



## Immunosuppressive therapy with anti-thymocyte globulin and cyclosporine A in selected children with hypoplastic refractory cytopenia

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### ABSTRACT

It is currently unknown whether immunosuppressive therapy or hematopoietic stem cell transplantation is the most appropriate treatment strategy for children with refractory cytopenia and normal karyotype or trisomy 8. We report on 31 children with hypoplastic refractory cytopenia treated with immunosuppressive therapy consisting of antithymocyte globulin and cyclosporine. At 6 months, 22 of 29 evaluable patients had a complete or partial response; a total of ten patients achieved a complete response at varying time points. Six patients subsequently received a transplant because of non-response, progression to advanced myelodysplastic syndrome or evolution of monosomy 7. Overall and failure-free survival rates at 3 years were 88% and 57%, respectively.

Key words: myelodysplastic syndrome, refractory cytopenia, immunosuppressive therapy, anti-thymocyte globulin, children.

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Refractory cytopenia (RC) is the most common subtype of myelodysplastic syndrome (MDS) in children.<sup>1</sup> In pediatric patients, the term RC is used instead of refractory anemia, since anemia is not always present.<sup>2,3</sup> Karyotype has a strong impact on outcome in RC.<sup>2</sup> Patients with monosomy 7 have a significantly higher risk of progression to advanced MDS than have patients with other chromosomal abnormalities or a normal karyotype. It has been suggested that autoimmunity contributes to the cytopenia of MDS, and there is a pathological overlap between aplastic anemia and hypoplastic MDS.<sup>4-7</sup> This concept led to the application of immunosuppressive therapy (IST) with anti-thymocyte globulin and/or cyclosporine A in adult MDS patients.<sup>8,9</sup> Since the role of IST in children with RC is unknown, we report the results of IST in selected children with RC.

### Design and Methods

#### Selection of patients for immunosuppressive therapy

Patients were enrolled in EWOG-MDS 98, a prospective observational study of the European Working Group of MDS in Childhood (EWOG-MDS) on MDS and juvenile myelomonocytic leukemia. Approval for the study was obtained from the institutional review board of each participating institution. Written informed consent was provided by each patient's parents, according to the Declaration of Helsinki. In the EWOG-MDS studies, patients with RC and monosomy 7, 7q-, or a complex karyotype are transplanted as soon as possible. For RC patients with other karyotypes, a watch and wait strategy can be applied in the absence of transfusion dependency or neutropenia (absolute neutrophil count [ANC] <1.0×10<sup>9</sup>/L). For patients with transfusion dependency or ANC <1.0×10<sup>9</sup>/L, hematopoietic stem cell trans-

plantation (HSCT) from a matched family member or suitable unrelated donor is recommended. However, in some patients with hypocellular bone marrow and a normal karyotype, IST is chosen. The choice of IST or HSCT from an alternative donor is influenced by the availability of a donor and the preference of physicians and parents.

Thirty-two children, who were initially diagnosed with RC in Germany (n=31) and Switzerland (n=1) were given IST as the first therapeutic intervention. One patient was excluded from the analysis because he was later diagnosed as having dyskeratosis congenita. The diagnosis of RC was confirmed in the other 31 children by central review of bone marrow and peripheral blood smears and bone marrow biopsies by one of us (IB).<sup>3,10</sup> At least bilineage morphological myelodysplasia was necessary for the diagnosis of RC. Fanconi's anemia was excluded by the chromosomal fragility test.

### Patients' characteristics

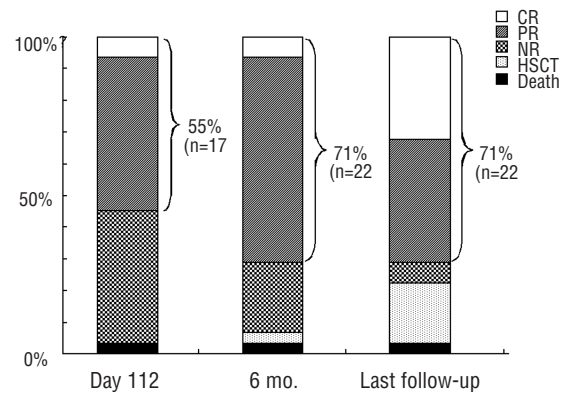
The median age at diagnosis was 10.1 (1.8-17.2) years. The median neutrophil count at IST was  $0.6 (0.07-1.3) \times 10^9/L$ . All patients were transfusion-dependent for platelets and 29/31 for red cells. Bone marrow cellularity was decreased for age in all patients. Standard metaphase cytogenetic analysis revealed a normal karyotype in 13 patients and an abnormal karyotype in one patient (47,XY,-2,+2mar). Interestingly, this abnormality was no longer detected after IST. In 17 patients, cytogenetic analyses failed because of insufficient metaphases; in 13 of these 17 patients, monosomy 7 and trisomy 8 could be excluded by fluorescence *in situ* hybridization (FISH).

### Immunosuppressive therapy

IST was given according to the protocol of study SAA 94 of the German/Austrian/Swiss Pediatric Aplastic Anemia Working Group.<sup>11</sup> It included antithymocyte globulin (horse: 0.75 mL/kg body weight for 8 days; Sangstat/Genzyme), cyclosporine A (5 mg/kg body weight, adjusted to blood levels (100-150 ng/mL by monoclonal assay or 200-400 ng/mL by polyclonal assay) until day >180, prednisolone (started at a dose of 1-2 mg/kg, tapered down from day 14 and stopped on day 28), and granulocyte colony-stimulating factor (5 µg/kg body weight until day 28) in cases of ANC  $<0.5 \times 10^9/L$ .

### Evaluation of response and statistic analysis

Response was evaluated by blood counts on day 112, at 6, 9, and 12 months, and every 6 months thereafter. A complete response was diagnosed in the presence of a hemoglobin level within the age-adjusted normal range, a platelet count  $> 150 \times 10^9/L$ , and neutrophil count  $> 1.5 \times 10^9/L$ . A partial response was diagnosed in patients with transfusion independency, platelet count  $> 20 \times 10^9/L$ , and neutrophil count  $> 0.5 \times 10^9/L$ . No response was present when neither the partial response nor the complete response criteria were met. Death, acquisition of chromo-

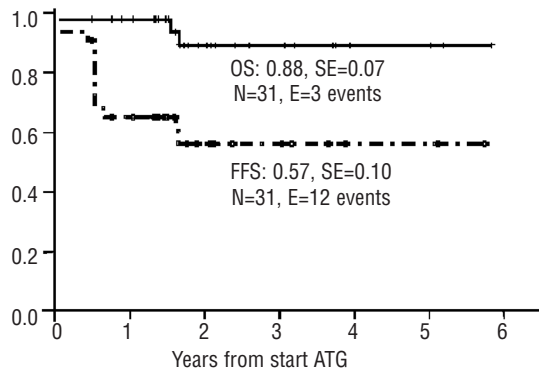


**Figure 1.** Response to immunosuppressive therapy at day 112, after 6 months, and at the last follow up for 31 children with hypoplastic refractory cytopenia. CR: complete remission, PR: partial remission, NR: no response, HSCT: hematopoietic stem cell transplantation. Six patients underwent subsequent HSCT because of NR (n=4), progression to RAEB (n=1), and clonal evolution of monosomy 7 without disease progression (n=1).

somal abnormality, progression to advanced MDS, a second course of IST, HSCT, no response at 6 months, and conversion to no response from partial or complete response (*relapse*) were considered to indicate treatment failure.<sup>12</sup> Overall survival was defined as the time from day 1 of treatment with IST to death or last follow-up. Failure-free survival was defined as the time from day 1 of treatment with IST to treatment failure or the last follow-up. The Kaplan-Meier method was used for survival analysis, and Fisher's exact test to examine the significance of the relationship between response to IST and categorized factors. A non-parametric rank test (Mann-Whitney U-test) was applied to evaluate the difference in quantitative factors between the different groups in terms of the response to IST.

## Results and Discussion

Between May 1998 and May 2005, IST was given as first-line treatment to 31 patients at a median of 70 (7-304) days after the diagnosis of RC. Response rates at day 112, after 6 months, and at the time of the last follow up are depicted in Figure 1. One patient died of a treatment-related cause (sepsis and intracranial bleeding) on day 14, and another child required early transplantation on day 148. Among the 29 patients evaluable for response to IST, 22 patients had responded to IST at 6 months (IST responders; complete response, n=2, partial response, n=20). Of these 22 responders, ten children were in complete remission at the last follow-up, while two had relapsed with transfusion-dependency, and one patient, with an initially normal karyotype, acquired monosomy 7 in the absence of an increased blast count on day 228. The latter patient and the two patients who relapsed underwent HSCT. Seven patients showed no response at 6 months (IST non-responders). Two of the seven IST non-responders



**Figure 2.** Overall survival and failure-free survival after immunosuppressive therapy in 31 children with hypoplastic refractory cytopenia. Death, acquisition of chromosomal abnormality, progression to advanced MDS, second course of IST, HSCT, no response at 6 months, and conversion to no response from partial or complete response were considered treatment failure. OS: overall survival, FFS: failure-free survival.

received a second course of antithymocyte globulin on days 233 and 241; one of them achieved partial remission and the other developed refractory anemia with excess blasts (RAEB) with monosomy 7 on day 366. Although most of the responses to IST were observed within 6 months, four additional patients became transfusion-independent at 9 to 18 months after IST (including one patient after a second course of IST); at the last follow up, three of the four children remained transfusion independent, and one had relapsed. Among the ten patients in complete remission at the time of the last follow-up, nine had discontinued cyclosporine A, while one was still receiving this drug. Similarly, among the 12 patients with partial remission at the time of the last follow-up, three were off cyclosporine A while nine were still receiving the drug. At a median of 484 (148-649) days after the start of IST, a total of six patients had undergone HSCT for non-response (n=4), progression to RAEB (n=1), or acquisition of chromosomal abnormality (n=1). The donor was an unrelated volunteer in five cases, and a newborn sibling in one case. With 28 patients alive, and three deaths after IST (n=1) or HSCT (n=2), the 3-year overall survival and failure-free survival rates were 88% and 57%, respectively (Figure 2).

Previous studies on IST in adult MDS patients elucidated some factors which appeared to favor a good response, including a younger age, shorter interval between diagnosis and IST, or a shorter period of transfusion-dependency, MDS subtype of refractory anemia, low platelet count, hypoplastic bone marrow, normal karyotype, and expression of HLA-DR15.<sup>8-9,13</sup> In this study, the median age at diagnosis (10.2 v 8.7 years) and the median interval between diagnosis and IST (75 v 70 days) did not differ significantly between responders and non-responders. However, patients enrolled in this study were children and the median interval between diagnosis and IST was

shorter than that in previous studies in adults.<sup>8,9</sup>

This study shows that some children with hypoplastic RC respond to IST and recover hematopoiesis, suggesting that the immune system plays a key role in the pathogenesis of the bone marrow failure in RC. However, the results should be interpreted with caution, because the patients enrolled represent a selected group of children with RC with hypoplastic bone marrow, no apparent cytogenetic abnormalities (with the exception of one patient), and a relatively short duration of disease, all of which may be favorable predictive factors for a response to IST. Moreover, the observational period of this study was short, and the long-term outcome remains unknown. A relevant limitation of the study is that conventional cytogenetic analysis failed in more than half of the patients.

The therapeutic aim in childhood MDS is a cure and not palliation. Children with MDS treated with IST have a high risk of relapse and progression to advanced MDS, as illustrated in this cohort. At the same time, HSCT in RC results in a cure rate of approximately 80%.<sup>2</sup> To avoid late complications, such as growth failure and infertility, preparative regimens with reduced intensity have been introduced.<sup>14</sup> Because of the favorable outcome of HSCT,<sup>14</sup> we suggest that IST should generally be applied only in patients with a low risk of disease progression, that is, in patients with hypocellular bone marrow, a normal karyotype, and a short duration of cytopenia. For patients with no response at 6 months we recommend HSCT if a 7/8 or 8/8 HLA antigen matched unrelated donor is available.

Molldrem *et al.* reported that IST non-responders among adult MDS patients have a high risk of disease progression.<sup>8</sup> Similarly, Kojima *et al.* noted that no response to IST at 6 months is a risk factor for secondary MDS in children with aplastic anemia.<sup>15</sup> Since the results of HSCT from matched unrelated donors have become comparable to those from matched sibling donors,<sup>14</sup> it is reasonable to recommend that, in the presence of a suitable alternative donor, IST non-responders should proceed to HSCT.

To conclude, IST can be a promising treatment option in selected patients with hypoplastic RC, specifically in patients without a suitable donor. Further evaluation of the long-term outcome after IST in comparison to HSCT is necessary to establish the most appropriate treatment strategy for children with RC.

#### Authors' Contributions

CN designed the study in co-operation with MF. AF was responsible for the data management. AY and CN contributed to the analysis and interpretation of the results and writing the manuscript draft. PN was responsible for the statistical analyses. CN, EB, UG, KWS, TK, and UGW recruited patients in the data base. MHE revised the paper critically for important intellectual content. IB was responsible for the morphological central review of the bone marrow of the enrolled patients. The manuscript was approved by all authors.

#### Conflict of Interest

The authors reported no potential conflicts of interest.

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