosynthesis class A (*PIGA*), *BCOR*/BCOR-like 1 (*BCORL1*), *DNMT3A* and *ASXL1*, suggesting mechanisms of clonal selection. Mutations in *PIGA* and *BCOR*/*BCORL1* are more specific to AA, while *DNMT3A* and *ASXL1* mutations are also found in MDS (Fig. 1). *PIGA* somatic mutations are found in up to 40% of patients with AA (16,32). PNH clones are detected in a higher proportion of patients with AA (up to 60%) and have been shown to escape T cell-mediated destruction. Blood cells with *PIGA* mutations are likely less immunogenic and thus may acquire a survival advantage (33). Somatic mutations in *JAK2/JAK3*, *RUNX1*, *TP53*, *TET2*, and CUB and sushi multiple domains 1 genes are less common in AA, and *SRSF2*, *U2AF1*, *MPL* and Erb-B2 receptor tyrosine kinase 2 mutations are rare (<3% of acquired AA cases) (34). Detected somatic mutations in AA have mostly variant allelic frequencies of <10% (23,34). Patients with AA and *PIGA*, *BCOR* or *BCORL1* mutations show a better response to IST, as well as improved progression-free survival (PFS) and OS rates, while *DNMT3A*, *ASXL1*, *JAK2/JAK3* or *RUNX1* mutations are associated with a worse IST response and survival rate. Notably, mutations in *DNMT3A* or *ASXL1* increase the risk of developing MDS from AA (23,34,35). Keel *et al* (36) detected pathological mutations in *MPL* and *TP53* genes in young patients with AA and MDS.

A high incidence of somatic mutations in MDS suggests that mutational profiling of myelodysplasia-related genes may help to distinguish AA from hMDS and may identify patients who are at risk for progression. Kulasekararaj *et al* (23) used targeted high-throughput DNA sequencing to determine somatic mutations in patients with acquired AA. Somatic mutations (*ASXL1*, *DNMT3A*, *BCOR*) were detected in 19% of patients with AA who had a longer disease duration and a higher risk of MDS transformation than those without mutations. Notably, the detection of *ASXL1*, *DNMTA*, *BCOR* and *TET2* mutations in the AA cohort coupled with published expression data provides a role for the potential association and cooperation between mutations in epigenetic regulators and immune-mediated BMF. Similarly, Huang *et al* (37) focused on a limited number of genes and found mutations
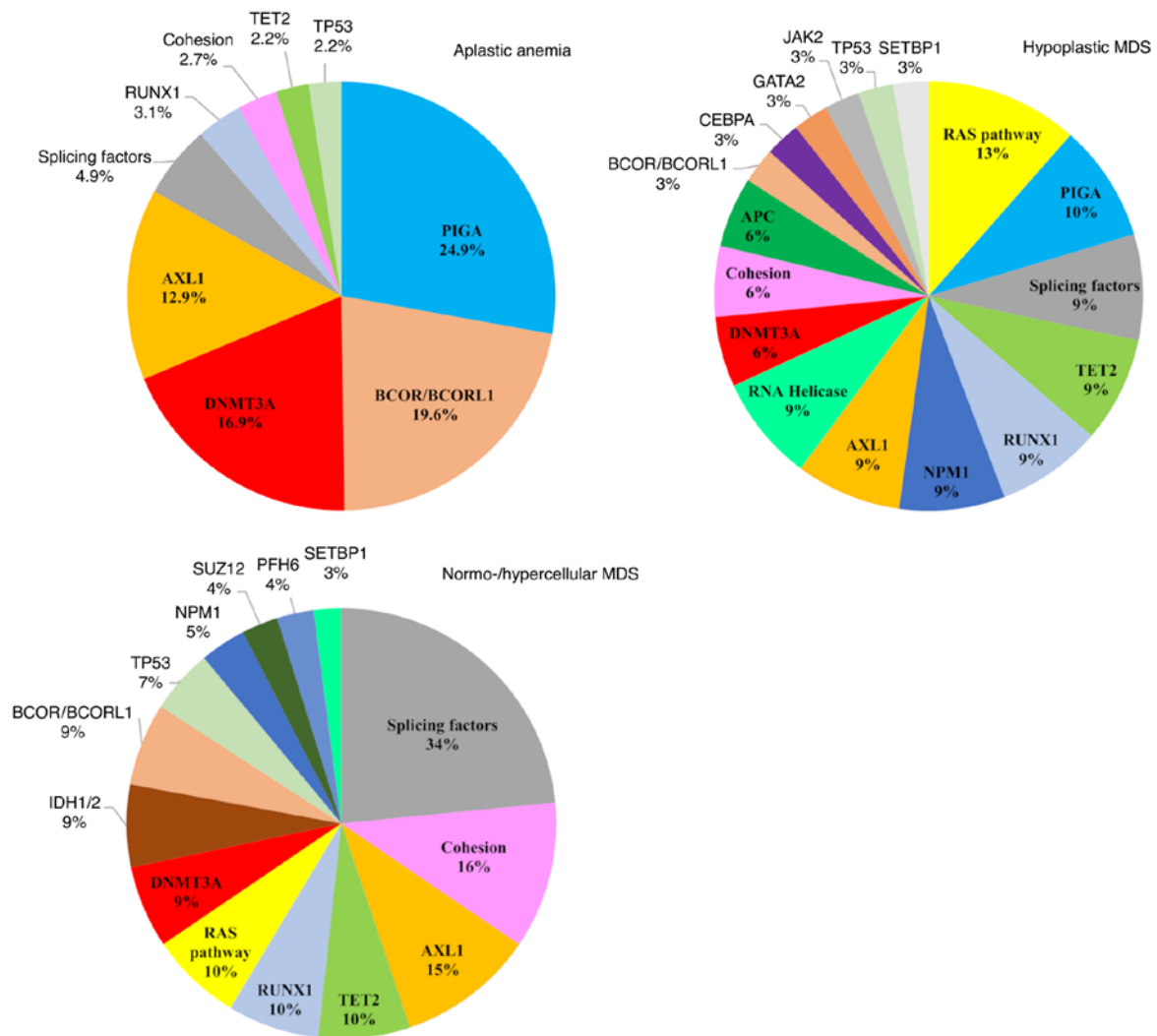
Figure 1. Relative frequency of the most common mutations in AA, hMDS and NH-MDS. Frequency of mutations in AA, hMDS and NH-MDS according to three comprehensive studies that focused on comparisons of these bone marrow failure syndromes (29,119,121). Only mutations with a frequency >2% are shown. AA, aplastic anemia; hMDS, hypoplastic myelodysplastic syndrome; NH-MDS, normo- or hypercellular MDS.

in epigenetic regulator genes, including *TET2* and *ASXL1,* in 17.4% of patients with AA. By contrast, Heuser *et al* (38) identified somatic mutations in only 5.3% of patients with AA and suggested that mutations in myeloid malignancy-related genes are rare in this disease.

3. Dysregulation of non-coding RNA (ncRNA)

MicroRNAs. In the last two decades, it has become increasingly evident that ncRNAs are important regulators of biological processes, including blood cell differentiation and immune response. There are several categories of ncRNAs, such as microRNAs (miRNAs), Piwi-interacting RNAs, small nucleolar RNAs and long ncRNAs (lncRNAs) (39). miRNAs are the most prolific class of ncRNAs and have been shown to play a role in the pathogenesis of MDS (40). Comprehensive data are available on expression miRNA profiles associated with MDS subtypes, disease stages and treatment response, as well as on dysregulation of specific miRNAs and their role in pathogenesis (Table II). As MDS originates in HSCs, a number of studies have been performed on CD34^{+} cells.

Abundantly expressed miRNAs in CD34^{+} cells of patients with MDS include, but are not limited to, *let-7b*, *miR-10a*, *miR-25*, the *miR-26* family, *miR-128a*, *miR-146*, *miR-155*, *miR-181a*, *miR-222* and *miR-223* (41). To date, no study has focused on the differential expression of miRNAs between hMDS and NH-MDS. In general, low-risk patients show distinctive expression profiles compared with high-risk patients (42,43). Sokol *et al* (43) defined a unique signature of 10 miRNAs (*miR-181a/b/c/d*, *miR-221*, *miR-376b*, *miR-125b*, *miR-155*, *miR-130a* and *miR-486-5p*) that accurately differentiated low-risk patients from high-risk patients. Notably, the 6-miRNA signature may distinguish RA/refractory cytopenias with multilineage dysplasia (RCMD) patients with a normal karyotype from those with trisomy 8, who usually show a good response to IST.

A cluster of 13 miRNAs, including *miR143*/*miR-145*, has been mapped in the deletion region del5q31-5q35 (44); these miRNAs are downregulated in a variety of human cancer types, such as colorectal and gastric cancer (45,46). Haploinsufficiency of these miRNAs and *miR-146a* (adjacent to the commonly deleted region) contributes to the 5q-syndrome

Table II. Dysregulated miRNAs and lncRNAs in MDS and acquired AA.

ncRNAs	MDS	Acquired AA
Upregulated miRNAs	miR-299-3p, miR-299-5p, miR-323-3p, miR-329, miR-370, miR-409-3p, miR-431, miR-432, miR-494, miR-654-5p and miR-665 (42) miR-34a, miR-148a, miR-199b, miR-451, miR-486, miR-125a, miR-151, miR-199a, miR-10a, miR-10b, miR-125b, miR-29c, miR-130a, miR-24, miR-126, miR-335 and miR-99b (48) miR-17-3p, miR-17-5p, miR-18a, miR-10a, miR-10b, miR-15a, miR-16, miR-222, miR-155, miR-181a, miR-21 and miR-126 (122) miRNAs clustered on 14q32 (123)	miR-34a-5p, miR-195-5p, miR-424-5p, miR-25-3p, miR-143-3p and miR-145-5p (52) miR-34a (56) miR-23a (66)
Downregulated miRNAs	miR-124, miR-146a, miR-150, let-7e, miR-143, miR-671-5p, miR-155, miR-182, miR-124a, miR-200c, miR-342-5p and let-7a (13) miR-196a*, miR-423-5p, miR-525-5p, miR-507, miR-583, miR-940, miR-1284 and miR-1305 (42) miR-222 and miR-10a (43) miR-145 and miR-146a (124)	miR-27b-3p, miR-130a-3p, miR-149-5p, miR-199a-5p, miR-382-5p, and miR-181d-5p (52) miR-126-3p, miR-145-5p, miR-223-3p and miR-199a-5p (55)
Upregulated lncRNAs	H19, EMCN-IT1, WT1-AS, MEG8 and PVT1 (59) TC07000551.hg.1, TC08000489.hg.1, TC02004770.hg.1 and TC03000701 (60) linc-RPIA, linc-ARFIP1-4, linc-TAAR9-1, lincC2orf85 and linc-RNFT2-1 (61) LOC101928834 (64)	
Downregulated lncRNAs	MEG3 (58) LEF1-AS1, TCL6, ST6GAL2-IT1 and U3 (59) LEF1-AS1 (65) lncENST00000444102 (125)	MEG3 (66) AC007556.1, AC007922.2, AC147651.1, C11l000.4, AC007991.2 and RHOXF1P1 (68)

AA, aplastic anemia; MDS, myelodysplastic syndrome; miR/miRNA, microRNA; lncRNA, long non-coding RNA.

phenotype (47,48). Furthermore, *miR-145* and *miR-146a* are implicated in the dysregulation of innate immune signaling in MDS HSPCs (49). *miR-146a* is a negative regulator of immune cell activation; it represses two targets, tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) and interleukin 1 receptor-associated kinase, which are signaling transducers upstream of nuclear factor κB (NF-κB) (40). NF-κB activation is regulated by *miR-125a*, which also plays a role in the regulation of innate immunity pathways and erythroid differentiation in MDS (50).

Compared with hMDS, acquired AA has more available miRNA data (Table II). Srivastava *et al* (51) recently found deregulated expression of *miR-126*, *miR-145*, *miR-155*, *miR-146* and *miR-150* in AA, and determined their target genes, phosphoinositide-3-kinase regulatory subunit 2 (*PIK3R2*), MYC proto-oncogene (*MYC*), suppressor of cytokine signaling 1, *TRAF6* and MYB proto-oncogene, respectively. In other recent study, Lu *et al* (52) integrated multiple expression profiles of miRNAs and mRNAs of BM T cells from patients with acquired AA and showed that *miR-34a-5p*, *miR-195-5p* and *miR-424-5p* may modulate T-cell differentiation and plasticity by targeting histone gene expression and histone modification. A similar approach was used in the study by Adhikari and Mandal (53), which identified significant upregulation of *miR-1202* in patients with AA compared with controls, and in which its putative targets, rap guanine nucleotide exchange factor 5 and mannosidase endo-α, were predicted. In the plasma of patients with acquired AA, Hosokawa *et al* (54) identified deregulation of *miR-150-5p*, *miR-146b-5p* and *miR-1*, which target immune pathways related to Toll-like receptors and TNF-α. Notably, the miRNA expression was restored to normal after successful IST. In particular, *miR-150-5p* showed a correlation with IST response, suggesting that it may serve as a biomarker for therapeutic monitoring (54). The same group analyzed miRNA levels in CD4$^+$ and CD8$^+$ T cells from patients with AA and detected downregulation of *miR-126-3p* and *miR-223-3p* in CD4$^+$ T effector memory cells, and of *miR-126-3p*, *miR-145-5p* and *miR-223-3p* in CD8$^+$ T effector memory and terminal effector cells. The expression levels of *miR-126-3p*, *miR-145-5p* and *miR-223-3p* became normal after successful IST. *MYC* and *PIK3R2* genes were shown to be targets of *miR-145-5p* and *miR-126-3p*, respectively (55). Sun *et al* (56) demonstrated that overexpression of miR-34a and downregulation of its target gene, diacylglycerol kinase ζ, enhanced T-cell activation in acquired AA. Giudice *et al* (57) analyzed exosomal miRNAs in severe AA and MDS, and found distinctive signatures between these BMF disorders. In patients with AA, *miR-126-5p* showed a negative correlation with IST response, and patients with high miRNA levels at diagnosis had the shortest PFS time compared with patients with lower or normal levels. Furthermore, *miR-4651* was exclusively present in severe AA responders to IST (57).

lncRNAs. lncRNAs represent another important class of ncRNAs whose role in hematopoietic disorders is being explored. Studies on lncRNAs in MDS display heterogeneity in experimental design (size of patient cohort, MDS subtypes, technologies used and analytical approaches), and thus far, no study has focused only on hMDS. The very first study by Benetatos *et al* (58) revealed hypermethylation of the maternally expressed 3 (*MEG3*) gene promoter in 34.9% of patients with MDS, which may confer a worse overall prognosis. Next, genome-wide studies defined the gene expression profiles of lncRNAs in various specific groups of patients with MDS, such as those with primary MDS (59,60), refractory anemia (RA) with excess blasts type 2 (RAEB-2) MDS (61), *de novo* MDS and MDS evolved from AA (62). Recently, Szikszai *et al* (59) analyzed lncRNA expression across all MDS subtypes and evaluated them in relation to disease subtypes, cytogenetic and mutational aberrations, and risk of progression. Comparative analysis between low- and high-risk patients determined 16 deregulated lncRNAs [e.g., downregulated *RP11-897M7.1* and long intergenic non-protein coding RNA 539, and upregulated T cell leukemia/lymphoma 6, long intergenic non-protein coding RNA 1013, LEF1 antisense RNA 1 (*LEF1-AS1*) and *CTC-436K13.2* in low-risk patients] (59). Yao *et al* (60) attempted to use lncRNA expression for the risk stratification of patients with MDS and integrated four lncRNAs (*TC07000551.hg.1*, *TC08000489.hg.1*, *TC02004770.hg.1* and *TC03000701.hg.1*) whose expression levels were associated with OS into a risk-scoring system. Higher lncRNA scores were associated with high-risk MDS, complex karyotype, and *RUNX1*, *ASXL1*, *TP53*, *SRSF2* and *ZRSR2* mutations. In relation to the skewed T cell repertoire in MDS, pathway analysis of differentially expressed genes between patients with the highest and lowest lncRNA risk scores determined T cell-related pathways [e.g., cytotoxic T-lymphocyte-associated protein 4 signaling in cytotoxic T lymphocytes and CD28 signaling in T helper (Th) cells] to be the most significant (60). Hung *et al* (63) recently identified an association between higher *KIAA0125* expression (BM mononuclear cells) and high-risk MDS, *ASXL1* and *NRAS* mutations, and poorer OS and leukemia-free survival. A recent study by Li *et al* (64) reported an association between a higher expression level of *LOC101928834* and a higher white blood cell count, a higher blast percentage, RAEB subtype and a shorter OS time in MDS. By contrast, *LEF1-AS1* expression has been shown to be significantly downregulated in patients with MDS compared with that in healthy controls (65).

There are limited data on lncRNAs in AA. Recently, Wang *et al* (66) demonstrated decreased expression of *MEG3* in CD4$^+$ T cells derived from patients with AA. *MEG3* can act as an miRNA sponge that sequesters *miR-23a* and induces T cell immunoreceptor with Ig and ITIM domains expression in CD4$^+$ T cells, leading to the expansion of Th17 and Th1 cells and increased serum TNF-α and interferon-γ (IFN-γ) levels. Jiang *et al* (67) reported that fibroblast growth factor 1 promoted the proliferation of BM mesenchymal stem cells from patients with AA by acetylation of lncRNA in the testis development related 1 gene promoter. Lu *et al* (68) recently analyzed differentially expressed lncRNAs and mRNAs between children with acquired AA and healthy controls. The study defined immune- or hematopoietic-related lncRNA/mRNA pairs [*AC007556.1*/dehydrogenase/reductase 9, *AC007922.2*/histamine receptor H4, *AC147651.1*/platelet derived growth factor subunit A, *AC111000.4*/growth factor independent 1B transcriptional repressor, *AC007991.2*/indoleamine 2,3-dioxygenase 1
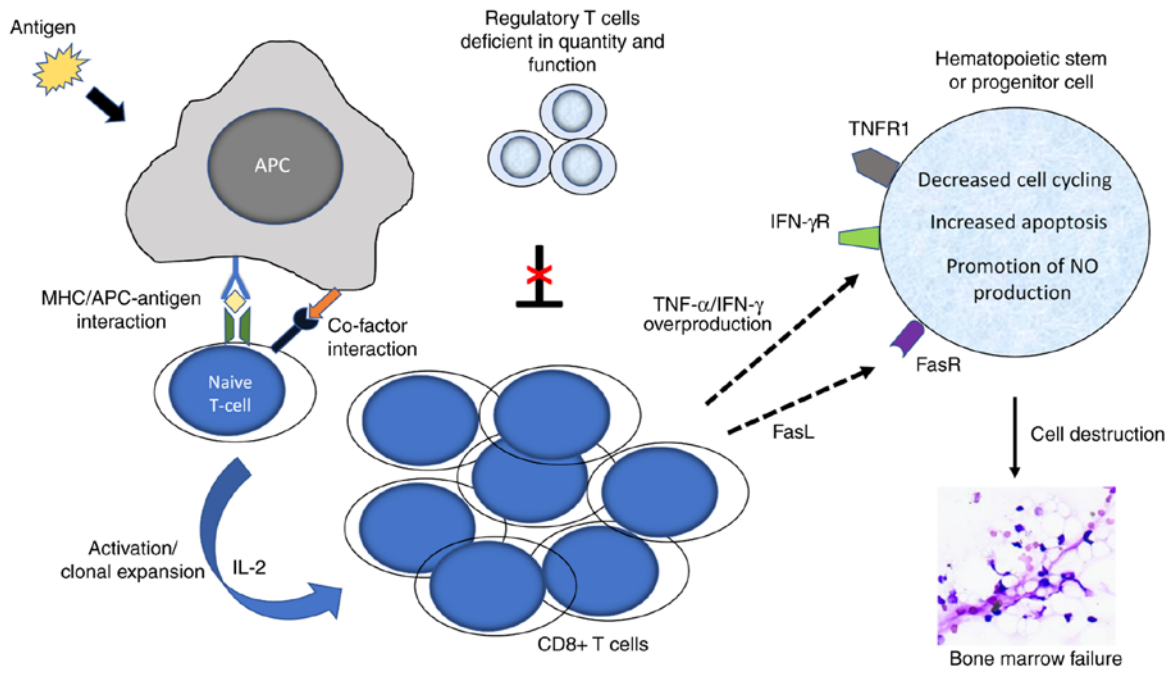
Figure 2. Proposed mechanism of hematopoietic stem cell destruction in acquired aplastic anemia. An unknown antigen that is presented by antigen-presenting cells triggers the activation of T cells that release IL-2. This results in clonal expansion of T cells overproducing proinflammatory cytokines. IFN-γ and TNF-α decrease cell cycling, increase apoptosis of HSPCs and promote the production of nitric oxide, which is toxic to other HSPCs. Regulatory T cells exhibit a decreased quantity and ability to suppress the proliferation of autologous T cells. Together, these events lead to HSPC damage and bone marrow failure. Adapted from (16). HSPC, hematopoietic stem or progenitor cell; APC, antigen-presenting cell; IL, interleukin; NO, nitric oxide; MHC, major histocompatibility complex; TNF-α, tumor necrosis factor α; IFN-γ, interferon γ; FasR, Fas receptor; FasL, Fas ligand.

and *RHOXF1P1*/semaphorin 7A (John Milton Hagen Blood Group)] that may be involved in the pathology of acquired AA (68).

Although there are no studies describing miRNA/lncRNA profiles exclusively in hMDS, there are reports demonstrating that RA and RCMD categories (typical for the majority of hMDS cases) show ncRNA expression patterns distinct from those of other MDS subtypes. Moreover, levels of specific ncRNAs have been successfully used for classification and stratification of patients with MDS (42,43,60). It may be assumed that hMDS is also associated with specific ncRNA profiles that differ from those of AA and could be used for differentiation. The dysregulation of ncRNAs detected in T cells derived from patients with AA and hMDS indicates that these regulators may contribute to the immunopathogenesis of these disorders.

4. Pathophysiology

MDS. The overlap of immunological features and the responsiveness of a significant proportion of patients with hMDS/AA to IST suggest that these distinctive clinical entities share an immune-mediated pathogenic mechanism. Clinical and experimental studies have provided compelling evidence that HSPCs are damaged by abnormally activated cytotoxic T cells (Fig. 2). Expanded CD4+ and CD8+ T cell clones have been observed in the BM of both patients with hMDS and those with AA (69,70). Melenhorst *et al* (71) analyzed the CD4+ and CD8+ T cell repertoires in patients with MDS by flow cytometry and PCR. Multiple T cell expansions (of both helper and cytotoxic T (Tc) cells) were observed, as well as

the functional differentiation *in vivo* of T cells from memory to effector T cells, in CD8+ cells. Similar findings were reported by Fozza *et al* (72), supporting the involvement of cytotoxic T cells either in antitumor immune surveillance or in autoreactive aggression toward hematopoietic precursors. Moreover, dominant T cell clones persist in patients with MDS that is unresponsive to immunosuppression and regress in responders (73). Strong polarization of BM CD4+ cells toward Th1 and of CD8+ cells toward Tc1 was observed in low-risk MDS compared with that in AA, suggesting T cell stimulation from clones of malignant hematopoietic cells (74). Regulatory T cells (Tregs) are deficient in quantity and function in patients with early MDS (75). The function of Tregs is to suppress the autoreactivity of other T cell populations to normal tissue; thus, their hypofunction may favour the autoimmune destruction of HSPCs (76).

The antigens that trigger the immune response in MDS are not known, but potential candidates [such as Wilms tumor protein 1 (WT1)] have been suggested. As patients with MDS and trisomy 8 often show a good response to IST, an immunological mechanism underlying BMF has been proposed. Trisomy 8 cells express high levels of *WT1*, and CD8+ T cells are able to recognize WT1 peptides and induce IFN-γ expression *in vitro*, suggesting that this antigen may contribute to the induction of an immune response (77). Sloand *et al* (78) further demonstrated that marrow HSCs with trisomy 8 may escape T cell-mediated destruction by overexpression of prosurvival protein cyclin D1 and survivin. Other neoantigens or overexpressed self-antigens [human leukocyte antigen (HLA)-A2-restricted nonameric peptide] may also elicit an immune response (79).

Abnormal overproduction of proinflammatory cytokines [such as TNF-α, IFN-γ and interleukin 17 (IL-17)] has been reported in patients with MDS and contributes also to ineffective hematopoiesis (80,81). In patients with low-risk MDS, the G/A polymorphism in the *TNF-α* promoter is associated with high levels of TNF-α produced by CD4^{+} and CD8^{+} T lymphocytes (82), suggesting its role in anemia. In a previous study, an increased frequency of CD4^{+} T cells producing IFN-γ was detected in hMDS, and *in vitro* decrease of interferon by cyclosporine led to improved hematopoiesis (10). The production of IFN-γ and TNF-α in low-risk MDS may be further enhanced by high levels of IL-17 (83). Based on the clinical/immunological/molecular features, Fattizzo *et al* (84) recently defined two hMDS phenotypes, namely, AA-like and MDS-like hMDS. The first is characterized by prevailing inflammation and immune activation, and a response to IST, and the second is characterized by genetic lesions, clonal selection and an increased risk of leukemic evolution.

AA. Up to 80% of patients with AA show a response to T cell-directed IST, supporting involvement of aberrant T cell populations in the pathogenesis. As in hMDS, BM T cells are also skewed toward oligo/polyclonal patterns in acquired AA (16,70,85). Giudice *et al* (85) observed oligoclonal characteristics in CD8^{+}CD57^{+} cells, as well as in total CD8^{+} T cells from patients with AA. de Latour *et al* (86) found an increased population of CD3^{+}CD4^{+}IL-17-producing T cells in patients with AA at presentation compared with that in controls, and this correlated with disease activity. Abnormally activated T cells destroy HSPCs through apoptosis [via Fas cell surface death receptor (Fas)/Fas ligand, granzyme, perforin] and the overproduction of proinflammatory cytokines. Extensive apoptosis of BM HSPCs has been observed in patients with AA, indicating that apoptosis is a major mechanism of cell destruction (87). BM CD34^{+} progenitor cells and lymphocytes of patients with AA overexpress Fas, which is involved in triggering the Fas-mediated apoptotic pathway (88). By contrast, normal expression of Fas has been observed in patients with AA in remission (89). Overproduction of cytokines may upregulate the expression of Fas (77).

As in hMDS, AA Tregs exhibit a decreased quantity and ability to suppress the proliferation of autologous T cells. Deep phenotyping of AA Tregs defined two specific Treg subpopulations, Treg A and Treg B, that may predict the response to IST. The Treg B subpopulation with a memory/activated phenotype was overrepresented in IST responders, while the Treg A subpopulation was significantly higher in non-responders. Furthermore, Tregs from patients with AA were IL-2-sensitive and could be expanded *in vitro* (90).

AA is strongly associated with PNH. PNH clones deficient in glycosyl-phosphatidylinositol (GPI)-anchored proteins appear to be spared by the immune attack mediated by T cells in BMF syndromes. PNH clones are frequently found in acquired AA (≤60%) and are also observed in MDS (10-20%, more common in low-risk cases) (91). The mechanism of this escape is not clear. It has been suggested that antigen targets of T cell attack or coregulators are GPI-linked proteins. Gargiulo *et al* (92) demonstrated that CD1d-restricted, GPI-specific CD8^{+} T cells are expanded in patients with PNH,

suggesting that the GPI may be targeted by autoreactive T cells and that these T cell clones are responsible for the BMF in PNH. Hanaoka *et al* (93) suggested that immunoselection of *PIGA* mutant cells is due to a deficiency in the stress-inducible GPI-linked membrane proteins UL16 binding protein 1 and 2, which activate natural killer and T cells. Furthermore, *PIGA* clones may acquire additional somatic mutations (*TET2*, SUZ12 polycomb repressive complex 2 subunit, *U2AF1* and *JAK2*), resulting in a proliferative advantage (94). Mechanisms and factors implicated in the immunopathogenesis of AA and hMDS are summarized in Table III.

In addition to the T cell-mediated immune response, aging, which is associated with numerous changes in the immune system, including chronic low-grade inflammation (known as inflammaging), may be involved in the development of AA and hMDS in elderly patients (95).

5. Genetic and molecular basis of an aberrant immune response

The genetic and molecular basis of an abnormal T cell response is being studied. Several polymorphisms in cytokine genes (e.g., IFN-γ, TNF-α and IL-6) have been linked to the high production of proinflammatory cytokines in AA and MDS (82,96). Furthermore, specific HLA haplotypes are associated with the AA phenotype and response to IST, suggesting that cytotoxic T cells may target the autoantigens presented on HSCs through these HLA class I molecules. HLA-DR15 (a serological split of HLA-DR2) is overrepresented in AA patients and MDS patients with RA compared with that in their healthy counterparts (97). The presence of this HLA allele is associated with a better response to IST in AA (98). Notably, patients with MDS bearing a PNH clone have a significantly higher HLA-DR15 allele frequency (99). Katagiri *et al* (100) demonstrated frequent loss of HLA alleles associated with copy number-neutral 6pLOH in acquired AA. Notably, the missing HLA alleles in 6pLOH(+) clones included HLA-A*02:01, A*02:06, A*31:01 and B*40:02, which were overrepresented in the germline of patients with AA. Osumi *et al* (101) suggested that *HLA-B*40:02* is one of the target antigens of T cells in idiopathic AA and that mutations in this HLA allele contribute to clonal escape. Babushok *et al* (102) screened patients with AA for somatic *HLA* class 1 loss and detected it in 17% of cases. Furthermore, the loss was correlated with a more severe disease course and more frequent evolution to MDS. Mutations in β2-microglobulin gene may represent another mechanism of MHC class I loss leading to defective CD4-8[+] cell-mediated cytotoxicity (103).

Defective telomere homeostasis is also suggested to play a role in the pathogenesis of AA and MDS. Approximately 35% of patients with AA show telomere length shortening in peripheral granulocytes and mononuclear cells. Patients with AA responsive to IST do not possess telomeres that differ in length compared with controls, while untreated patients and non-responders show marked telomere shortening (104). The degree of telomere erosion has been correlated with the severity of AA, risk of relapse, overall survival rate and risk of clonal evolution to MDS (105). In MDS, telomere shortening is mostly linked to disease progression and leukemic transformation into AML. A decrease in telomere length was also observed in

Table III. Immunopathological mechanisms and genetic factors involved in the immune dysregulation of AA and hMDS.

Factors	AA	hMDS	(Refs.)
Immunological			
Cytotoxic T cells	Highly increased oligoclonal and polyclonal	Increased oligoclonal and clonal	(84,85,127)
T helper cells	Highly increased and polarized toward Th1 cells (clonal) Increased Th17 cells in severe AA	Increased polyclonal and polarized toward Th1 cells	(74,84,86,126,127)
Regulatory T cells	Deficient in quantity and function	Deficient in quantity and function	(76,84,90)
PNH clone	Up to 60%	Up to 40%	(84,121)
LGL	Increased	More increased oligoclonal and polyclonal	(3,84,129)
B cells	More reduced	Decreased	(84,129)
Macrophages	Increased TNFα producing intermediate monocytes	Increased TNFα producing macrophages	(84)
Cytokine production	Highly increased	Increased	(80,84)
Cytokine levels			(26,80,84,130)
TNF-α, IFN-γ, TGF-β, and G-CSF	Highly increased	Increased	
IL-10	Increased	Decreased	
Putative antigens	HLA class I molecules (HLA-DR15 and HLA-B*40:02) GPI-linked proteins	WT1, HLA-DR15	(77,92,93,97,101,127)
Genetic			
Polymorphisms in cytokine genes	IFN-γ, TNF-α, and IL-6	IFN-γ, TNF-α, TGF-β and IL-10	(96,127,130)
Mutations	STAT3 mutations	STAT3 mutations; AXL1 mutations	(115,116)
Dysregulated gene/protein expression	Downregulated miR-126-3p, miR-145-5p, and miR-223-3p; overexpression of miR-34a; downregulated MEG3; overexpression of FasR	Overexpression of WT1 mRNA/protein; overexpression of FAS-L and TRAIL	(55,56,66,79,88,127)

AA, aplastic anemia; hMDS, hypoplastic myelodysplastic syndrome; Th, T helper; Th17, T helper 17; PNH, paroxysmal nocturnal hemoglobinuria; LGL, large granular lymphocyte; HLA, human leukocyte antigen; GPI, glycosyl-phosphatidylinositol.

low-risk MDS patients with RA (42%) and patients with low to intermediate-1 risk (43.1 and 30.8%, respectively), according to the International Prognostic Scoring System, compared with age-matched controls (106-108). Bouillon *et al* (109) found significantly shortened age-adapted telomere length in both patients with hMDS and those with AA, but patients with AA showed more accelerated telomere shortening compared with patients with hMDS.

Mutations in telomerase complex genes (telomerase RNA component and telomerase reverse transcriptase) have been reported in AA and MDS (110,111); however, the mutations are considered risk factors for BMF rather than genetic determinants (112). Genetic variants of other telomerase genes, i.e., telomeric repeat binding actor 1/2, may be associated with risk for AA; however, they are rare (113). Furthermore, the presence of pathogenic regulator of telomere elongation helicase 1 variants, resulting in telomere erosion, has been associated with AA and hMDS (114).

In MDS, *AXL1* mutation appears to be relevant to immune-mediated BMFF, since patients with this mutation show an upregulation of the immune response pathway compared with patients with wild-type *ASXL1* (115). Furthermore, acquired signal transducer and activator of transcription 3 mutations have been found predominantly in acquired AA and MDS with hypoplastic features, suggesting that they may result in self-reactivation of cytotoxic T lymphocytes (116). Notably, some immunodeficiencies, such as cytotoxic T lymphocyte associated antigen deficiency and deficiency of adenosine deaminase 2, are associated with iAA (117). *GATA2* deficiency is also associated with AA and its clonal evolution to myeloid malignancies (118).

Potential implications of ncRNAs in the immunopathogenesis of BMF were demonstrated the study by Hosokawa *et al* (55), which detected downregulation of *miR-126-3p* and *miR-223-3p* in CD4$^+$ T effector memory cells and downregulation of *miR-126-3p, miR-145-5p* and *miR-223-3p* in CD8$^+$ T effector memory and terminal effector cells in AA. *miR-126-3p* and *miR-145-5p* targeted *MYC* and *PIK3R2*, which were upregulated in the CD4$^+$ and CD8$^+$ T cells of the patients with AA. Notably, successful IST was associated with the recovery of miRNA levels.

6. Conclusions

Although acquired AA and hMDS represent distinct clinical entities, they show considerable clinicopathological similarities and are difficult to distinguish from each other. The overlaps likely originate from a common pathogenic mechanism based on cytotoxic T cell-mediated attack against certain antigens located on stem or more lineage-restricted progenitor cells. Despite the overlaps, these disorders differ in some characteristics that are an important part of the differential diagnosis. However, the cytological/morphological differences may be subtle due to severe hypocellularity in some cases and need to be evaluated carefully in the context of other findings.

Deep phenotyping has proposed that hMDS is a mixed phenotypic entity comprising of two phenotypes, one resembling AA (non-malignant BMF) and one closer to that of NH-MDS (BMF prone to malignant transformation). A similar situation likely exists also in AA, in which a small proportion of patients transform to MDS and/or AML, even after successful IST in some cases. Identifying patients at risk of disease progression is a crucial step for early intervention and appropriate follow-up.

The NGS era has increased our knowledge of genetic lesions in these disorders and improved the diagnostic specificity of identifying malignant myelodysplasia; however, there are no specific mutations that clearly separate AA from hMDS. Mutations in *BCOR/BCORL1, PIGA, DNMT3A* and *ASXL1* genes are prevalent in AA, but *DNMT3A* and *ASXL1* mutations are also found in MDS. Clones with *DNMT3A* and *ASXL1* mutations usually increase in size and predict a poorer response to IST and progression to MDS/AML. By contrast, *BCOR, BCORL1* and *PIGA*-mutated clones remain small or disappear and predict a better response to IST and favourable outcomes of AA. High diversity of mutational profiles, driver vs. passenger mutations and infrequently mutated genes of unclear pathogenetic relevance are challenging aspects of NGS testing. The role of other molecular factors in BMF, such as ncRNAs, is also being explored. With the development of RNA interference technology and miRNA-inhibitory agents, these RNAs may provide novel therapeutic approaches in autoimmune disorders.

In conclusion, the diagnostic criteria defining boundaries between AA and hMDS remain the focus of debate and will surely be refined by the incorporation of molecular features into classification schemes.

Availability of data and materials

Not applicable.

Authors' contributions

HV wrote the manuscript. MB critically revised the manuscript. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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