Immunological derangement in Hypocellular Myelodysplastic Syndromes

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Abstract - Hypocellular or hypoplastic myelodysplastic syndromes (HMDS) are a distinct subgroup accounting for 10–15% of all MDS patients, that are characterized by the presence of bone marrow (BM) hypocellularity, various degree of dysmyelopoiesis and sometimes abnormal karyotype.

Laboratory and clinical evidence suggest that HMDS share several immune-mediated pathogenic mechanisms with acquired idiopathic aplastic anemia (AA).

Different immune-mediated mechanisms have been documented in the damage of marrow hematopoietic progenitors occurring in HMDS: they include oligoclonal expansion of cytotoxic T lymphocytes (CTLs), polyclonal expansion of various subtypes of T helper lymphocytes, overexpression of FAS-L and of the TNF-related apoptosis-inducing ligand (TRAIL), underexpression of Flice-like inhibitory protein long isoform (FLIPL) in marrow cells as well as higher release of Th1 cytokines, such as interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α). It has also been documented that some HMDS patients have higher frequency of polymorphisms linked both to high production of proinflammatory cytokines such as TNF-α and transforming growth factor-β and to the inhibition of T-cell mediated immune responses such as interleukin-10, further suggesting that immune-mediated mechanisms similar to those seen in AA patients may also operate in HMDS.

Clinically, the strongest evidence for immune–mediated hematopoietic suppression in some HMDS is the response to immunosuppression including mainly cyclosporine, antithymocyte globulin and/or cyclosporine, or alemtuzumab.

Here we review all these immune mechanisms as well as the influence of this deranged cellular and humoral immunologic milieu on the initiation and possible progression of MDS. All these observations are pivotal not only for a better understanding of MDS pathophysiology, but also for their immediate clinical implications, eventually leading to the identification of MDS patients who may benefit from immunosuppression.

Keywords: hypocellular myelodysplastic syndrome, immune system, bone marrow microenvironment

1. OVERLAPPING AND DIFERENTIAL FEATURES BETWEEN APLASTIC ANEMIA, HYPOCELLULAR AND NORMO/HYPERCELLULAR MDS

Myelodysplastic syndromes (MDS) are a heterogeneous group of diseases characterized by impairment of cellular differentiation (also defined as ineffective hematopoiesis) progressive peripheral cytopenias and increased risk of developing acute myeloid leukemia (AML) [1]. Although the French-American-British (FAB) and World Health Organization (WHO) classification systems do not take into account hypoplastic or hypocellular myelodysplastic syndromes (HMDS) as a defined category of MDS, being HMDS probably considered the expression of a transitional status of other MDS categories, they appear to be a distinct clinicopathologic entity [2].

HMDS accounts for 10–15% of all MDS [3-5] and are characterized by the following features: age-corrected bone marrow hypoplasia (ie cellularity less than 30% under age of 60 years or cellularity less than 20% for older than 60 years) [4], marked dyserythropoiesis, both dysgranulopoiesis and dysmegakaryopoiesis [4,5], macrocytosis, severe neutropenia and thrombocytopenia [3-6], frequent abnormal karyotype [6-8], low rate of progression to acute leukemia, and poor response to conventional therapeutic approach for MDS [6].

Recently, Bennett and Orazi described several others marrow morphological criteria, detectable by bone biopsy histological analysis, useful to help HMDS diagnosis, such as the presence of dysplastic megakaryocytes [4-5] within the disorganized microarchitecture of MDS marrow [4], the detection of fibrosis [4,8], and the immunohistochemical identification of aggregates or clusters of blasts in the central intertrabecular region of marrow, also defined as abnormally localized immature myeloid precursor cells (ALIP) [4,5,9-10].

According to FAB and WHO classification systems, the majority of HMDS cases fall into refractory anemia (RA) and refractory cytopenias with multilineage dysplasia (RCMD) categories [3-8]. In comparison to RA and RCMD, HMDS patients typically are younger, more frequently display a severe neutropenia and thrombocytopenia and a lower percentage of blasts, as well as even less frequently show karyotypically abnormal dysplastic marrow cells. Although Tüzuner et al. documented no difference in prognosis between HMDS and normo-/hypercellular MDS [11], several other studies have reported a more favorable overall survival in the subgroup of HMDS patients [6,8,12].

The distinction between HMDS and AA is even more problematic than that with RA and RCMD when marrow is sparsely cellular with an overall cellularity less than 20% and when these findings are associated with the presence of mast cells and reactive lymphocytes, sometimes organized in small lymphoid clusters, similar to those observed in AA bone marrow biopsies [4,5].

The presence of a clear dysmegakaryopoiesis and dysgranulopoiesis, but not of a mild isolated dyserythropoiesis, usually also found in AA, the detection of karyotypic and fluorescent in situ hybridization (FISH)
abnormalities, as well as the identification of any sideroblast, of clusters of blasts, and of an increased number of marrow fetal hemoglobin (HbF)-positive erythroblasts, distinctly address toward a diagnosis of HMDS [13].

However, recognized HMDS karyotypic and FISH abnormalities, such as trisomy 8, trisomy 1q, 20q deletion and monosomy 7, can be also be found, although less frequently, in AA patients in particular throughout their clinical course [6-8]. Additional clonal molecular defects such as mutations in the RNA component of telomerase (TERC) or in the telomerase reverse transcriptase enzyme (TERT) genes and several other microdeletions, assessed by single nucleotide polymorphism (SNP) array-based karyotyping, have been documented in both HMDS and AA patients [14].

The separation of AA and HMDS is even more difficult when clonal cytogenetic markers are absent. Quantification of marrow CD34+ cells by immunohistochemistry and flow-cytometry has been reported to help in distinguishing between AA and HMDS [15-20]. Matsui et al. have demonstrated that the mean percentage of CD34+ cells in AA patients is significantly lower than those of HMDS patients [15]. Noteworthy, we previously documented that, in addition to the defect in more mature committed progenitor cells, also most immature hematopoietic stem cells, measured as secondary colony-forming cells (CFC) after 5 weeks of long-term bone marrow culture (LTBMC), were affected by disease process in HMDS [18,19]. However, although marrow and circulating CD34+ cells and secondary CFC numbers were significantly higher in HMDS than in AA, we found that there was a high degree of overlap between these two diseases, clearly demonstrating that secondary CFC numbers in either marrow and peripheral blood could not help to distinguish AA from HMDS in an individual patient [17,18].

Recently, Tripathi et al. also reported that circulating blood lymphocytes of AA patients had significantly lower S-phase fraction (SPF) and aneuploidy in comparison to HMDS patients, suggesting that SPF and aneuploidy could be a further parameter to differentiate AA from HMDS patients [21].

In addition, we documented that HMDS and RA patients show a severe deficit in marrow and circulating committed (CD34+ cells and primary CFC), and immature progenitor cells (such as secondary CFC), compared to normal donors, implying that immature hematopoietic stem cell compartment is affected by disease process in HMDS. However, despite the dramatic difference in marrow cellularity, both more committed and immature progenitor cells showed no significant differences between HMDS and RA patients (Figure 1) [17].

II. IMMUNOLOGIC DERANGEMENT IN MDS AND HMDS

It is widely recognized that genetic, epigenetic, apoptotic and differentiation abnormalities characterizing MDS hematopoietic progenitors may be initiated and/or supported by a significant derangement of immunological microenvironment in some MDS patients. The immune effector mechanisms involved in the complex physiopathology of MDS include not only marrow and circulating immune cell changes, but also dysregulation in their cytokine expression and release. The role of the
main changes in the immune cell compartment and in the cytokine profile in MDS, including HMDS, is discussed below.

**Effect of immune cell changes**

Similar to AA, it has been recently documented by several authors, using different T-cell-receptor (TCR) repertoire molecular analysis techniques, that oligoclonal expansion of cytotoxic T cells (CTL) expressing specific TCR variable beta (Vβ) chain, with unique hypervariable complementarity determining region 3 (CDR3), is detectable in blood and marrow of some MDS patients, regardless of the presence of a hypocellular or normohypercellular MDS marrow [23,24].

It has been reported that some MDS patients may show a decrease of such immunodominant CTL clonotypes after immune-suppressive treatment concurrently with blood count improvement, suggesting that these CTL clonotypic-specific assays may be used for monitoring disease activity and response to immunosuppressive therapy [25,26].

The presence of these dominant T-cell clonotypes in blood and marrow of about 90% of MDS patients, as well as the documentation that the number of TCR-Vβ families with skewed CDR3 constitutes about 10% of the total T-cell population in MDS patients, suggest that these dominant clonal CTL in MDS are likely the result of an antigen-driven dominant immune response, and hypothesize their pivotal pathophysiologic role in T-cell mediated inhibition of hematopoietic progenitors in MDS patients [24-26]. Formal proof of this hypothesis has been achieved in AA patients, showing that these dominant T-cell clonotypes exert potent cytotoxicity against AA autologous marrow progenitor cells, but have not been demonstrate in MDS so far due to the difficulty of obtaining sufficient numbers of autologous target MDS cells [22,23].

The antigens driving the immune attack on hematopoietic progenitors of AA and possibly of some MDS patients could be similar, but unfortunately they are remain unknown, as well as the possible primary abnormalities of hematopoietic progenitors leading to the breaking of immune self-tolerance [24,27].

Recently, Sloand et al. have documented increased numbers of Vβ CTL subfamilies in marrow and blood of MDS with trisomy 8, suggesting that these immunodominant CTL, able to selectively kill trisomy 8 MDS cells in vitro, may account for the higher responsiveness of MDS patients with trisomy 8 to immunosuppressive therapy, compared with other MDS subtypes [6,28,29].

Furthermore, Sloand et al. have demonstrated, by microarray analysis [30], that CD34+ MDS cells with trisomy 8 overexpress the Wilms tumor protein 1 (WT1) and that circulating CD8+ CTL cells, recognizing WT1126-154 peptides, may be detected, by tetramer analysis, in this MDS population, suggesting that WT1 may operate as a neoantigen on trisomy 8 MDS cells triggering the expansion of WT1-specific CD8+ T cells and leading to autoimmune suppression of MDS clone and likely of residual normal marrow cells [27,30,31]. However, it is not possible to exclude that other neoantigens or the overexpression of other self-antigens presented by trisomy 8 MDS cells, such as neutrophil elastase (NE), proteinase 3 (P3) and the human leukocyte antigen (HLA)-A2 restricted nonameric peptide (PR1), detectable also in other myeloid malignant cells, might elicit the expansion of such antigens-specific CD8+ CTL [29,31].

Two main pathways in CTL-mediated cytotoxicity have been described: Ca2+-dependent perforin/granzyme-mediated apoptosis, and Ca2+-independent Fas ligand (FAS-L)/Fas-receptor (Fas-R) mediated apoptosis [32-37]. Previously, we have reported that Fas-L is slightly increased on CD3+ cells of HMDS and RA patients, compared with normal controls [17]. Moreover, we and others have documented that CD34+ cells of HMDS, as well as low/intermediate-1 risk and trisomy 8 MDS patients, overexpress Fas-R further suggesting a central role of Fas-L/Fas-R-mediated apoptosis in ineffective hematopoiesis of some MDS [17,28,32]. However, we did not find any differential sensitivity of circulating and marrow hematopoietic progenitors patients to Fas-L between RA and HMDS in vitro, supporting the hypothesis that similar mechanisms for CD34+ cell impairment may be involved in these two diseases [17].

Recently, it has also been reported that decreased expression of Fas-R, or Fas-associated via death domain (FADD), detected when low-risk MDS progress to AML, may be related to FAS gene silencing mediated by DNA methylation at nuclear factor KB (NF-kB) binding sites [38].

Several abnormalities have been also reported within various CD4+ subsets in MDS patients, including HMDS [3,6,39]. Similar to CTL, increased number of individual Vβ subfamilies have been detected in CD4+ T cells of MDS patients, independent of their International Prognostic Scoring System (IPSS) category; conversely to CTL, CD4+ T cells do not show increased frequencies of CDR3 immunodominant clones, indicating that expanded Vβ subfamilies are polyclonal in MDS patients [40].

We and others previously found that CD4+ T helper (Th) cells producing interferon-gamma (IFN-γ), playing a central role in promoting and maintaining CTL responses in several immune-mediated diseases, are increased in HMDS and low-risk MDS patients, compared to healthy controls; however, a high degree of overlap in IFN-γ producing CD4+ Th1 cells was present between these two diseases [17,41].

Other CD4+ T helper cell subsets, such as regulatory T-cells (Treg), Th17 and Th22, regulating CTL activity, have been described contributing to derangement of immunological microenvironment in MDS [42-46].

Impaired proliferative capacity and bone marrow trafficking of Treg, implying an enhancement of autoimmune processes, have been documented in low-risk MDS [47-51]. By contrast, high-risk MDS show increased Treg numbers, resulting in deficient anti-tumor immunity,
Figure 2. Main pathways involved in immune-mediated apoptosis of hematopoietic progenitor cell compartment in lower risk MDS and in immune-escape of MDS clone in higher risk MDS. See paragraph II, immunologic derangement in HMDS and MDS for details.

**Abbreviations.** APAF-1 = apopotic protease activating factor-1; CM Treg = central memory T regulatory cell; CTL = cytotoxic T lymphocyte; DC = dendritic cell; EM Treg = effector memory T regulatory cell; FADD = Fas-Associated protein with Death Domain; FLICE = FADD-like interleukin-1 beta-converting enzyme; FLIP = Flice-like inhibitory protein; HSC = hematopoietic stem cell; FAS-L = FAS ligand; INF-γ = interferon gamma; INFsR = interferon receptor; iNOS = inducible nitric oxide synthase; IRF-1 = interferon regulatory factor-1; KIR = Killer Ig-like receptors; MDSC = myeloid derived suppressor cell; MHC = major histocompatibility complex; NF-kB = nuclear factor kB; NK = natural killer cell; NO = nitric oxide; TCR = T cell receptor; TGFβ = transforming growth factor b; TGBR = TGFβ receptor; TH1 = lymphocyte T helper 1; TH17 = lymphocyte T helper 17; TNF-α = tumor necrosis factor alpha; TRAIL = TNF–related apoptosis-inducing ligand; TRAIL-R = TNF–related apoptosis-inducing ligand receptor; TRAF2 = TNF receptor associated factor 2.
favoring MDS progression into AML [43,45]. reported to be inversely related with CD8+ cytotoxic T-cell recruitment, degree of dyserythropoiesis, and the need for erythropoietin treatment [51]. In addition, it has been recently described that also high-risk MDS patients with normal number of total Treg may often show expansion of effector memory Tregs (TregEM: CD3+, CD4+, FOXP3+, CD25+, CD127dim, CD27+, CD45RA-), a highly suppressive Treg subset arising from central memory Treg (TregCM: CD3+, CD4+, FOXP3+, CD25+, CD127dim, CD27+, CD45RA+), which may promote MDS clone escape from immunosurveillance and finally AML progression [50]. Indeed, increased Treg EM frequency in MDS patients was reported independently associated with a higher number of marrow blast cells and decreased overall survival [48].

The role of Th17 cells in MDS is still controversial. Kordasti et al. reported higher levels of Th17 cells in low-risk MDS patients and their correlation with increased apoptosis of marrow hematopoietic cells [41]. Conversely, Bouchlou documented decreased numbers and functional impairment of Th17 cells in patients with low/intermediate-1 risk MDS, and increased numbers in patients with intermediate-2/high risk MDS [47]. By contrast, concomitant expansion of peripheral Th22 and Th17 population has been more recently documented in high-risk MDS, as compared to low-risk MDS patients. This finding suggests that these two CD4+ Th subsets, which mediate the suppression of immune anti-tumor responses, may favor MDS clone immune escape during MDS progression (Figure 2) [42].

In addition to the above mentioned effector cells, that are all involved in adaptive immune responses, also cells of innate immune responses seem equally contribute to the pathogenesis of MDS; they include natural killer (NK) cells and myeloid-derived suppressor cells (MDSCs).

NK cells may mediate inhibition of marrow hematopoietic progenitors in low-risk MDS through direct killing and production of cytokines, such as IFN-γ and TNF-α [52-55]. Higher frequencies of NK cells have been documented in low-risk MDS. Moreover, in HMDS patients, we found a decreased frequency of long cytoplasmic tail of killer cell immunoglobulin-like receptor KIR2DL3, mediating inhibitory signals [56,57].

Only recently, it has also been documented that Lin- HLA-DR CD33+ MDSCs, which play an important role in suppressing T-cell responses during inflammation and in cancer, were markedly expanded in blood and marrow of MDS patients, contributing both to ineffective granulopoiesis of MDS patients, through the production of inflammatory molecules, such as TGF-β, NO, IL-10 and arginase, and to promoting T cell tolerance favoring MDS progression [58-61].

Cytokine dysregulation

Expression and secretion profile of multiple cytokines, such as tumor necrosis factor alpha (TNF-α), TNF-related apoptosis-inducing ligand (TRAIL), IFN-γ, Flice-like inhibitory protein (FLIP), transforming growth

Concordantly, total marrow Treg levels have been factor beta (TGF-β), IL-4, IL-6, IL10 and IL17, produced by the above described immune effector cells and other components of microenvironment, have been found dysregulated in some MDS, further suggesting their contribution to the pathophysiology of this disease [3,6,17,62].

CD4+ and CD8+ T lymphocytes, mainly from low-risk MDS patients, consistently show higher expression of TNF-α, as compared to healthy controls; in addition, increased serum levels of this cytokine has been documented in lower risk MDS [17,63,64]. Furthermore, TRAIL, usually not expressed by healthy marrows cells, is overexpressed in MDS cells inducing preferentially apoptosis of cytogenetically dysplastic cells by upregulating their agonistic receptors 1/2 and likely downregulating expression and/or function of long isoform of the cytoplasmic inhibitor of apoptosis FLIP [65,66]. Interestingly, when we analyzed polymorphisms at positions -308 of the promoter region of TNF-α, we found in MDS patients a high frequency of G/A genotype, which has been associated with increased expression and production of TNF-α [56].

IFN-γ, has been frequently found overexpressed in marrow and peripheral blood cells from low-risk MDS patients, as above described; in addition, IFN-γ has been even more frequently found increased in the serum of these patients. On the other hand, we have previously documented that in vitro blockade of IFN-γ improves autologous marrow colony formation in HMDS and RA patients [17,56]. Furthermore, elevated IL-12 and IL-17 levels, enhancing IFN-γ production by CD4+ and CD8+ T lymphocytes, were recently described in lower-risk MDS patients [67]. Noteworthy, IFN-γ, in case of inhibition of interferon regulatory factor-1 (IRF-1), a tumor suppressor gene frequently inactivated in high risk MDS, may induce aberrant stimulatory signals in high risk MDS, may induce aberrant stimulatory signals in MDS clone [18,53,54]. Conversely, high levels of IRF-1 have been found associated with a favorable prognosis and an increase of autoimmune phenomena in MDS [68]. However, in contrast to AA patients, we did not find, using single nucleotide polymorphisms (SNPs) analysis, increased frequencies of the hypersecretory genotype T/T of the IFN-γ both in HMDS and normo/hypercellular MDS [56].

In addition to IFN-γ and TNF-α, TGF-β is reported to be another potent inhibitor of hematopoiesis triggering apoptosis of both early and late hematopoietic progenitors. Both TNF-α and IFN-γ, as well as, TGF-β may stimulate the expression of inducible nitric oxide synthase (iNOS) by normal MDS cells and marrow microenvironment, contributing to ineffective hematopoiesis in MDS [17,52,53,69]. IFN-γ, TNF-α, and TGF-β can all induce apoptosis of primary human hematopoietic progenitors mediated by p38 mitogen-activated protein kinase (MAPK), which has been found overexpressed in low-risk MDS [69-71].

Moreover, direct evidence of hyperactivation of the TGF-β pathway in MDS has been recently proven by
showing that smad2, a downstream mediator of TGF-β receptor I kinase (TBR1) activation, is overexpressed in gene expression profiles of MDS CD34+ cells and is constitutively activated in marrow progenitors of low-risk MDS patients [69]. Noteworthy, as previously documented for AA patients, when we examined the frequency of TGF-β polymorphisms, MDS population showed a higher rate of GG codon 25 variant genotype and of TT codon 10 variant, consistent with a high secretory phenotype [56]. When MDS patients were subdivided according to marrow cellularity, we found that hypocellular MDS were characterized by a higher prevalence for G/G genotype, further suggesting that immune-mediated mechanisms similar to those seen in AA patients may also operate in hypoplastic MDS patients, leading to depletion of early and late hematopoietic progenitor cells [56].

In HMDS patients, but not in AA patients, analysis of promoter polymorphisms of IL-10 showed increased prevalence of G/A genotype variant at position –1082, functionally associated with decreased production of IL-10 levels [56]. Defective IL-10 levels frequently described in low-risk MDS may allow enhanced production of Th1 cytokines responsible for increased apoptosis and decreased progenitor cell differentiation and proliferation in MDS patients. Conversely, high serum levels of IL-10, inhibiting T-cell mediated immune responses, have been reported in high-risk patients (Figure 2) [72].

### III. CURRENT IMMUNOSUPPRESSIVE STRATEGIES IN MDS

Immunosuppression with horse antithymocyte globulin (ATG) and cyclosporine A (CyA) is the standard of care for AA patients lacking a low-risk transplant procedure, resulting in a durable overall response rate (ORR) in about 60-70% of patients [73].

Immunosuppression was first used with some success in HMDS patients and then also applied to normo- or hyper-cellular MDS [74,75]. Since about half of all deaths in low-risk MDS are related to complications of cytopenia rather than leukemic evolution, immunosuppression in such studies has been widely used in these subtypes of MDS patients [3,6].

Main studies with current immunosuppressive treatments in MDS patients are summarized in Table 1. However, as a complete discussion on the results of the immunosuppression in MDS patients is beyond the scope of this article, the reader is referred to others extensive review on this topic [3,6,76-78].

CyA-based treatment studies have documented that CyA induces, both in hypocellular and normo-/ hyper-cellular MDS patients, fast (cumulative time to response 2 months) and sustained (about 2 years) hematological improvement in about 60% of MDS (cumulative ORR: 62%, range 8-82%) with an estimated overall survival (OS) of 28 months. In addition, CyA allows to achieve

| TRIALS | N | AGE (median years [range]) | TREATMENT REGIMENS | HMDS | ORR | TRANSFUSION INDEPENDENCE | TIME TO RESPONSE | REMISSION DURATION |
|--------|---|-----------------------------|--------------------|------|-----|-------------------------|------------------|-------------------|
| Molidrem, 1997 | 25 | 56 (24-81) | ATG | NR | 44 | 44 | NR | 10 |
| Jonasova, 1998 | 17 | 57 (25-85) | CyA | 53 | 82 | 94 | NR | 10.2 |
| Raza, 2001 | 83 | 67 (42-87) | Thalidomide | 15 | 19 | 12 | 4 | 2.5 |
| Molidrem, 2002 | 61 | 60 (27-81) | ATG | 38 | 34 | 34 | 3.5 | 36 |
| Selleri, 2002 | 11 | 54 (33-78) | CyA | 100 | 73 | 54 | 2.3 | 58.5 |
| Yaci, 2003 | 32 | 59 (28-79) | ATG+CyA | 26 | 6 | 3 | 2.5 | 12 |
| Killek, 2003 | 30 | 54.5 (31-73) | ATG | 27 | 33 | 33 | NR | 15.5 |
| Shimamoto, 2003 | 50 | 55 | CyA | 20 | 60 | 50 | 1.8 | NR |
| Deege, 2004 | 15 | 67 (32-81) | ATG + etanercept | 7 | 46 | 33 | NR | (24-36) |
| Raza, 2004 | 37 | 68 | Remicade | 22 | NR | NR | (6-12) |
| Stadler, 2004 | 35 | 63 (41-75) | ATG | 11 | 34 | 20 | 3 | 9 |
| Brolden, 2006 | 25 | 62 (43-82) | ATG+CyA | 20 | 24 | 34 | 2 | 7 |
| Slouw, 2008 | 129 | 60 (NR) | ATG | 33 | 30 | 31 | 4 | 36 |
| 74 | CyA | 24 |
| 13 | | 8 |
| 42 | ATG+CyA | 48 |
| Garay, 2009 | 15 | 63 (42-80) | ATG+CyA | 33 | NR | NR | 3.7 | NR |
| Scott, 2010 | 25 | 64 (53-85) | ATG+etanercept | 56 | NR | 2 | 5.36 |
| Slouw, 2010 | 31 | 57 (23-72) | Alemtuzumab | 68 | 29 | 3 | NR |
| Xu, 2010 | 37 | NR | CyA+Thalidomide | 38 | NR | 19.5 | NR |
| Passweg, 2011 | 45 | 62 (23-75) | ATG+CyA | 20 | NR | 16.4 |
| Xiao, 2011 | 37 | 44 (21-70) | CyA+Thalidomide | 57 | 41 | 1.8 | 22 |
| Xiao, 2011 | 80 | 41 (12-75) | CyA | 48 | 77.5 | 1.5 | 24 |
| Kadila, 2012 | 24 | 62 (41-78) | ATG+CyA | 25 | NR | 4 | NR |

**Abbreviations.** ATG = antithymocyte globulin; CyA = cyclosporine A; HMDS = hypoplastic myelodysplastic syndrome; NR = not reported; ORR = overall response rate, resulted from complete remission + partial remission + hematological improvement; OS = overall survival. Ref. [67-86].
transfusion independence in 58% of MDS patients. CsA was generally well tolerated, requiring drug withdrawal in a minority of patients, most of whom due to renal toxicity. However, we and others documented even higher hematological responses in hypocellular MDS [17,75,79-81].

Various clinical trials have investigated the role of horse (h) or rabbit (r) ATG alone [74,80,82-84] and in combination with CsA in MDS [80,85-89]. Approximately 30% of younger patients with lower risk MDS, expressing the HLA-DR15 allele and showing hypocellular bone marrow, achieved a rapid (time to response about 2.8 months) and prolonged hematological response with an estimated OS of 41 months [74,80,82-89]. As reported by the largest ATG-based treatment study of National Institutes of Health (NIH) on MDS, combination therapy of ATG with CsA, in comparison to ATG alone, is able both to further improve hematological responses allowing to achieve approximately 44% of ORR and to decrease the relapse rate [80]. In this and other ATG-based treatment study it has been reported that MDS patients relapsing post-ATG may be again responsive to CsA treatment [3,6].

In contrast to AA, although the comparative efficacy of hATG versus rATG has not been formally studied in MDS, it seems that there is no significant difference between the two sources of ATG in terms of hematological responses or adverse effects [84,90]. In addition, both CSA- and ATG-based treatments appeared do not increase progression to acute leukemia.

Alemtuzumab is an anti-CD52 monoclonal antibody, which has been successfully used in the treatment of AA patients failing initial immunosuppression with ATG and not eligible for transplant [91]. Recently, alemtuzumab monotherapy was used in a pilot study of 32 MDS patients, selected on the basis of likely responsiveness to immunosuppression due to younger age, low IPSS score, and the presence of HLA-DR15. A sustained ORR was documented among 77% and 57% of patients with intermediate-1 and intermediate-2 MDS, respectively, with a median time to response of about 3 months. Noteworthy, four of seven patients with karyotypic abnormalities at diagnosis had complete cytogenetic remission, including one patient with monosomy 7 [92].

In addition, we should mention that the therapeutic TNF-α blockade with anti-TNF-α monoclonal antibodies, soluble TNF-α receptors, and chemical inhibitors of TNF, such as thalidomide, have been disappointing when used as monotherapy [93-97]. More recently, combination of thalidomide with CsA [98,99], as well as of soluble TNF-α receptor etanercept with ATG [100,101] in MDS patients seems to increase hematological responses in MDS patients.

IV. CONCLUSION

Distinguishing HMDS from AA is clinically relevant since the incidence of progression to acute leukemia is higher in HMDS. Differential diagnosis between these two diseases is still challenging, especially when dysplastic cells are difficult to detect due to marked hypocytoleularity of specimens and karyotypic abnormalities are not found. Also the more advanced SNP technology do not help to differentiate between these two diseases given that similar molecular abnormalities may be detected both in AA and MDS, including HMDS.

The autoimmune pathogenesis of MDS, including HMDS, is multifactorial and still unraveled. Several laboratory and clinical studies have provided evidence that some MDS seem likely to be related to derangement in the complex cross-talk between immunological microenvironment and marrow hematopoietic stem cells, resulting on one side in ineffective hematopoiesis related to immune-mediated apoptosis of normal hematopoietic progenitors, and on the other side in triggering clonal expansion of dysplastic progenitor cells leading to acute leukemia development.

Mechanisms of immune damage of hematopoietic progenitors, mediated by increased numbers of CTL, IFN-γ producing CD4+ cells and Th17 cells, increased levels of pro-apoptotic cytokines, as well as by decreased numbers of Treg, are predominantly involved in HMDS and low-risk MDS. By contrast, mechanisms developing an immunosuppressive narrow microenvironment favoring MDS clone escape, mainly associated with increased numbers of TregEM, MDSCs and anti-apoptotic cytokines, as well as with NK cell dysfunction, may contribute to high-risk MDS and acute leukemia progression.

Clinically, the best evidence for immune-mediated impairment of hematopoietic progenitor cell compartment in MDS patients is the improvement of peripheral cytopenia and survival after CsA- and ATG-based regimens, or alemtuzumab. Although the response to immunosuppression has been documented more often in HMDS patients, it may occurs also in lower-risk MDS patients. Other predictive factors recognized for response to immunosuppression in MDS include mainly younger age, HLA-DR15 expression, shorter duration of red cell transfusion dependence [102] and trisomy 8.

Based on the mechanisms described above, such better understanding of innate and adaptive immune responses involved in MDS pathophysiology, may pave the way for the development of novel immunotherapeutic approaches. Indeed, therapeutic strategies interfering with immune-mediated apoptosis of hematopoietic progenitor cell compartment in lower risk MDS and with immune-evasion of MDS clone in higher risk MDS, are already under evaluation in phase I/II clinical trials and are available for investigation in MDS patients [72,103-106].

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