Antithymocyte globulin and cyclosporine A as combination therapy for low-risk non-sideroblastic myelodysplastic syndromes

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The present study evaluated the combination of antithymocyte globulin (ATG) and cyclosporine A (CsA) in patients with low-risk myelodysplastic syndromes. Twenty patients (17 with refractory anemia and 3 with refractory anemia with excess blasts) received treatment with rabbit-ATG plus CsA. The overall response rate was 30% (6/20); three of the six responders had a complete response. The responses lasted 2-58 months, with two patients still being in complete remission at 42 and 58 months. Short-lasting cytogenetic remissions were achieved in two patients. ATG was poorly tolerated in patients over 70 years of age. Four out of 20 patients progressed to acute myeloid leukemia within a year. We conclude that immunosuppressive treatment may be a therapeutic option for selected patients with myelodysplastic syndrome.

Key words: MDS, ATG, cyclosporine A, treatment.

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Bone marrow apoptosis and ineffective hematopoiesis are key features of low-risk myelodysplastic syndromes (MDS), although the underlying pathogenetic mechanisms vary between different subgroups.[1-3] The chain of stimuli leading to apoptosis can basically be divided into an intrinsic pathway involving mitochondria-mediated cell death, and an extrinsic pathway including death-receptor-mediated apoptosis as well as autoimmune mechanisms with T-cell-mediated cell death.[4-6] Treatment with antithymocyte globulin (ATG) has been evaluated for low-risk MDS in a series of phase II trials.[7-12] While studies encompassing relatively young and selected patients have shown responses rates of up to 64% in patients with refractory anemia (RA),[13] other studies have shown only modest efficacy,[10-11] and the response rate in sideroblastic anemia (RARS) is dismal. According to multivariate analysis, duration of transfusion need, age, and HLA DR15 phenotype predict for a response to treatment.[14] Major responses to ATG seem to be sustained, as is observed in patients with aplastic anemia. Other studies have reported that cyclosporine (CsA) alone may induce responses in patients with RA.[15] Since ATG in combination with CsA is an established treatment for aplastic anemia,[16] we evaluated this combination in patients with low-risk, non-sideroblastic MDS.

Design and Methods

Patients and study protocol

Patients were included in a prospective, open-label multicenter phase II study between July 1999 and April 2002. The aim was to evaluate the efficacy of treatment with ATG plus CsA, and to assess long-term outcome. Eligibility criteria were a diagnosis of French-American-British (FAB)[17] classified RA or RAEB with less than 10% myeloblasts and no ringed sideroblasts, low or intermediate-1 International Prognostic Scoring System (IPSS) score,[18] and significant anemia (hemoglobin <100 g/L or transfusion dependency), and/or thrombocytopenia (platelet counts <30×10^9/L). An upper age limit of 70 was introduced after the first ten treated patients, due to the occurrence of severe adverse events in two patients >70 years of age. The study followed the guidelines of the Regional Ethical Committees. Only a few patients underwent HLA typing, since the report about the predictive value of a HLA DR15 phenotype was published after the onset of this trial.

Fluorescence in situ hybridization analyses

Interphase fluorescence *in situ* hybridization (FISH) analyses of -Y (males only), -5/del(5q), -7/del(7q), and trisomy 8 were performed on bone marrow aspirates obtained before the start of treatment and at 6 months, using the probes CEP Y, LSI EGR1/D5S23, LSI D7S486/CEP 7, and CEP 8, respectively (Vysis, Stockholm, Sweden). Between 200 and 350 nuclei were analyzed for each probe. The cut-off values (median + 3SD) were 8% for -Y, 6% for -5, 7% for del(5q), 8% for -7, 9% for del(7q), and 5% for +8.

Drugs and treatment

ATG-Fresenius (generously provided by Fresenius AG, Germany) was administered as intravenous infusion. The initial ATG dose was 10 mg/kg/day of ATG for 4 days, but after a lower than expected response rate in ten patients, the dose was increased to 20

Table 1. Patients with a response to treatment with ATG + CsA.

FAB/age/sex	Cytopenia[1]	Cellularity	Cytogenetics	Response (type)[3]	Cytogenetic response	Time to response	Response duration	Progression of disease
RA/54/M	A (transf)	normo	normal	CR / A		1 month	CR + 58 months	–
RA/72/M	T (transf)	hyper	+8, 50%[2]	PR / T	46 XY	1 month	2 months	AML at 8 months
RAEB/53/M	ATL	hyper	normal	PR / ATL		3 months	5 months	AML at 9 months
RA/43/M	ATL	hypo	normal	CR / ATL		10 months	CR + 42 months	–
RA/67/F	T	hypo	7q-, 13%[2]	PR / T	46 XX	1 month	2 months	Transfusion need
RA/58/M	A (transf)	hyper	normal	CR / A		2 months	9 months	Transfusion need

[1]*A: anemia; T: thrombocytopenia; L: leukopenia; AML: acute myeloid leukemia;* [2]*shown by conventional cytogenetics and FISH. Percentage of positive cells demonstrated by FISH;* [3]*CR: complete response; PR: partial response; improvement of A: anemia, T: thrombocytopenia, L: leukopenia.*

mg/kg/day for 3 days. Oral treatment with CsA was initiated on day 4 and continued for 32 weeks; the dose was adjusted to achieve a target serum concentration of 200 ng/mL. Patients not considered eligible for ATG treatment could receive only CsA. Prophylactic treatment with prednisone (day 1-10), trimethoprim-sulfamethoxazole, fluconazol and acyclovir (6 months) was given during the study.

Response criteria

A complete response was defined as a hemoglobin level >115 g/L, without needing transfusions, a neutrophil count >1.5×10^9/L a platelet count >150×10^9/L, and <5% marrow blasts.[8] A partial response was defined as an increase in hemoglobin of 15 g/L, transfusion independence for eight consecutive weeks, or a >100% increase of granulocyte or platelet counts in case of pre-treatment neutropenia and thrombocytopenia.

Follow-up

Patients were followed up for acute myeloid leukemia evolution and survival for 33 months after inclusion of the last patient.

Statistical analysis

Data are expressed as mean±SD, or median plus range, when appropriate. Survival was depicted using a Kaplan-Meier plot, and curves were compared using the log-rank test.

Results and Discussion

Patients

Twenty-five patients, 18 males and 7 females, with a median age of 62 (43-82) years and a median disease duration of 6 months (range 1-67), were included in the study. Twenty patients had RA and 5 had RAEB. According to the IPSS, 18 had low-risk disease and 7 had intermediate-1-risk disease. Bone marrow cellularity was high, normal, and low in 13, 7, and 5 patients, respectively. Conventional chromosome analysis revealed aberrations in nine out of the 20 patients tested; deletion 5q- (n=4), +8 (n=2), del 7q (n=2), and complex karyotype. Three patients aged between 72 and 82 were treated with CsA only (no response), and two were excluded before the start of ATG treatment due to disease progression. Hence, 20 patients (17 with RA and 3 with RAEB) were treated with ATG and CsA.

Results of treatment

Five patients with RA and one with RAEB (6/20, 30%) responded to ATG+CsA. Of these six responders, three had a complete response and three a partial response. The median time to response was 2 months (1-10 months) (Table 1). The response rate to ATG + CsA or CsA alone according to an intention-to-treat analysis was 24% (6/25). The median age of the responding patients was 56 (43-72) years, compared with 64 (44-76) years in the non-responding group. Only one of nine patients responded to the lower dose of ATG, whereas five of 11 patients who were treated with the higher dose responded.

Adverse events

Two patients (aged 76 and 79 years) were withdrawn from the study during ATG treatment due to severe but non-fatal side effects (atrial fibrillation and hypotension requiring intensive care). The frequency and severity of side effects were not related to the dose of ATG. Four patients were withdrawn from CsA treatment before 6 months. One of these developed a rapidly growing colon cancer 2 months after treatment.

FISH results

The two responding patients treated because of isolated thrombocytopenia are of particular interest (Table 1). One of them had transfusion-dependent steroid-refractory thrombocytopenia and trisomy 8 in 50% of the cells before treatment. He showed a partial response to ATG + CsA with platelet counts above 10×10^9/L for 3 months. At the 6-month evaluation, thrombocytopenia had recurred, but neither at this occasion, nor at leukemic transformation two months later, was the +8 clone detectable. The other patient had a small 7q- clone (13% of cells), which normalized after treatment, leading to a partial response with increased platelet counts for 2 months.

Long-term outcome

The median duration of the responses was 7 (2-58) months, with two patients still in remission at 42 and 58 months. One of these is dependent on CsA treatment in order to maintain a normal hemoglobin level. Four of the 20 patients, of whom two responded to treatment, developed acute myeloid leukemia within a year. The median

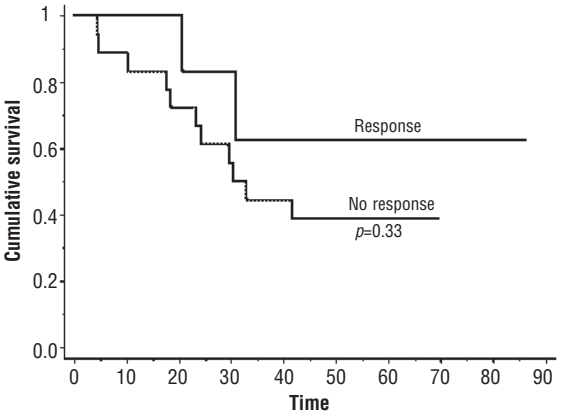

Figure 1. Survival of patients who responded (n=6) or did not respond (n=14) to treatment with ATG+CsA. There is no significant difference between the curves (p=0.33).

survival of evaluable patients was 33 months. The median survival of the non-responders was 30 months, while the median survival was not reached for the responders (Figure 1). There was no significant difference in survival between responding and non-responding patients.

The aim of the present study was to evaluate the effects of treatment with ATG plus CsA in patients with low-risk MDS who had transfusion-dependent refractory anemia or severe thrombocytopenia. The study evaluated the efficacy and toxicity profile of immunosuppression in a relatively unselected cohort of patients. Hence, the decision to withdraw patients from ATG treatment was initially based only on clinical evaluation.

The response rate to the combined treatment with ATG and CsA was not different from that obtained in previous studies using ATG alone.[7-12] However, considering the long-term outcome of one of the responders, it is possible that maintenance treatment with CsA may be indicated in some cases. The three patients who were considered too fragile for ATG treatment did neither respond to CsA alone, nor did they tolerate CsA treatment well. The overall response rate to ATG + CsA was 30% in the evaluable cohort, which is in line with results of previously published European studies.[8-9] However, three of these responses were partial and relatively short-lasting, with questionable benefit for the patients. The three patients who achieved complete responses were young (43-58 years) and had normal karyotypes. Two of them, 10% of the cohort, had long-lasting responses of significant clinical value. One of these was HLA-DR15 positive (*data not shown*). Hence, we conclude that there is a relatively small subset of MDS patients who are responsive to immunosuppressive treatment. Our data indicate that the initial dose of ATG used in this study was probably too low. We also observed that the overall tolerance to both ATG and CsA was lower in patients above the age of 70 years, suggesting that immunosuppressive treatment should not be used routinely in elderly patients. Besides the risk of severe adverse events it is also possible that immune-mediated cytopenia is a less common disease mechanism in aged individuals.

Cytogenetic remissions were seen in two cases, both with short-lasting responses. The clinical significance of this is uncertain, but the findings support previous studies indicating that unbalanced chromosomal changes in MDS may be secondary rather than primary events.[18]

Our long-term results raise the question of whether immunosuppressive treatment may increase the risk of progression to acute myeloid leukemia. Although four cases of acute myeloid leukemia in 20 patients with low-risk MDS are not more than could be expected,[17] we cannot exclude the possibility that progression was related to treatment with ATG in some of these individuals. All four patients had intermediate-1 IPSS scores, including one patient with a complex aberration, and had been diagnosed with MDS within 2 months before entering the study. Thus, a certain observation time to allow detection of spontaneous progression may be indicated before treatment.

In conclusion, immunosuppressive treatment is a therapeutic alternative for selected patients with MDS, in particular younger individuals with RA, normal karyotype, and HLA DR15 phenotype. Responses in this cohort may be long-lasting and stable.

PAB: study co-ordinator, has collected and completed all study data, and wrote the first version of the manuscript: I-MD, RH, EJ, LK, MS, J-MT, BU, GÖ: national and regional coordinators in the Nordic MDS Group. They discussed and wrote the protocol, applied to local authorities for ethical/MPA permission, pursued the study and actively took part in writing and commenting the manuscript: AP-MD: in charge of morphological review, and the corresponding part of the manuscript; BJ: in charge of molecular genetics, and the corresponding part of the manuscript; EH-L: in charge of the Nordic MDS Group and thus of all clinical studies.

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