

Immune dysregulation in myelodysplastic syndrome

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Abstract

Myelodysplastic syndrome (MDS) represents one of the most challenging health-related problems in the elderly. Characterized by dysplastic morphology in the bone marrow in association with ineffective hematopoiesis, pathophysiological causes of this disease are diverse including genetic abnormalities within myeloid progenitors, altered epigenetics, and changes in the bone marrow microenvironment. The concept that T-cell mediated autoimmunity contributes to bone marrow failure has been widely accepted due to hematologic improvement after immunosuppressive therapy (IST) in a subset of patients. Currently, IST for MDS primarily involves anti-thymocyte globulin (ATG)-based regimens in which responsiveness is strongly associated with younger (under 60 years) age at disease onset. In such cases, progressive cytopenia may occur as a consequence of expanded self-reactive CD8⁺ cytotoxic T lymphocytes (CTLs) that suppress hematopoietic progenitors. Although most hematologists agree that IST can offer durable hematologic remission in younger patients with MDS, an international clinical study and a better understanding of the molecular mechanisms contributing to the expansion of self-reactive CTLs is crucial. In this review, data accumulated in the US, Europe, and Asia will be summarized to provide insight and direction for a multi-center international trial.

Overview of myelodysplastic syndrome

Incidence

Idiopathic MDS, as opposed to treatment-related MDS, occurs with higher incidence in older individuals in the Western world.^{1,2} In the United States, the incidence of MDS from 2001 to 2003 was recently established from population-based cancer registries from the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Program and North American Association of Central Cancer Registries (NAACCR) databases and showed

that the overall incidence is 3.4 per 100,000 (4.5 in men and 2.7 in women). The median age at diagnosis was 76 years, with 86% of cases over 60 years of age.^{3,4} Among racial groups in the US, white individuals had the highest incidence rate (3.5/100,000 in Whites) followed by African Americans (3.0/100,000), native Americans (1.3/100,000) and Asians and Pacific Islanders (2.6/100,000).^{3,4} Compared to the median age in the US, the median age of patients with MDS in Asian and African countries is younger (50 and 60 years old, respectively).⁵⁻¹¹

Current classification systems

To account for heterogeneity within this disease, in the past few decades the MDS classification system was evolved. In 1982, the French-American-British (FAB) classification system was established,¹² which was later refined to the World Health Organization (WHO) subtyping.^{13,14} And recently, the WHO classification was revised as the following groups: (1) refractory cytopenia with unilineage dysplasia (RCUD) that includes refractory anemia (RA), refractory neutropenia (RN), and refractory thrombocytopenia (RT); (2) refractory anemia with ringed sideroblasts (RARS); (3) refractory anemia with multilineage dysplasia (RCMD); (4) refractory anemia with excess blasts-1 (RAEB-1); (5) refractory anemia with excess blasts-2 (RAEB-2); (6) myelodysplastic syndrome, unclassified (MDS-U); (7) MDS associated with isolated del(5q); and (8) refractory cytopenia of childhood (RCC) as a group of provisional entities. Major points of the modification are the following: (1) patients who lack convincing morphological dysplasia but have specific MDS-related cytogenetic abnormalities have presumptive evidence of MDS; (2) refractory neutropenia and thrombocytopenia are added, along with refractory anemia, to the category of RCUD; (3) patients with dysplasia and <5% bone marrow (BM) blasts but ≥2% in the peripheral blood (PB) are classified RAEB1, and (4) a provisional entity, Refractory Cytopenia of Childhood (RCC) has been added; distinguishing RCC from aplastic anemia (AA) can be difficult.

The International Prognostic Scoring System (IPSS) was introduced as a universal risk stratification system for treatment decision making.¹ IPSS considers the percentage of marrow blasts, the number of cytopenias, and bone marrow cytogenetics to predict survival and progression to AML. Patients with untreated MDS are categorized into four IPSS prognostic risk groups; low, intermediate-1 (Int-1), intermediate-2 (Int-2), and high-risk with median survival estimates of 5.7, 3.5, 1.2, and 0.4 years, respectively. The risk of AML evolution of 25% of patients was determined to

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be 9.4 (low-risk), 3.3 (Int-1), 1.1 (Int-2), and 0.2 (high-risk) years, respectively.

The IPSS system was designed to be applied only at diagnosis in patients with *de novo* MDS and may not be suitable for serial assessment of patients whose disease evolves over time. Recently, the WHO classification-based prognostic scoring system (WPSS) was proposed to estimate survival and risk of AML transformation at multiple time points during the natural course of MDS. The WPSS score integrates the three most important prognostic factors in MDS patients classified according to WHO criteria: karyotype, WHO subgroup, and requirement for RBC transfusion as an indicator of symptomatic anemia. In contrast with the four risk groups identified by the IPSS, the WPSS stratifies MDS patients into five different risk categories: very low (score=0), low (1), intermediate (2), high (3-4), or very high (5-6). Significant differences can be seen among the five groups in overall survival (P<0.0001) and risk of AML transformation (P<0.0001). In an independent cohort of patients with MDS, median survival ranged from 140 months for patients with a score of 0 to ten months for those with a score of 5 or more. The most significant improvement in prognostic stratification compared with IPSS is observed in patients without excess blasts, primarily due to the strong impact of the number of dysplastic lineages and transfusion dependency.

Kantarjian *et al.* also proposed a new risk model in MDS that accounts for events not considered in the original IPSS. They analyzed 1,915 patients with MDS including secondary MDS and CMML. Only 507 patients (26%) could be classified by the IPSS.¹⁵ A multivariate survival analysis identified a myelodysplastic syndrome risk score (Table 1). Four prognostic risk groups are proposed for the new risk model: (1) low-risk (score 0-4: 16% of patients), in which patients have an estimated median survival of 54 months and a 3-year survival rate of 63%; (2) intermediate-1 risk (score 5-6; 24% of patients),

in which patients have an estimated median survival of 25 months and a 3-year survival rate of 34%; (3) intermediate-2 risk (score 7-8; 24% of patients), in which patients have an estimated median survival of 14 months and a 3-year survival rate of 16%; and (4) high-risk (score ≥ 9 ; 36% of patients), in which patients have an estimated median survival of six months and a 3-year survival rate of 4%. The proposed model was validated in the separate test group and proved to be applicable at any time during the course of disease.

Immunological pathophysiology

Overview

Various pathological disturbances have been linked to the etiology of MDS including genes that are involved in cell cycle control, DNA repair, gene transcription, telomere length and other chromosome abnormalities, mitotic checkpoints, cell growth, apoptosis, and cell differentiation and maturation.¹⁶⁻²¹ In response to this pleiotropic biology, several therapeutic options have been developed in recent years including azacitidine,²² decitabine,^{23,24} and lenalidomide.²⁵⁻²⁷ Information from the treatment of patients with these newer agents has influenced the opinion of investigators about the underlying pathogenesis of MDS.

Dysregulation of immunity in trisomy 8 myelodysplastic syndrome

T-cell immune dysregulation is definitely associated with bone marrow failure in AA and large granular lymphocyte leukemia (LGLL), which share several clinical features with MDS. Historically, IST in MDS has been applied in cases with bone marrow hypocellularity; a clinical feature that is commonly associated with AA and observed in approximately 15% of MDS cases.²⁸ Numerous abnormalities in immune function appear to contribute to impaired hematopoiesis including elevated plasma levels of several cytokines such as tumor necrosis factor- α (TNF- α)^{29,30} and interferon- γ (IFN- γ),³¹ which are well-known contributors to AA pathogenesis. Many investigators have now confirmed that cytopenias are corrected by T-cell depleting immunotherapy in some MDS patients.³²⁻³⁵ From data *in vitro* and *in vivo*, the current consensus is that a few autologous T cells directly suppress erythroid (CFU-E) and granulocytic (CFU-GM) progenitor cell differentiation.³⁶

A hallmark of T-cell dominant autoimmunity is the expansion of T-cell clones with restricted diversity that possess a limited T-cell receptor (TCR)-V β repertoire. Limitation in TCR-V β complementary-determining region 3 (CDR3)

Table 1. Simplified myelodysplastic syndrome risk score (0-15 Points).

Prognostic Factor	Coefficient	Points
Performance status ≥ 2	0.267	2
Age, y		
60-64	0.179	1
≥ 65	0.336	2
Platelets, $\times 10^9/L$		
< 30	0.418	3
30-49	0.270	2
50-199	0.184	1
Hemoglobin < 12 g/dL	0.274	2
Bone marrow blasts, %		
5-10	0.222	1
11-29	0.260	2
WBC $> 20 \times 10^9/L$	0.258	2
Karyotype: Chromosome 7 abnormality or complex ≥ 3 abnormalities	0.479	3
Prior transfusion, yes	0.107	1

From Kantarjian¹⁵

diversity and clonal expansion of T cells in association with bone marrow suppression suggests that escape from peripheral tolerance and recognition of self-antigens may contribute to suppressive hematopoiesis. Peripheral tolerance may be averted by chromosomal aberrations, or other disease-specific changes that occur in the microenvironment. While the mechanisms that underlie the generation of T-cell clonal expansion remains elusive, Epling-Burnette *et al.* showed that clonal T-cell expansion occurs in 50% of MDS patients compared to 5% in healthy individuals of a similar age range. Importantly, T-cell expansion was not restricted to specific IPSS, WHO subtypes, age, transfusion history, or karyotypes³⁷ implying that T-cell expansion occurs commonly among all MDS prognostic subgroups. Sloand *et al.* has confirmed the identity of one antigen that drives abnormal T-cell clonal expansion that contributes to impaired hematopoiesis in patients with a trisomy 8 cytogenetic abnormality.³⁸ This abnormality is seen in both MDS and AA and is associated with favorable prognosis. Indicative of underlying immune pathology, patients with trisomy 8 display an increased frequency of response to IST compared to patients with other chromosomal abnormalities.³⁸ Clonally expanded CD8⁺ cells with reduced TCR-V β repertoire diversity and direct cytotoxicity of autologous trisomy 8 hematopoietic progenitors was observed *in vitro*.³⁹ CD8⁺ T-cell self-reactivity was not observed in MDS patients with 5q- and monosomy 7 chromosomal abnormalities.³⁹ Bone marrow progenitors with a trisomy 8 abnormality were more sensitive to Fas-mediated apoptosis and more likely to express activated apoptotic markers such as caspases.⁴⁰ In addition, a small number of trisomy 8 cells were

present in bone marrow by fluorescence *in situ* hybridization (FISH) long before cytogenetic conversion by standard metaphase karyotyping, suggesting that trisomy 8 aneuploidy may be an early event in MDS. It was hypothesized that the CTL response was initially triggered by abnormal stem cells but the bone marrow failure was subsequently mediated by a 'bystander' CTL response against healthy hematopoietic cells. The Wilms' tumor-1 (WT1) protein, which is localized on chromosome 8, and thus overexpressed in MDS patients with trisomy 8, represents a well-defined target of self-reactive CTLs in IST responsive patients.⁴¹ T cells may undergo TCR-dependent activation in response to the overproduction of the WT1 antigen, differentiate into effector and memory cells that elicit a WT1-specific CTL response, and as a consequence lead to bone marrow failure through the destruction of both trisomy 8 and normal hematopoietic progenitors (HPCs). Long-term survival of WT1-specific T cells has previously been linked to the expression of c-myc, CD1, and survivin (an inhibitor of apoptosis).^{38,42}

While the linkage between immune-mediated pathogenesis and trisomy 8 is mechanistically established, this chromosomal abnormality occurs in only 5-10% of MDS patients and more often in those that evolve from AA. Response to IST is clearly not restricted to this limited subpopulation of MDS patients suggesting that the mechanism of immune pathogenesis in patients without trisomy 8 must be explored further. The role of aberrant T-cell homeostasis and the relationship between skewed TCR-V β repertoires in patients without a trisomy 8 is yet to be defined. In summary, distinguishing features of trisomy 8 positive bone marrow cells may represent a distinct

pathophysiological entity associated with the direct stimulation of CD8⁺ T cells that may receive benefit from IST (Figure 1).

Search for predictive biomarkers of immune pathophysiology

Since the mechanisms contributing to immune pathophysiology and patient selection criteria for IST remain elusive in patients without trisomy 8, a search for predictive biomarkers is critically needed. Classic paroxysmal nocturnal hemoglobinuria (PNH) is an acquired disease characterized by intravascular hemolysis that results from the clonal expansion of *PIG-A* mutant hematopoietic stem cells (HSCs) producing blood cells deficient in glycosylphosphatidylinositol-anchored proteins (GPI-AP), such as CD55 (decay accelerating factor, DAF) and CD59 (membrane inhibitor of reactive lysis, MIRL). Such GPI-AP deficient (paroxysmal nocturnal hemoglobinuria (PNH) - type) cells are often detectable in the peripheral blood of patients with bone marrow failure syndromes including AA and RA as defined by the FAB group.⁴³⁻⁴⁵ How PNH-type cells arise and increase in patients with BM failure remains obscure. *PIG-A* mutation itself does not confer a proliferative advantage to HSCs. The most widely accepted mechanism for clonal expansion of PNH-type cells in patients with BM failure is the “escape hypothesis”, which states that the relative number of *PIG-A* mutant HSCs increases by avoiding immunological attacks by T cells or NK cells.^{46,47} A murine study demonstrated that GPI-AP-deficient hematopoietic cells can evade a T-cell attack due to either the absence of the target peptide recognized by GPI-AP specific T cells, or the absence of accessory GPI-AP molecules, which are required for HSCs to be attacked by cytotoxic T cells.⁴⁸ In MDS patients, the expansion of PNH-type cells have been reported in about 20% of the patients with lower-risk MDS and that phenomenon has been linked in some studies to IST responsiveness.^{43,44} The assay for PNH detection has not been standardized across institutions in the US and differs from that of Europe and Asia making the association between presence of PNH-type cells and response to IST difficult to interpret.⁴⁹ Standardization of the assay around the world will likely improve the accuracy of this measure as a reliable biomarker of disease.

Recently, Zou *et al.* analyzed the age-adjusted CD4/CD8 ratio in 54 MDS patients compared to 37 healthy controls which revealed that inadequate CD4⁺ rather than expansion of CD8⁺ T cells was associated with a lower ratio in a group that included both lower and higher risk MDS patients defined by the International Prognostic Scoring System (IPSS).⁵⁰ Inversion of the CD4/CD8 ratio was strongly associated with response to therapy (P<0.001). The loss

Possible Mechanisms of Autoimmunity in MDS

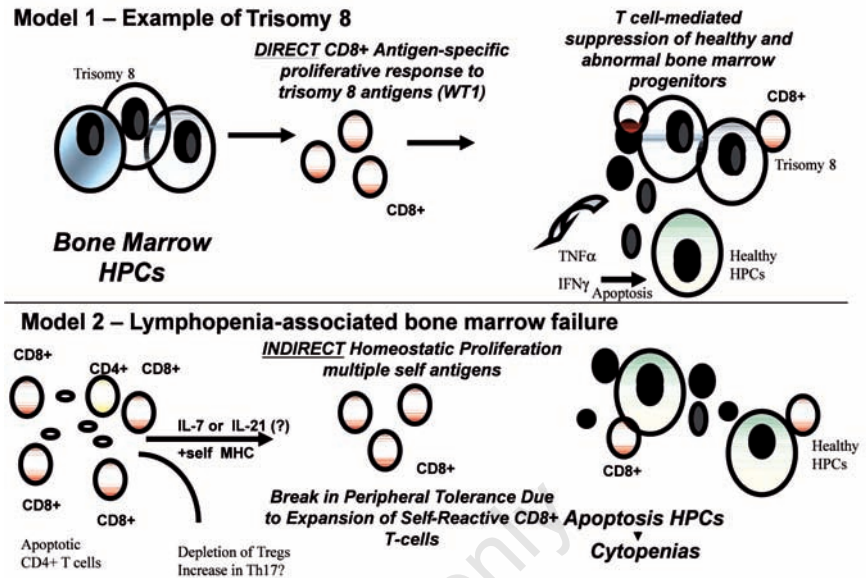


Figure 1. Molecular model of T-cell pathogenesis in MDS. Described in this review are the supporting data that link T-cell abnormalities to multiple abnormal events that include apoptotic response and the presence of inflammation in the bone marrow microenvironment. The mechanism controlling the expansion of self-reactive CD8⁺ T-cells appears to be two fold described by two models (DIRECT and INDIRECT); 1) there may be direct activation of CD8⁺ T cells in response to abnormally expressed bone marrow antigens as exemplified by patients with trisomy 8, and 2) there may be expansion of destructive CD8 T cells due to homeostatic cytokines of the IL-2R $\beta\gamma$ common cytokines which include IL-7, IL-2, IL-15, and IL-21. From previous results, lymphopenia-associated autoimmunity has been linked to antigen-specific CD8⁺ T cells with variable self-antigen reactivity. Survival advantage and clonal expansion dominates from CD8⁺ self-reactive T cells that respond to antigen in the context of self-MHC class I bypassing peripheral tolerance mechanisms. Results suggest that self-reactive CD8⁺ T-cells are involved in the suppression of bone marrow progenitors through direct cytotoxicity of the MDS clones such as trisomy 8⁺ HPCs or the release of cytokines such as TNF α and IFN γ . There is also evidence that depletion of CD4⁺ regulatory T cells (Tregs) and accumulation of IL-17-secreting CD4⁺ T cells (Th17) may be imbalanced. Evidence supports the role of these T-cell abnormalities in cytopenias and increased apoptosis observed in lower-risk MDS. HPC=Hematopoietic Progenitor Cells, WT1=Wilms' tumor 1 antigen.

of CD4-cells was inversely correlated to the proliferative T-cell index before treatment in IST-responsive patients suggesting that proliferation and accelerated CD4⁺ T-cell turnover may be important in disease pathogenesis. In many autoimmune diseases, there is a correlation between the loss of CD4⁺ T cells and the expansion of autoreactive CD8⁺ T cells.⁵¹ This abnormality supersedes the simple loss of CD4⁺ regulatory T cells (Tregs) from the peripheral or tissue compartment. Reduction in CD4⁺ T cells may initiate a cytokine-dependent pathway known as “homeostatic proliferation” in which the interleukin (IL)-2R γ common cytokines such as IL-2, IL-7, IL-15 and/or IL-21 non-specifically expand antigen-specific and self-reactive T cells leading to an increased risk for evasion of peripheral tolerance mechanisms leading to autoimmunity.⁵² Results by Zou *et al.* are the first to demonstrate involvement of CD4⁺ T cells and

response to IST. Since the loss of CD4⁺ T cells is strongly associated with impaired T-cell homeostasis and accumulation of self-reactive CD8⁺ T cells contributes to autoimmunity in many models of human disease, this mechanism may explain immune pathogenesis from a global perspective. The highest homeostatic proliferative index is afforded to T cells with the highest affinity to self-antigens, explaining not only trisomy 8 autoimmunity, but also autoimmune pathogenesis in other settings. A discovery of T-cell receptor (TCR) antigen reactivity in patients with aggressive homeostatic proliferation, without trisomy 8, is needed. Kordasti *et al.* recently showed that the numbers of CD3⁺ CD4⁺ IL-17 producing T cells (Th17) were markedly increased and CD4⁺ CD25^{high} FoxP3⁺ regulatory T cells (Tregs) inversely decreased in lower risk MDS compared with higher risk MDS.⁵³ Tregs from MDS patients suppressed IFN- γ secretion by effector

CD4⁺ T cells but had no effect on IL-17 production. In addition, the serum levels of IL-7, IL-12, RANTES and IFN- γ are significantly elevated in lower risk MDS, while inhibitory factors, such as IL-10 and soluble IL-2 receptor, are significantly higher in high-risk disease. They concluded that the *unfavorable* Th17:Tregs ratio in low-risk MDS may explain the higher risk of autoimmunity and the improved response to immune suppression in patients with low-risk MDS compared to those with high-risk disease.⁵³ In general, more work should be performed to determine the precise relationship between CD4⁺ T cells, HLA DR15, and homeostatic turnover as an alternative mechanism of autoimmunity in MDS (Figure 1).

Therapy

Response criteria

In line with changes in the various classification systems, therapeutic response standards have also evolved. In 2000, the International Working Group (IWG) recom-

mended specific criteria for response that defined four treatment goals including: (1) treatment that alters the natural history of the disease; (2) cytogenetic response; (3) hematologic improvement (HI); and (4) quality of life (QOL).⁵⁴ The HI responses are particularly relevant in patients treated with IST since patients generally have lower risk disease, possess fewer bone marrow blasts, and suffer from long-standing cytopenia. The IWG criteria for HI define specific response to cytopenia in the three hematopoietic lineages; erythroid (HI-E), platelet (HI-P), and neutrophils (HI-N). In 2006, the IWG proposed modifications to the response for altering natural history of MDS and hematologic improvement. These are summarized in Table 2.⁵⁵

ATG based regimens

The goals of MDS therapy are to prolong survival, delay or prevent AML progression, and to improve QOL. Although allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative treatment in MDS, eligibility for HSCT is limited due to the need for an HLA-matched donor and the procedure-related co-

morbidities, which is particularly important in this older patient population.⁵⁶ With the success of ATG in AA, there have been several studies of not only ATG but other ATG-combination therapies in MDS.⁵⁷⁻⁵⁹

ATG is a mixture of purified polyclonal IgG from the sera of rabbits (rATG, Thymoglobulin, Genzyme, Cambridge, MA) or horses (eATG) immunized with human thymocytes (Lymphoglobulin, and Atgam) or the Jurkat cell line (ATG Fresenius, Fresenius SE, Bad Homburg, Germany).⁶⁰ ATG has diverse effects on the immune system including; (1) T-cell depletion in blood and peripheral lymphoid tissues through complement-dependent lysis and apoptosis;⁶¹ (2) modulation of key cell surface molecules that mediate leukocyte/epithelium interactions;⁶² (3) induction of apoptosis in B-cell lineages;⁶³ (4) interference with dendritic cell functional properties;⁶⁴ (5) induction of regulatory T⁶⁵ and natural killer T cells,⁶⁶ (6) direct effect on hematopoietic progenitor cell colony formation from primitive hematopoietic cells;⁶⁷ and (7) possibly, elimination of the Fas-Fas-L interactions.⁶⁸ Each product has shown differential effects on the immune microenvironment and homeostasis. Table 3 summa-

Table 2. Proposed modified International Working Group response criteria.

Category	For altering natural history of myelodysplastic syndrome Response Criteria (responses must last at least 4 wk)
Complete remission	Bone marrow: $\leq 5\%$ myeloblasts with normal maturation of all cell lines Persistent dysplasia will be noted Peripheral blood Hgb ≥ 11 g/dL Platelets $\geq 100 \times 10^9/L$ Neutrophils $\geq 1.0 \times 10^9/L$ Blasts 0%
Partial remission	All CR criteria if abnormal before treatment expect: one marrow blasts decreased by $\geq 50\%$ over pre-treatment but still $> 5\%$ Cellularity and morphology not relevant
Marrow CR	Bone marrow: $\leq 5\%$ myeloblasts and decrease by $\geq 50\%$ over treatment Peripheral blood: if HI responses, they will be noted in addition to marrow CR
Stable disease	Failure to achieve at least PR, but no evidence of progression for > 8 wks
Hematologic improvement	For hematologic improvement Response Criteria (responses must last at least 8 wk)
Erythroid response (pretreatment, < 11 g/dL)	Hgb increase by ≥ 1.5 g/dL Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusions/8 wk compared with the pre-treatment transfusion number in the previous 8 wk. Only RBC transfusions given for a Hgb of ≤ 9.0 g/dL pre-treatment will count in the RBC transfusion response evaluation
Platelet response (pre-treatment, $< 100 \times 10^9/L$)	Absolute increase of $\geq 30 \times 10^9/L$ for the patients starting with $> 20 \times 10^9/L$ platelets Increase from $< 20 \times 10^9/L$ to $> 20 \times 10^9/L$ and by at least 100%
Neutrophil response (pretreatment, $< 1.0 \times 10^9/L$)	At least 100% increase and an absolute increase $> 0.5 \times 10^9/L$
Progression or relapse after HI	At least one of the following: At least 50% decrement from maximum response levels in granulocytes or platelets Reduction in Hgb by ≥ 1.5 g/dL Transfusion dependence

From Zou⁵⁰

Table 3. Clinical trials of ATG based regimen.

Author (Published year) ref	Country	N	Age (Median)	Treatment	Formulation	RA (%)	HR (%)
Molldrem (2002) ⁶⁹	US	61	60	eATG	Atgam	61	34
Sauntharajah(2002) ⁷⁰	US	72	59	eATG and/or CsA	Atgam	46	29
Yazji (2003) ⁷¹	US	31	59	eATG and CsA	Atgam	58	23
Steensma (2003) ⁷²	US	8	69	eATG	Atgam	25	0
Stadler (2004) ⁷³	Germany	35	63	eATG rATG	Lymphoglobulin Thymoglobulin	60 80	40 27
Broliden (2006) ⁷⁴	Sweden	20	64	rATG and CsA	ATG Pharmacia	85	30
Lim (2007) ⁷⁵ ¶	UK, Germany, and Italy	96 (UK, 65; Germany, 13; Italy, 18)	56	eATG	Lymphoglobulin	84	42
Sloand (2008) ⁸⁷⁶	US	116 (eATG and CsA, 42: eATG, 74)	60	eATG and CsA eATG	Atgam	67	48 24

RA, refractory anemia; HR, hematological response; eATG, equine antithymocyte globulin; rATG, rabbit antithymocyte globulin; CsA, cyclosporine. ¶Responses were defined by IWG criteria. §Responses were the same when the response classification of the IWG criteria was retrospectively applied.

izes the results of clinical trials with ATG-based regimens in MDS.

The first pilot study investigating ATG in patients with MDS was conducted by investigators at the National Institute of Health (NIH) in 1997⁷⁷ and the mature data (median follow-up: 30 months) with a larger sample size was reported in 2002.⁶⁹ In this single-arm prospective study, 61 transfusion-dependent patients with MDS (with <20% blasts) were treated with one cycle of eATG (Atgam) at 40 mg/kg/d for four days. The response criteria were as follows; (1) independence from transfusion for a minimum of six weeks with a sustained increase in hemoglobin, or (2) stable hemoglobin within eight months of ATG treatment, as measured from the time the patient last received a transfusion until reinstatement of RBC transfusions. Within eight months of treatment, 21 of 61 patients (34%) no longer required red blood cell transfusions. Independence from transfusion was maintained in 17 of the 21 responders (81%) for a median of 36 months (range 3-72 months). Ten of 21 patients (47.5%) with severe thrombocytopenia had sustained increase in platelet counts, and 6 of 11 patients (55%) with severe neutropenia had sustained neutrophil counts of greater than 1×10^9 cells/L. The clinical characteristics associated with response included younger age (Odds ratio, 0.45, $P=0.005$) and lower platelet counts (median $68 \times 10^9/L$ vs. $135 \times 10^9/L$, $P=0.038$).

Sauntharajah *et al.* reported that human leukocyte antigen (HLA)-DR15 is overrepresented and is predictive of response to IST in the patients with MDS.⁷⁰ It is well known that HLA-DR15 is overrepresented in patients with AA and PNH.⁷⁸⁻⁸⁰ Sloand *et al.* recently confirmed these findings in 129 MDS patients who received equine antithymocyte globulin (eATG, Atgam, Pfizer, New York, NY) with or without cyclosporine (CsA, Novartis, Basel, Switzerland) at the National Heart Lung Blood

Table 4. Comparison of response to CsA studies.

Author (Published year)	Country	N	Age (median)	RA (%)	HR (%)
Jonasova (1998) ⁸³	Czech	17	53	94	82
Catalano (2000) ⁸⁴	Italy	9	63	100	55
Atoyebi (2002) ⁸⁵	UK	6	70	100	0
Shimamoto(2003) ⁸⁶ ¶	Japan	50	55	96	60
Dixit (2005) ⁸⁷	India	19	48	68	58
Chen (2006) ⁸⁸	China	32	46	78	63
Ishikawa (2007) ⁸⁹ ¶	Japan	20	52	85	53

¶Responses were defined by IWG criteria.

Institute (NHLBI).⁷⁶ Of 129 patients treated with IST, 39 (30%) achieved a hematologic response with a median follow-up of three years (0.03-11.3 years). Factors associated with response to IST were age and HLA-DR15 expression with the strongest linkage observed in patients under 61 years of age.

Yazji *et al.* reported a durable response rate of 23% with at least hematologic improvement.⁷⁶ In comparison to the initial NIH studies, a wider spectrum of MDS patients including RA/RARS subtypes constituted 54% of the cohort and only 35% had normal cytogenetics (vs. 54% in studies by Molldrem *et al.*^{68,77}) Another study conducted by Steensma *et al.* was terminated early due to a lack of clinical efficacy.⁷² The early closure was restricted to a small sample size of 8 subjects. No hematologic response was observed in any study subject. Different factors might have contributed to this disappointing result. This cohort shared unfavorable characteristics: all with a Hgb below 9 g/dL (in comparison to 10 g/dL in all the other studies), older age (range 62-74 years), 6 of 8 patients had RAEB-1 and only 2 of 8 subjects had an RA subtype. Based on the IPSS, 63% were Int-1 and 36% were Int-2. Four patients had complex cytogenetics and only 2

had a normal karyotype. Hence, this cohort did not represent the low-risk MDS population that was predicted to respond to IST based on NIH criteria. The small sample size (with 95% confidence interval of 0-37%) could not statistically exclude the 35-45% hematologic response reported in the literature.

In a non-randomized, single-arm trial by Killick *et al.*,⁸¹ the efficacy of eATG (lymphoglobulin) was studied in a multi-institutional trial. Valuable data with a median follow-up of 34 months was reported in 2007.⁷⁵ A total of 96 patients from centers in the UK (n=65), Germany (n=13), and Italy (n=18) were treated with one cycle of lymphoglobulin at 15mg/kg/d for five days. The International Working Group (IWG) response criteria were applied to evaluate response. In this study, a total of 40 patients (42%) achieved a hematologic response, of which 30 patients (75%) displayed a durable hematologic response lasting a median duration of 31.5 months (range 6-92 months). In a multivariate analysis, both low IPSS ($P=0.018$) and bone marrow hypocellularity ($P=0.012$) were independent predictors of response to ATG. In addition, IPSS was the sole predictor of overall survival, with Int-2 and high-risk patients having a significantly poor-

er survival outcome ($P < 0.01$).

Stadler *et al.* compared the efficacy of eATG (lymphoglobulin, 15 mg/kg/d for five days, $n=20$) to rATG (thymoglobulin, 3.75 mg/kg/d for five days, $n=15$), which is commonly used for the treatment of AA. There was no significant difference between the two ATG products with regard to either the clinically relevant responses or frequency of adverse effects.⁷³

Broliden *et al.* reported the efficacy of ATG and CsA therapy in MDS patients,⁷⁴ since this combination therapy is well known to be more effective in the patients with AA.⁸² Twenty patients (17 RA, 3 RAEB) received treatment with rATG (ATG-Fresenius) plus CsA. The overall response rate was 30% (6/20); 3 of the 6 responders had a complete response. The responses lasted 2-58 months, with 2 patients still being in complete remission at 42 and 58 months, respectively.

CsA usage in MDS

Table 4 summarizes previous clinical trials of CsA in MDS.⁸³⁻⁸⁹ CsA has a strong immunosuppressive effect mediated by inactivation of the protein phosphatase calcineurin, resulting in the inhibition of IL-2 gene activation.⁹⁰ Inhibition of proliferative CTL expansion may be critical for autoimmunity in MDS. The first prospective trial with CsA was designed by Janasova *et al.*⁸³ Seventeen cytopenic patients with MDS were treated and a durable response was observed in 82%. Of note, 16 out of the 17 subjects had the RA subtype but varying degrees of bone marrow cellularity. Most patients had a normal karyotype or a single cytogenetic abnormality of 5q-, which both showed positive association with response in this study although 5q- was not associated with response to ATG in the recent NHLBI study.⁷⁶ The selection of cohorts with only favorable profiles might contribute to the surprisingly high response rate. Catalano *et al.* treated 9 patients with RA and also demonstrated a favorable response.⁸⁴ In contrast, another study reported by Atoyebi *et al.*⁸⁵ showed no substantial response to CsA but an unacceptable toxicity profile. An older median age of the treatment group (70 years) may have impacted the therapeutic response and likely increased the risk of CsA-related nephrotoxicity.

There have been several reports of immunosuppression use from Asian countries. A large multicenter study of Japanese MDS patients was reported by Shimamoto *et al.*⁸⁶ Fifty low-risk MDS patients were treated with CsA (6 mg/kg/d) with variable marrow cellularity. Of these patients, 30 patients (60%) showed hematologic improvement. Comparison of the clinical characteristics in responders and non-responders demonstrated that patients with a favorable karyotype, 0 or

0.5 IPSS score, and HLA-DRB1*1501 were more responsive than patients with intermediate or poor risk karyotypes, 1.0 or 1.5 IPSS score, and other HLA types, respectively. There was no significant difference between response to immunosuppressive therapy and age, BM cellularity, BM blast percentage, or dose and blood concentration of CsA. Moreover, they found that bone marrow dysplastic features were present regardless of hematologic response. This group demonstrated a significant association between hematologic improvement and an inverted CD4/CD8 ratio and TCR-V β and $-\gamma$ gene rearrangements as biological predictors of response.⁹¹ Although correlative studies were carried out in only a few patients ($n=4$), these results corroborate the findings by Zou *et al.* using samples from patients at the NHLBI.⁵⁰ Recently, Ishikawa *et al.* suggested the significance of the PNH clone in patients with low-risk MDS in a prospective study.⁸⁹ They treated 20 patients with MDS with CsA (4 mg/kg/d). Among 19 patients evaluated, 10 showed hematologic improvement, most of the toxicity associated with CsA usage was manageable, and no patient had progressed to AML at the latest follow-up (median 30 months). A short duration of illness, RA with minimal bone marrow dysplasia, and the presence of the PNH clone were all significantly associated with a platelet response. A minority of RA patients who did not possess such predictive variables achieved an isolated erythroid response.

Summary

MDS is the one of the most challenging problems in hematology. Although the current classification and prognostic schemes have improved over the past decade, these pathological classification-based schemes are ineffective to distinguish pathological diversity and therapeutic response. Importantly, substantial clinical benefit is observed with IST treatment in the US, Europe, and Asia. Response rates of ATG therapy ranged from 20-40% and CsA response ranged from 50-60% using IWG response criteria. ATG therapy, however, has not been used in Asia and few trials of CsA therapy have been performed in Western countries creating an uncertainty about whether there are specific pathobiological features of the disease in Asia that predispose patients to IST response or whether CsA therapy is superior to ATG therapy. For the most effective application of IST, definitive mechanisms of pathophysiology and biological predictors of response must be established. A large clinical trial that includes international centers and compares ATG to CsA will clarify the features

associated with immune-mediated MDS. This study should include not only clinical response assessment but also correlative laboratory analyses of variables shown previously to predict for hematologic improvement such as T-cell clonality, T-cell homeostasis, HLA type, microarray expression profiling, and PNH analysis. To detect PNH-type cells, a sensitive assay must be applied to detect small expansions of these cells. In conclusion, the concept of autoimmunity in MDS patients is widely accepted but, clarification of the autoimmune mechanism contributing to bone marrow myelodysplasia and impaired hematopoiesis must be obtained. In the future, the focus should be on developing an international coordinated clinical trial to define geographical and therapeutic differences, newer approaches to immune modulation, and basic studies to understand the pathophysiological mechanisms.

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