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**Stem Cell Disorders**

# Prolonged response to cyclosporin-A in hypoplastic refractory anemia and correlation with *in vitro* studies

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ABSTRACT

***Background and Objectives.*** **Lymphocyte abnormalities in myelodysplastic syndromes (MDS) have been widely described, but the role of the immune system in the pathogenesis of these clonal disorders remains controversial. An active role of lymphocytes in suppressing normal hematopoiesis may be implicated in MDS with hypoplastic marrow. We have studied *in vitro* and *in vivo* activity of cyclosporin-A (CSA) on hematopoiesis in patients affected by hypoplastic MDS without blast excess.**

***Design and Methods.*** **Nine consecutive patients with hypoplastic refractory anemia (RA), followed up in our out-patient unit, were treated with CSA at daily doses of 1-3 mg/kg for at least three months. Low dose steroids or danazol were transiently added in 7/9 patients. Differences between pre- and post-treatment parameters were studied by the Student's t-test. *In vitro* effect of CSA on circulating hematopoietic progenitors was studied by the methylcellulose colony assay.**

***Results.*** **Before treatment, fewer circulating hematopoietic progenitors were found in all patients as compared to normal subjects. The number of $CD34^+$ cells was about halved, while circulating erythroid and myeloid colony-forming cells (CFC) were reduced to one-fifth. After a mean period of 22 months of CSA treatment (median: 14.5 months), hemoglobin was significantly and persistently increased in two patients, platelets in one, platelets and hemoglobin in two. Two patients showed transient responses, one patient did not tolerate the treatment and one patient is close to a significant response. At *in vitro* CSA concentrations similar to those achieved *in vivo* after oral administration the drug significantly increased cell colony growth in hypoplastic RA. This test correctly predicted a positive clinical response to CSA in 3/5 cases and treatment failure in 4/4 cases.**

***Interpretation and Conclusions.*** **About one half of hypoplastic RA patients benefited from CSA treatment. A larger study could verify whether *in vitro* culture of hematopoietic progenitors in the presence of CSA can predict the clinical response and whether this treatment could prolong patients' survival.**


Key words: cyclosporin-A, myelodysplastic syndromes, refractory anemia, hypoplastic MDS

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Myelodysplastic syndromes (MDS) can be considered as indolent clonal expansion with a high rate of ineffective hematopoiesis, which is attributable to the coexistence of increased proliferation and a tendency to apoptosis.[1-4] The role of the immune system in the pathogenesis of MDS is still poorly defined; there is no clear evidence of clonal involvement of B- or T-lymphocytes. It is possible that, at least in some cases, dysregulation of the immune system may favor a clonal substitution of the hematopoietic tissue, as hypothesized for paroxysmal nocturnal hemoglobinuria.[5] Indeed, activated CD8 T-cell clones spontaneously producing inhibitory cytokines were reported to circulate in peripheral blood and bone marrow of MDS patients,[6-8] and over-expression of interferon-$\gamma$ and tumor necrosis factor-$\alpha$ was proposed to be involved in the pathogenesis of MDS.[8-11] The apparent contradiction between clonal expansion and marrow failure is more evident in a particular subset of MDS: that with hypoplastic marrow from presentation. In this subgroup, accounting for about 15% of all MDS,[12,13] there is a stronger suspicion of an active role of the immune system in the pathogenesis of marrow failure, as typically observed in aplastic anemia (AA).[10] The higher expression of Fas-R on $CD34^+$ cells of hypoplastic MDS patients may mediate the *in vivo* pressure exerted by the immune system on both normal and clonal MDS hematopoiesis and may account for the ineffective hematopoiesis and exaggerated apoptosis frequently seen in this disease.[1,14-17] In addition, Fas-L expression in marrow cells of MDS patients was reported to correlate with anemia.[18]

After anecdotal clinical reports,[19] successful immunosuppressive therapy in MDS patients has more recently been attributed to *in vivo* reduction of cytotoxic T-lymphocytes, based on a significant suppression of normal myeloid progenitor growth *in vitro*.[20] Although the deficiency of the stem cell compartment is less severe in MDS than in AA,[21] the above reported clinical and laboratory findings suggest that similar pathogenetic mechanisms may operate in both diseases, leading to depletion of the early hematopoietic progenitor cell pool.

Starting from these data, we tried to verify whether non-intensive immunosuppression with cyclosporin-A (CSA) could improve cytopenia in nine consecutive patients affected by hypoplastic MDS without blast excess, and whether there is a relationship between the effect of CSA on circulating MDS progenitor growth *in vitro* and clinical response to CSA *in vivo*.

## Design and Methods

### Patients

From September 1995 to March 1999 nine patients (6 males, 3 females, median age 60 years, range 37-78) were given a diagnosis of hypoplastic refractory anemia (RA). They represented about 15% of all cases of MDS diagnosed in the same period in our Division. Diagnostic criteria were based upon the FAB classification and were supported by bone marrow histologic evaluation in most cases. Marrow cellularity under 30% was considered hypoplastic, while a reduction of a single maturative lineage to 5%, or less, of bone marrow nucleated cells allowed diagnosis of monolinear hypoplasia. Table 1 shows diagnosis and main hematologic data at presentation. Cases of MDS with blast excess were excluded from this study, as were cases of hypoplastic evolution of typical normo- or hypercellular MDS. After giving informed consent, hypoplastic MDS patients received immunosuppression by CSA at a daily oral dose of 1-3 mg/kg. Low dose prednisone (maximum daily dose 25 mg) or danazol (50-400 mg daily) were added if no beneficial effect was seen after one month of treatment. The rationale of this treatment plan was to maintain a prolonged immunosuppression using a non-myelotoxic immunosuppressant and to try to synergize this treatment by the addition of steroids or immunomodulating agents. Danazol was employed particularly in thrombocytopenic patients, with the aim of reducing peripheral mechanisms of platelet clearance by interfering with macrophage Fc receptors.[22] The treatment lasted for at least three months. In cases of favorable effects, daily doses of CSA were reduced, and withdrawal of prednisone or danazol was attempted. Table 2 shows the treatment received by each patient. Differences between means of hematologic parameters before and after the treatment were analyzed by the Student's t-test, taking into consideration the values recorded during the last three months before treatment.

### Specimen collection

Peripheral blood (PB) samples were obtained after informed consent from 37 normal donors and 10 hypoplastic MDS patients; nine of them were subsequently treated *in vivo* with CSA (Table 1, 2, 3 and 5). Patients #1 to #5 performed the *in vitro* study within 3 months of beginning CSA; none of these patients had received CSA for at least one week before sampling. The others had not received CSA before sampling at all.

### Cytogenetic study

Cytogenetic analysis was performed using the standard GTG-banding technique.

### Flow cytometry analysis

Phycoerythrin (PE)-conjugated monoclonal antibodies (moAb) to CD34 (clone HPCA-1; Becton Dickinson, Mountain View, CA, USA) were used to identify $CD34^+$ cells. PB samples were stained with the moAb for 20 minutes at 4°C. After staining, samples were subjected to red blood cell lysis with ammonium chloride buffer (Ortho, Raritan, USA) and washed with phosphate-buffered saline. Flow cytometry was performed using a flow cytometer (FACS, Becton-Dickinson). During acquisition, a threshold was set on forward light scatter to exclude cell debris from analysis. A region on sideward light scatter was drawn to include all mononuclear cells (MNCs) for determinations of CD34 expressing cells, and live gate acquisition was performed on cellular events that fell into that region. Ten thousand events were acquired in the live gate, with a minimum of $6\times10^4$ events in the entire population.

### Hematopoietic colony assay

Circulating mononuclear cells were isolated by density gradient centrifugation using lymphocyte separation medium (Organon, Durham, NC, USA). After washing with Hanks' balanced salt solution (HBSS), cells were resuspended in Iscove's modified Dulbec-



**Table 1. Hypoplastic MDS patients: hematological data at diagnosis.**

| Case | Gender | Age (yrs) | Diagnosis | Hb (g/dL) | MCV (fL) | WBC ($10^9$/L) | ANC ($10^9$/L) | Plt ($10^9$/L) | Bone marrow | Cytogenetics | Transfusions | Previous therapy |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 73 | RA | 11.4 | 106 | 3.5 | 2.1 | 185 | Erythroblastopenia | ND | Yes | Folic acid |
| 2 | F | 44 | RA | 9.6 | 108 | 4.1 | 2.5 | 589 | Erythroblastopenia | 46XX | No | Danazol |
| 3 | F | 43 | RA | 6.5 | 103 | 3.0 | 1.2 | 34 | Hypoplasia | 46XX t(1;3) | Yes | Prednisone and danazol |
| 4 | F | 38 | RA | 12 | 107 | 4.9 | 2.5 | 227 | Erythroblastopenia | ND | No | Prednisone |
| 5 | M | 78 | RA | 7.3 | 107 | 3.9 | 2.4 | 114 | Erythroblastopenia | 47XY +8 | Yes | Folic acid |
| 6 | M | 37 | RA | 14.2 | 97 | 3.3 | 0.5 | 27 | Amegakaryocytosis | 46XY del(5q) | No | Folic acid |
| 7 | M | 66 | RA | 7.5 | 109 | 3.0 | 1.5 | 227 | Hypoplasia | 46XY | Yes | Erythropoietin, folic acid |
| 8 | M | 69 | RA | 13.4 | 104 | 3.5 | 0.8 | 61 | Amegakaryocytosis | 46XY | No | Danazol |
| 9 | M | 60 | RA | 10.9 | 122 | 7.6 | 5.8 | 40 | Hypoplasia | ND | No | None |
| 10 | F | 74 | RA | 8.1 | 115 | 2.6 | 1.3 | 95 | Hypoplasia | ND | Yes | None |

*RA: refractory anemia; ND: not done; Hb: hemoglobin; MCV: mean cell volume; WBC: white blood cells; ANC: absolute neutrophils; Plt: platelets.*

**Table 2. *In vivo* treatment of hypoplastic MDS patients.**

| Case | CS-A duration (months) | Associated drugs | Side effects |
|---|---|---|---|
| 1 | 31.1 | Prednisone | Paresthesias |
| 2 | 10.2 | Prednisone and danazol | None |
| 3 | +32.2 | Prednisone | Gingival hypertrophy, tremor |
| 4 | +25.7 | None | Paresthesias |
| 5 | 3.9* | Prednisone | Nephrotoxicity and gastric intolerance |
| 6 | +36.4 | Danazol | Gingival hypertrophy |
| 7 | +14.5 | Prednisone and danazol | None |
| 8 | +11.7 | Prednisone and danazol | None |
| 9 | +10 | None | None |

*+Still under treatment; *drug withdrawn because of toxicity.*

**Table 3. Treatment results.**

| Case | Mean Hb (g/dL) | | Mean WBC ($10^9$/L) | | Mean Plts ($10^9$/L) | | EE | LSE (mos) |
|---|---|---|---|---|---|---|---|---|
| | Pre* | Post | Pre* | Post | Pre* | Post | | |
| 1 | 8.7 | 9.5 | 3.8 | 3.4 | 163 | 125 | Transient | 2.1 |
| 2 | 8.4 | 8.8 | 5.6 | 6.0 | 647 | 702 | Transient | 4.2 |
| 3 | 10.2 | 12.3 | 6.1 | 5.5 | 23 | 95 | Yes | 0.4 |
| 4 | 10 | 11.5 | 4.7 | 6.6 | 247 | 258 | Yes | 8 |
| 5 | 8.1 | 8.6 | 4.3 | 7.0 | 114 | 128 | No | |
| 6 | 14.2 | 14.9 | 3.1 | 3.4 | 25 | 76 | Yes | 3.5 |
| 7 | 7.9 | 10.1 | 3.3 | 5.4 | 280 | 330 | Yes | 2.4 |
| 8 | 12.3 | 11.5 | 4.1 | 3.8 | 38 | 53 | No | |
| 9 | 10.9 | 12.1 | 6.4 | 4.3 | 48 | 68 | Yes | 1.2 |
| $\bar{X}$ | 10.2 | 11 | 4.6 | 5.0 | 176 | 204 | | |
| *p* | 0.007 | | 0.2 | | 0.01 | | | |

*EE: efficacy evaluation; LSE: latency to significant effect.*
**Average over three months prior to treatment.*

co's Medium (IMDM) supplemented with 5% fetal calf serum (FCS). HBSS, IMDM and FCS were purchased from Life Technologies, Gaithersburg, MD, USA. Isolated MNC cells were plated in methylcellulose (Stem Cell Technologies, Vancouver, Canada) at a concentration of $5\times10^5$ cells/mL of medium (35 mm dishes; 1 mL of medium/dish), in basal condition and after 2-hour incubation with 500 ng/mL of CSA (Sandoz, Basel, Switzerland). The growth factor cocktail consisted of 10 ng/mL IL-3, 50 ng/mL G-CSF, 50 ng/mL GM-CSF, 20 ng/mL stem cell factor (SCF) and 2 U/mL erythropoietin (EPO) (Amgen, Thousand Oaks, CA, USA). Groups of more than 50 cells at day 14 of incubation at 37°C in 5% $CO_2$ were counted as colonies derived from a single granulocyte-macrophage (CFU-GM) or erythroid (BFU-E) progenitor. The number of CFC was obtained by adding together CFU-GM and BFU-E. All cultures were performed in quadruplicate. Values were expressed as mean value ± standard error of mean (SEM). All the experimental procedures were carried out in endotoxin-free plastic ware. For estimation of circulating $CD34^+$ and CFC per milliliter, the following equations were used: $CD34^+/mL = CD34^+/10^5 \times WBC/\mu L$ divided by $10^2$; $CFC/mL = CFC/10^5$ MNC $\times$ WBC/µL $\times$ % MNC divided by $10^4$.

### *Statistical analysis*

Student's t-test was employed for the analysis and *p* values ≤ 0.05 were considered as statistically significant.

## Results

### *In vivo effect of CSA*

Hb level and platelet number significantly increased during the treatment (mean pre-treatment Hb 10 g/L, mean post-treatment Hb 11.1 g/L, $p$=0.007; mean pre-treatment Plt $176\times10^9$/L, mean post-treatment Plt $203\times10^9$/L, $p$=0.01). CSA treatment produced a persistent significant improvement of abnormal parameters in five patients: Hb in two cases, platelets in one case, platelets and hemoglobin in two cases; these patients are still under treatment after a mean period of 22 months (range 4-36+). Two anemic patients experienced a moderate transient benefit (for ten and thirty months of treatment). In one case CSA was withdrawn because of renal toxicity; in one patient a trend toward a favorable effect has been observed over twelve months of treatment. These results are summarized in Table 3. The response to treatment occurred after a mean of 3.5 months (range 0.6-8; median 2.4); in the two cases in which CSA was not combined with steroids, the response was observed after 3.5 and 8 months. Only one of the five responsive patients (#6) is still taking danazol (50 mg every other day); the others are on CSA alone. The response was never complete: blood counts did not return to normal values, nor did signs of dysplasia disappear. The treatment was generally well tolerated: only one patient discontinued CSA, because of serum creatinine increase (2 mg/dL) and gastrointestinal side effects. In some cases, modest gingival hypertrophy, weight gain and paresthesias were observed, but these did not lead to treatment interruption. CSA was administered without negative consequences even to an HBsAg-positive patient. Plasma levels of polyclonal CSA were measured every two weeks, and toxicity ranges were not reached in any case (mean: 277 ng/mL, range 30-1100, n=9).

### *Circulating $CD34^+$ and CFC in hypoplastic MDS patients*

Quantitative assessment of bone marrow $CD34^+$ and CFC may be affected by a variable degree of contamination with peripheral blood and different distributions of cellularity within the aspirated bone marrow area. Thus, we analyzed circulating $CD34^+$ and CFC from MDS patients in comparison with those of normal subjects. Circulating total nucleated cells (TNC) were used for flow cytometry evaluation of $CD34^+$ cells, while mononuclear cells were assayed for the measurement of CFC. We also evaluated the absolute number of $CD34^+$ and CFC per milliliter of blood, by adjusting the measured numbers of $CD34^+$ and CFC for white blood cell count and the proportion of blood mononuclear cells at the time of testing, as described in the *Design and Methods* section. The

**Table 4. CD34⁺ cells and hematopoietic progenitors in peripheral blood of hypoplastic MDS patients.**

| | *CD34⁺* | | | *CFC* | | |
|---|---|---|---|---|---|---|
| | *n* | *x10⁵ TNC* | *x mL* | *n* | *5x10⁵ MNC* | *x mL* |
| Normal subjects | 23 | 97.9±8 | 5656±474 | 20 | 64±4.7 | 277±27 |
| Hypoplastic MDS | 10 | 64±18 | 2930±1007 | 10 | 13.6±3 | 52±13 |
| *p** | | 0.05 | 0.009 | | <0.001 | <0.001 |

**Student's t test.*

**Table 5. *In vitro* effect of CSA on circulating hematopoietic progenitors.**

| *Case* | *BFU-E 5x10⁵ mononuclear cells plated* | | *CFU-GM 5x10⁵ mononuclear cells plated* | |
|---|---|---|---|---|
| | *without CSA* | *with CSA* | *without CSA* | *with CSA* |
| 1 | 2.0±1.0 | 4.0±2.0 | 6.0±0.5 | 9.0±1.0 |
| 2 | 7.0±1.0 | 20.5±7.0 | 8.0±0.5 | 12.0±4.0 |
| 3 | 2.0±4.0 | 4.0±1.5 | 3.0±1.0 | 19.0±6.0 |
| 4 | 7.0±1.5 | 9.5±2.5 | 9.5±2.5 | 22.5±3.5 |
| 5 | 1.5±0.5 | 1.5±0.5 | 1.5±0.5 | 1.5±0.5 |
| 6 | 17.0±2.5 | 23.5±2.5 | 14.5±2.5 | 28.5±8.5 |
| 7 | 2.5±1.5 | 1.5±1.0 | 2.0±2.0 | 2.5±2.5 |
| 8 | 0.5±0.5 | 0.5±0.5 | 1.0±0.5 | 1.0±0.5 |
| 9 | 17.0±4.0 | 26.5±3.5 | 15.5±3.5 | 30.0±1.5 |
| 10 | 10.0±1.0 | 14.0±2.5 | 8.0±1.0 | 19.0±4.0 |
| $\bar{X}$ | 6.6±1.9 | 11.1±3.2 | 6.9±1.6 | 13.0±3.2 |

number of circulating CD34⁺ cells was significantly lower in hypoplastic MDS patients as compared in normal donors (x ± SEM: 64±18/10⁵ total nucleated cells vs 97.9±8.4; *p*=0.05); the reduction was even more pronounced when CD34⁺ cell number was expressed per unit volume of blood (2930±100/mL of blood vs 5,656± 474; *p*=0.009) (Table 4). Hematopoietic progenitor cells of MDS patients may show an abnormal pattern of growth in methylcellulose culture, i.e. a high cluster/colony ratio. As described in the *Design and Methods* section, in order to assess the number of CFC in MDS patients we counted only morphologically normal colonies. Compared to normal controls, circulating CFC were significantly decreased in hypoplastic MDS (13.6± 3/5×10⁵ MNC cells plated vs 64±4.7; *p* <0.001; 52 ±13/mL of blood vs 277±27; *p*=0.001) (Table 4). The number of CD34⁺ cells and of CFC did not predict the clinical response to CSA.

### *In vitro sensitivity of circulating progenitors to CSA and clinical correlations*

At concentrations comparable to those reached after *in vivo* administration, CSA significantly potentiated circulating CFC colony growth of MDS patients. CFC colony number increased from a mean value ± SEM of 13.6±3 to 25.5±6 without and with CSA, respectively (*p*=0.036). When CFC were subgrouped into BFU-E and CFU-GM, the enhancing effect of CSA was observed for both types of colonies (BFU-E: 6.6±1.9 vs 11.1±3.2, *p*=0.01; CFU-GM: 6.9±1.6 vs 13.0±3.2, *p*=0.007) (Table 5). By analyzing the patients one by one, it appeared that *in vitro* CSA addition significantly increased (at least 50% increase) the number of erythroid colonies in 4/10 and that of myeloid colonies in 7/10 (Figure 1). When we tried to correlate *in vitro* and *in vivo* CSA effects, we found that the *in vitro* test correctly predicted the response in 6/9 patients when CFU-GM were considered, and in 4/9 patients when BFU-E were considered (Table 6).

## Discussion

At variance with acute myeloid leukemia, MDS are generally characterized by poor response to intensive chemotherapy and a clinical course sufficiently prolonged to allow the effect of non-intensive treatment approaches to be studied.[23,24] In the present study we

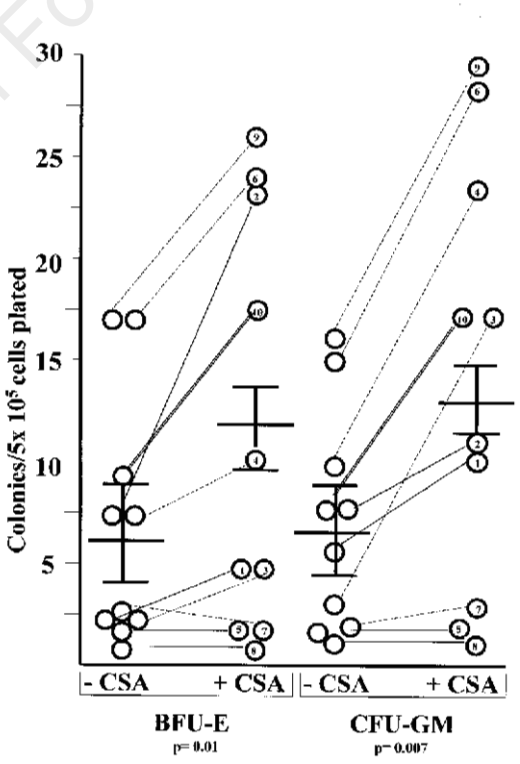


**Figure 1. Effect of *in vitro* CSA pretreatment (500 ng/mL) on circulating hematopoietic progenitors of hypoplastic refractory anemia patients. Horizontal bars are mean values and vertical bars are standard errors of mean. *p*: Student's T test. Numbers within circles: patient number as in tables. Dotted line: patients who responded to CSA treatment. Continuous line: non-responder patients. Double line: untreated patient.**

**Table 6. Correlation between *in vitro* and *in vivo* CSA effect.**

| | | *In vitro BFU-E* | | *In vitro CFU-GM* | |
|---|---|---|---|---|---|
| | | *Effective* | *Ineffective* | *Effective* | *Ineffective* |
| *In vivo* | Effective | 2 | 3 | 4 | 1 |
| | Ineffective | 2 | 2 | 2 | 2 |
| | *p** | 0.5 | | 0.002 | |

*Statistical analysis: Chi-square test.*

aimed to verify a possible improvement of hematologic status in hypoplastic MDS without directly targeting the dysplastic clone. Although in principle hypoplastic MDS could be considered as a transitional stage towards normocellular or hypercellular MDS, clinical and laboratory findings suggest that this form is a distinct variety of MDS.[14,15] It has been hypothesized that the immunologic abnormalities found in MDS[25] may represent a misdirected response of the immune system toward the normal hemopoietic tissue[6-11,20] elicited by the abnormal clone itself or by another unknown trigger.[26] Immune-mediated damage to normal hematopoiesis sparing the abnormal clone may give a relative growth advantage to the latter, leading to a monoclonal hypo-, normo- or hypercellular marrow, causing a variable degree of peripheral cytopenia.[5] Although it has not been established whether the hypothetical immune aggression occurs mainly via cytotoxic or cytokine-mediated mechanisms, immunosuppressive treatments such as corticosteroids, CSA and antithymocyte globulin (ATG) have occasionally been used with success in patients with MDS.[27-29] A hematologic response to ATG in patients with refractory anemia was recently found to be associated with disappearance of T-cell mediated suppression of *in vitro* myeloid progenitor growth.[20] In this preliminary study, we decided not to use intensive immunosuppression with ATG or high dose corticosteroids in order to avoid the risk of severe infections, and preferred to use low-dose CSA associated with potentially synergistic drugs, such as low-dose steroids and danazol. In a previous study, we did not detect any stimulatory effect of danazol either *in vitro* on the growth of all committed hematopoietic progenitors or *in vivo* in a cohort of MDS patients.[30] With the present schedule only one patient, elderly and in poor cardiovascular condition, dropped out of the study because of renal and gastrointestinal side effects. As compared to other reports,[27,29] in our patients treatment duration was longer, which allowed us to detect even delayed responses and to verify response duration. Consistent with their hypoplastic bone marrow, all our patients showed significantly decreased numbers of circulating CD34$^+$ cells and CFC compared to those in normal controls. The decrease of circulating CFC was consistently more pronounced than that of the CD34$^+$ cell compartment, suggesting the existence of damaging mechanisms selectively affecting the committed progenitors. An alternative hypothesis is impairment of proliferation and differentiation of CD34$^+$ MDS cells.[31] Since in our culture studies we counted only well formed colonies and neglected MDS cell clusters, it is likely that in our patients the number of CFC mainly reflects residual normal hematopoiesis. Indeed, circulating CD34$^+$ cells and CFC in hypoplastic MDS were significantly higher than those observed in patients with severe aplastic anemia.[32,33] In our small series, neither CD34$^+$ cell nor CFC number correlated with the clinical response to CSA. We found that *in vitro* pre-treatment of circulating mononuclear cells with CSA significantly enhanced hematopoietic progenitor growth in several cases, and this prompted us to look for a correlation between *in vitro* effect of CSA on hematopoietic progenitor growth and clinical response to CSA. Such a correlation could be documented only in some patients; the discrepancies can be attributed to the complexity of *in vivo* MDS hematopoiesis as compared to the simplification of the *in vitro* culture system.

In conclusion, our study suggests that a trial with immunosuppressive drugs is indicated for unilinear or multilinear hypoplastic MDS. The study of a larger number of patients is needed to ascertain whether *in vitro* tests in the presence of CSA are able to predict the response to immunosuppression. A prospective randomized study could evaluate treatment influence on patients' survival.

### Contributions and Acknowledgments

*LC and CS designed the clinical and in vitro studies and wrote the paper. LC, CC, SR were involved in the clinical assessment of the patients and in data collection. MV performed the statistical analyses. GV and PR perfomed the hematopoietic progenitor assays and flow cytometry. LL performed the cytogenetic studies. BR supervised the study, revised the manuscript and gave final approval for submission. The order of authorship takes into account the contribution given to the study.*

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### Disclosures

*Conflict of interest: none.*

*Redundant publications: no substantial overlapping with previous papers.*

### Manuscript processing

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### Potential implications for clinical practice

- Colony formation from peripheral blood of hypoplastic MDS patients was improved upon *in vitro* addition of CSA, paralleling the clinical results.
- A trial with immunosuppression is recommended in patients with hypoplastic MDS.

## References

1. Yoshida Y. Hypothesis: apoptosis may be the mechanism responsible for the premature intramedullary cell

death in the myelodysplastic syndrome. Leukemia 1993; 7:144-6.
2. Rajapaksa R, Ginzton N, Rott LS, Greenberg PL. Altered oncoprotein expression and apoptosis in myelodysplastic syndrome marrow cells. Blood 1996; 88:4275-87.
3. Parker JE, Mufti GJ. Ineffective haemopoiesis and apoptosis in myelodysplastic syndromes. Br J Haematol 1998; 101:220-30.
4. Aul C, Bowen DT, Yoshida Y. Pathogenesis, etiology and epidemiology of myelodysplastic syndromes. Haematologica 1998; 83:71-86.
5. Luzzatto L, Bessler M, Rotoli B. Somatic mutations in paroxysmal nocturnal hemoglobinuria: a blessing in disguise? Cell 1997; 88:1-4.
6. Smith MA, Smith JG. The occurrence subtype and significance of haemopoietic inhibitory T cells (HIT cells) in myelodysplasia: an in vitro study. Leuk Res 1991; 15:597-601.
7. Sugawara T, Endo K, Shishido T, et al. T-cell mediated inhibition of erythropoiesis in myelodysplastic syndromes. Am J Hematol 1992; 41:304-5.
8. Maciejewski JP, Hibbs JR, Anderson S, Katevas P, Young NS. Bone marrow and peripheral blood lymphocyte phenotype in patients with bone marrow failure. Exp Hematol 1994; 22:1102-10.
9. Verhoef GE, Schouwer P, Ceuppens JL, Van Damme J, Goossens W, Boogaerts MA. Measurement of serum cytokine levels in patients with myelodysplastic syndromes. Leukemia 1992; 6:1268-72.
10. Young NS. Pathophysiology II: Immune suppression of hematopoiesis. In. Young NS, Alter BP,eds: Aplastic anemia, acquired and inherited. Philadelphia: WB Saunders; 1994. p. 68-99.
11. Mundle SD, Ali A, Cartlidge JD, et al. Evidence for involvement of tumor necrosis factor-alpha in apoptotic death of bone marrow cells in myelodysplastic syndromes. Am J Hematol 1999; 60:36-47.
12. Maschek H, Kaloutsi V, Rodriguez-Kaiser M, et al. Hypoplastic myelodysplastic syndrome: incidence, morphology, cytogenetics, and prognosis. Ann Hematol 1993; 66:117-22.
13. Tuzuner N, Cox C, Rowe JM, Watrous D, Bennett JM. Hypocellular myelodysplastic syndromes (MDS): new proposals. Br J Haematol 1995; 91:612-7.
14. Maciejewski JP, Selleri C, Sato T, Anderson S, Young NS. Increased expression of Fas antigen on bone marrow CD34+ cells of patients with aplastic anaemia. Br J Haematol 1995; 91:245-52.
15. Lepelley P, Grardel N, Erny O, et al. Fas/APO-1 (CD95) expression in myelodysplastic syndromes. Leuk Lymphoma 1998; 30:307-12.
16. Selleri C, Sato T, Anderson S, Young NS, Maciejewski JP. Interferon-gamma and tumor necrosis factor-alpha suppress both early and late stages of hematopoiesis and induce programmed cell death. J Cell Physiol 1995; 165:538-46.
17. Maciejewski J, Selleri C, Anderson S, Young NS. Fas antigen expression on CD34+ human marrow cells is induced by interferon gamma and tumor necrosis factor alpha and potentiates cytokine-mediated hematopoietic suppression in vitro. Blood 1995; 85: 3183-90.
18. Gupta B, Niehans GA, LeRoy SC, et al. Fas ligand expression in the bone marrow in myelodysplastic syndromes correlates with FAB subtype and anemia, and predicts survival. Leukemia 1999; 13:44-53.
19. Bagby GC Jr, Gabourel JD, Linman JW. Glucocorticoid therapy in the preleukemic syndrome (hemopoietic dysplasia): identification of responsive patients using in-vitro tecniques. Ann Intern Med 1980; 92:55-8.
20. Molldrem JJ, Jiang YZ, Stetler-Stevenson M, Mavroudis D, Hensel N, Barrett AJ. Haematological response of patients with myelodysplastic syndrome to antithymocyte globulin is associated with a loss of lymphocyte-mediated inhibition of CFU-GM and alterations in T-cell receptor Vß profiles. Br J Haematol 1998; 102:1314-22.
21. Sato T, Kim S, Selleri C, Young NS, Maciejewski JP. Measurement of secondary colony formation after 5 weeks in long-term cultures in patients with myelodysplastic syndrome. Leukemia 1998; 12:1187-94.
22. Mylvaganam R, Ahn YS, Garcia RO, Kim CI, Harrington WJ. Very low dose danazol in idiopathic thrombocytopenic purpura and its role as an immune modulator. Am J Med Sci 1989; 298:215-20.
23. Cazzola M, Anderson JE, Ganser A, Hellstrom-Lindberg E. A patient-oriented approach to treatment of myelodysplastic syndromes. Haematologica 1998; 83: 910-35.
24. Kouides PA, Bennett JM. Advances in the therapy of the myelodysplastic syndromes. Cancer Treat Res 1999; 99:335-62.
25. Enright H, Miller W. Autoimmune phenomena in patients with myelodysplastic syndromes. Leuk Lymphoma 1997; 24:483-9.
26. Raza A. Hypothesis: myelodysplastic syndromes may have a viral etiology. Int J Hematol 1998; 68:245-56.
27. Molldrem JJ, Caples M, Mavroudis D, Plante M, Young NS, Barrett AJ. Antithymocyte globulin for patients with myelodysplastic syndrome. Br J Haematol 1997; 99:699-705.
28. Biesma DH, van den Tweel JG, Verdonck LF. Immunosuppressive therapy for hypoplastic myelodysplastic syndrome. Cancer 1997; 79:1548-51.
29. Jonasova A, Neuwirtova R, Cermak J, et al. Cyclosporin A therapy in hypoplastic MDS patients and certain refractory anaemias without hypoplastic bone marrow. Br J Haematol 1998; 100:304-9.
30. Selleri C, Catalano L, De Rosa G, Fontana R, Notaro R, Rotoli B. Danazol: in vitro effects on human hemopoiesis and in vivo activity in hypoplastic and myelodysplastic disorders. Eur J Haematol 1991; 47:197-203.
31. Sawada K. Impaired proliferation and differentiation of myelodysplastic CD34+ cells. Leuk Lymphoma 1994; 14:37-47.
32. Maciejewski JP, Selleri C, Sato T, Anderson S, Young, NS. A severe and consistent deficit in marrow and circulating primitive hematopoietic cells (long-term culture-initiating cells) in acquired aplastic anemia. Blood 1996; 88:1983-91.
33. Selleri C, Maciejewski JP, De Rosa G, et al. Long-lasting decrease of marrow and circulating long-term culture initiating cells after allogeneic bone marrow transplantation. Bone Marrow Transplant 1999; 23:1029-37.