Comparison of horse and rabbit antithymocyte globulin in immunosuppressive therapy for refractory cytopenia of childhood

Ayami Yoshimi,[1] Marry M. van den Heuvel-Eibrink,[2] Irith Baumann,[3] Stephan Schwarz,[4] Ingrid Simonitsch-Klupp,[5] Pascale de Paepe,[6] Vit Campr,[7] Gitte Birk Kerndrup,[8] Maureen O'Sullivan,[9] Rita Devito,[10] Roos Leguit,[11] Miguel Hernandez,[12] Michael Dworzak,[13] Barbara de Moerloose,[14] Jan Starý,[15] Henrik Hasle,[16] Owen P. Smith,[17] Marco Zecca,[18] Albert Catala,[19] Markus Schmugge,[20] Franco Locatelli,[21] Monika Führer,[22] Alexandra Fischer,[1] Anne Guderle,[1] Peter Nöllke,[1] Brigitte Strahm,[1] and Charlotte M. Niemeyer[1]

[1]Department of Pediatrics and Adolescent Medicine, Division of Pediatric Hematology and Oncology, University of Freiburg, Germany; [2]Sophia Children's Hospital, Erasmus Medical Centre, Rotterdam, and Dutch Childhood Oncology Group, the Hague, the Netherlands; [3]Department of Pathology, Boeblingen Hospital, Clinical Centre South West, Boeblingen, Germany; [4]Department of Pathology, University Medical Center Erlangen, Germany; [5]Clinical Institute of Pathology, Medical University of Vienna, Austria; [6]Department of Pathology, University Hospital Ghent, Belgium; [7]Department of Pathology, University Hospital in Motol, Prague, Czech Republic; [8]Department of Pathology, Vejle Hospital, Denmark; [9]Histology Laboratory, Our Lady's Hospital for Sick Children, Dublin, Ireland; [10]Department of Pathology, Bambino Gesù Children's Hospital, Rome, Italy; [11]Department of Pathology, University Medical Centre Utrecht, and Dutch Childhood Oncology Group, the Hague, the Netherlands; [12]Department of Pathology, Hospital Universitario La Fe, Valencia, Spain; [13]St. Anna Children's Hospital and Children's Cancer Research Institute, Department of Pediatrics, Medical University of Vienna, Austria; [14]Department of Pediatric Hemato-Oncology, Ghent University Hospital, Belgium; [15]Department of Pediatric Hematology and Oncology, Charles University and University Hospital Motol, Prague, Czech Pediatric Hematology Working Group, Czech Republic; [16]Department of Pediatrics, Aarhus University Hospital Skejby, Aarhus, Denmark; [17]Paediatric Oncology and Haematology, Our Lady's Hospital for Sick Children Crumlin, Dublin, Ireland; [18]Pediatric Hematology-Oncology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; [19]Department of Hematology, Hospital Sant Joan de Déu, Barcelona, Spain; [20]Department of Hematology and Oncology, University Children's Hospital, Zurich, Switzerland; [21]Department of Pediatric Hematology and Oncology, Bambino Gesù Children's Hospital, Rome, University of Pavia, Italy; and [22]Dr von Haunersches Kinderspital, Children Hospital of the Ludwig-Maximilians-University of Munich, Germany on behalf of the European Working Group of MDS in Childhood

ABSTRACT

Refractory cytopenia of childhood is the most common subtype of myelodysplastic syndrome in children. In this study, we compared the outcome of immunosuppressive therapy using horse antithymocyte globulin (n=46) with that using rabbit antithymocyte globulin (n=49) in 95 patients with refractory cytopenia of childhood and hypocellular bone marrow. The response rate at 6 months was 74% for horse antithymocyte globulin and 53% for rabbit antithymocyte globulin (P=0.04). The inferior response in the rabbit antithymocyte globulin group resulted in lower 4-year transplantation-free (69% *versus* 46%; P=0.003) and failure-free (58% *versus* 48%; P=0.04) survival rates in this group compared with those in the horse antithymocyte globulin group. However, because of successful second-line hematopoietic stem cell transplantation, overall survival was comparable between groups (91% *versus* 85%; P=ns). The cumulative incidence of relapse (15% *versus* 9%; P=ns) and clonal evolution (12% *versus* 4%; P=ns) at 4 years was comparable between groups. Our results suggest that the outcome of immunosuppressive therapy with rabbit antithymocyte globulin is inferior to that of horse antithymocyte globulin. Although immunosuppressive therapy is an effective therapy in selected patients with refractory cytopenia of childhood, the long-term risk of relapse or clonal evolution remains. (*ClinicalTrial.gov identifiers: NCT00662090*)

Introduction

Refractory cytopenia of childhood (RCC) is a provisional entity of the pediatric myelodysplastic syndrome (MDS) in the WHO classification and is characterized by persistent cytopenia with dysplasia and <5% blasts in the bone marrow.[1] It accounts for more than half of all children with MDS. In contrast to adults with refractory anemia, the majority of children with RCC have bilineage or trilineage cytopenia, with approximately 80% having a hypocellular bone marrow.[2] Laboratory and clinical findings in studies of myelodysplasia in adults suggest that autoimmunity directed against hematopoietic stem cells contributes to the development of cytopenia in MDS.[3-7] In addition, it is generally believed that there is a pathophysiological overlap between aplastic anemia and hypocellular MDS.[8] These concepts have led to the use of immunosuppressive therapy (IST), which has proven to be effective in some adults with MDS.[9-13]

Recently, IST has also been used in children with RCC.[14,15] In 2007, we reported the preliminary results of IST in 31 RCC patients, who were registered in the European Working Group of MDS in Childhood (EWOG-MDS) study;[14] the response rate after 6 months of IST was 76% and the overall survival rate at 3 years was 88%. In the same year, Lymphoglobulin® [horse antithymocyte globulin (ATG), Genzyme], which was used for IST in patients with aplastic anemia and MDS in Europe and Japan, was withdrawn from the market. In the absence of another available, licensed

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Correspondence: ayami.yoshimi@uniklinik-freiburg.de

horse-ATG, Lymphoglobulin® was replaced by rabbit-ATG in many countries. However, a randomized controlled trial on aplastic anemia conducted by Scheinberg *et al.* reported an inferior response and decreased survival after rabbit-ATG (Thymoglobulin®, Genzyme) compared with those after horse-ATG (ATGAM®, Pfizer).[16] In addition, a number of non-randomized studies showed inferior results for rabbit-ATG in adults and children with aplastic anemia.[17-19] No published series of patients with aplastic anemia has shown that rabbit-ATG is superior to horse-ATG in the context of first-line IST.[16-22] To date, there have only been a few reports on the efficacy of IST with rabbit-ATG in patients with MDS.[10,23,24]

In this study, we compared the outcome of IST using horse-ATG with that of IST using rabbit-ATG in a large series of children with RCC.

Methods

Selection of patients for immunosuppressive therapy

Bone marrow and peripheral blood smears and bone marrow biopsies were centrally reviewed by reference pathologists in each country and the diagnosis of RCC was made according to the WHO criteria.[1,25] Fanconi anemia was excluded in all patients. Patients with RCC aged ≤18 years were enrolled in the prospective studies EWOG-MDS-98 (05/1998-12/2006) and EWOG-MDS-2006 (01/2007-08/2011, *ClinicalTrial.gov identifier: NCT00662090*). These studies were approved by the institutional review board of each participating institution. Written informed consent was provided by the patients' parents according to the Declaration of Helsinki.

Patients with RCC are treated according to a risk-based strategy (Figure 1). Because of a high risk of disease progression, all patients with monosomy 7/7q- or three or more chromosomal aberrations undergo allogeneic hematopoietic stem cell transplantation (HSCT).[26] For patients without these unfavorable karyotypes, a watch-and-wait strategy is applied in the absence of transfusion dependency or neutropenia. For patients with transfusion dependency or an absolute neutrophil count <1.0×10^9/L, HSCT is recommended. Alternatively, IST can be applied for patients with a hypocellular bone marrow. The choice of IST or HSCT is influenced mainly by the availability of a matched family or unrelated donor and the preference of physicians and parents.

Immunosuppressive regimen

IST was administered as previously reported,[14,27] and included horse-ATG (15 mg/kg/day × 8 days; Lymphoglobulin®, Genzyme),

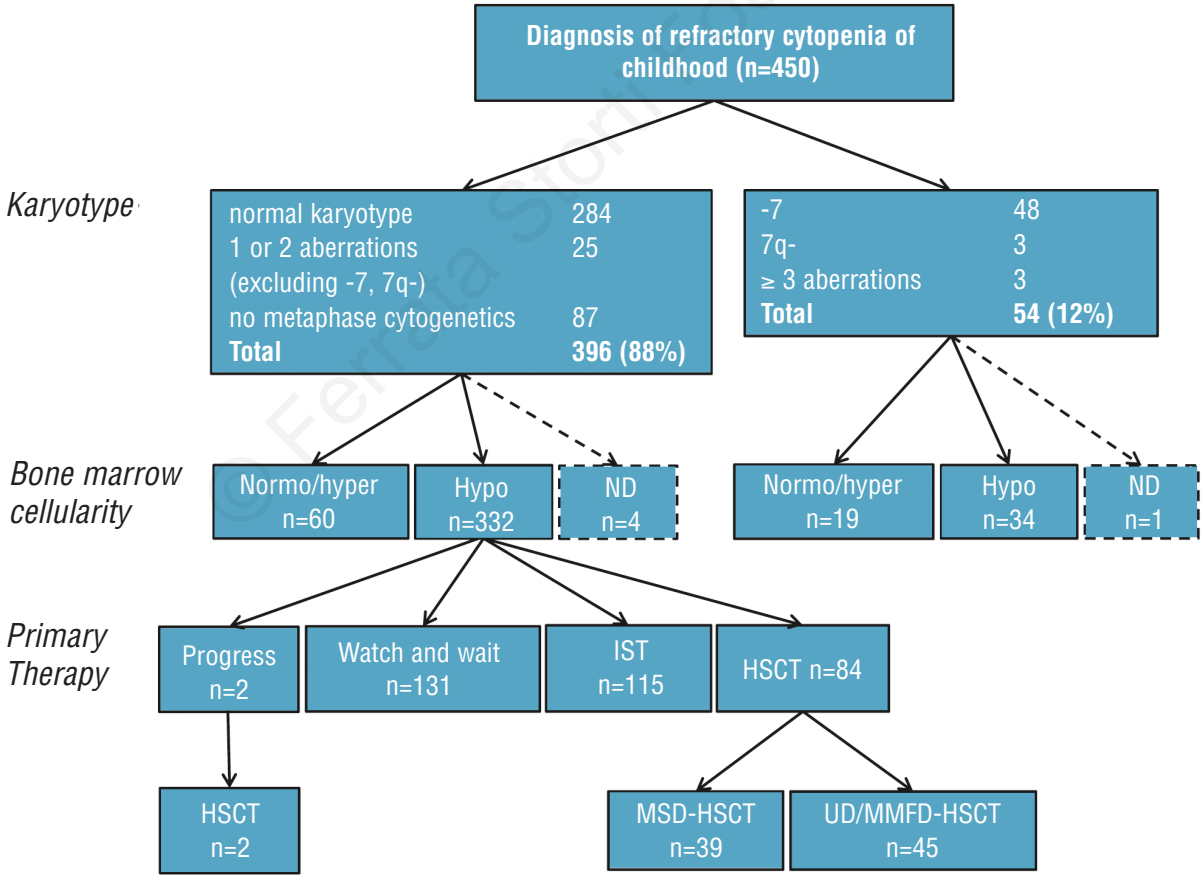

Figure 1. Overview of the 450 patients with refractory cytopenia of childhood. The patients were subdivided according to karyotype and bone marrow cellularity. Of the 332 patients with a normal karyotype, one or two cytogenetic aberrations (excluding 7/7q-), or no result of metaphase cytogenetics and a hypocellular bone marrow, two exhibited progression to refractory anemia with excess blasts before hematopoietic stem cell transplantation (HSCT), 131 received neither immunosuppressive therapy (IST) nor HSCT within 180 days after diagnosis (watch and wait strategy), 115 received IST, and 84 received HSCT from a matched sibling donor (MSD; n = 39), an unrelated donor (UD; n = 44), or a mismatched family donor (MMFD; n = 1) as primary therapy. ND: no data available.

cyclosporine A (5 mg/kg/day, adjusted to maintain blood levels of 100–150 ng/mL by monoclonal assay or 200–400 ng/mL by polyclonal assay) for at least 180 days, and prednisolone (initiated with 1–2 mg/kg/day, tapered from day 14 and stopped at day 28). In patients with an absolute neutrophil count <0.5×10⁹/L, granulocyte colony-stimulating factor (5 μg/kg/day until day 28) was also given. Because of the unavailability of horse-ATG (Lymphoglobulin®) since 2007, this was replaced by rabbit-ATG (Thymoglobulin®, 3.75 mg/kg/day for 5 days, Genzyme).

Definitions and statistical analysis

A complete response was defined as a hemoglobin level within the age-adjusted normal range, a platelet count of ≥150×10⁹/L, and an absolute neutrophil count of ≥1.5×10⁹/L. A partial response was diagnosed in patients who did not qualify for complete response and exhibited transfusion independency, a platelet count of ≥20×10⁹/L, and an ANC of ≥0.5×10⁹/L. No response was defined as not meeting either the criteria for either partial or complete response. Relapse was defined as conversion from partial or complete response to no response.[14]

Survival curves were calculated using the Kaplan–Meier method and compared using the two-sided log-rank test.[28] For estimation of failure-free survival, death, acquisition of a chromosomal abnormality, disease progression, development of paroxysmal nocturnal hemoglobinuria, a second course of IST, HSCT, and relapse were classified as treatment failure. To calculate the cumulative incidence of relapse and clonal evolution, death and HSCT were considered to be competing risks.[29] Categorical variables were compared using the χ^2 test. Continuous variables were compared using the Mann–Whitney test or the Kruskal–Wallis rank test with an adjacent post-hoc Mann–Whitney U-test.[30,31] Logistic regression modeling was used for multivariate analyses.[32]

Results

Characteristics of patients with refractory cytopenia of childhood

A total of 471 consecutive RCC patients were registered in the EWOG-MDS studies between January 1998 and August 2011. Data for at least 180 days of follow-up after diagnosis were available for 450 patients. Of these, 54 (12%) had monosomy 7/7q- or three or more chromosomal aberrations, while the remaining 396 (88%) had either a normal karyotype (n=284), one or two chromosomal aberrations other than monosomy 7/7q- (n=25), or no karyotype result because of insufficient metaphases (n=87; Figure 1). In the latter group, 332 patients had a hypocellular bone marrow; 115 received IST as primary therapy, 84 received a transplant from either a matched sibling donor (n=39), an unrelated donor (n=44), or a mismatched family donor (n=1), two exhibited progression to refractory anemia with excess blasts, and 131 received neither IST nor HSCT within 180 days after diagnosis. The median age of the patients who received IST (9.7 years) was lower than that of patients who received HSCT as primary therapy (matched sibling donor-HSCT: 12.4 years, unrelated/mismatched family donor-HSCT: 11.4 years, *Online Supplementary Table S1*). There was no significant difference in blood counts at diagnosis between the IST and HSCT groups. The median interval between diagnosis and initiation of therapy was shortest in the IST group and longest in the unrelated/mismatched family donor-HSCT (IST: 60 days, matched sibling donor-HSCT: 84 days, unrelated/mismatched family donor-HSCT: 134 days, *P*=0.01; *Online Supplementary Table S1*).

Characteristics of the study cohort

Of the 115 patients in the IST group, data analysis was performed in 95 patients from the following countries who were treated with the recommended dose of either horse-ATG (n=46, Lymphoglobulin®) or rabbit-ATG (n=49, Thymoglobulin®), with at least 6 months of follow-up of treatment: Austria (n=5), Belgium (n=5), Czech Republic (n=4), Denmark (n=1), Germany (n=72), the Netherlands (n=4), and Switzerland (n=4).

The preliminary results for the first 31 patients who received IST have been reported previously.[14] Two patients from this previous report were excluded from the current analysis because they received a different type of ATG (Tecelac®, Biotest Pharma) or had a normocellular bone marrow. Comparison of the 95 patients who received horse-ATG and rabbit-ATG showed no significant differences in age, sex, blood counts at diagnosis, and interval between diagnosis and initiation of IST (Table 1). The follow-up duration was significantly shorter in the rabbit-ATG group than in the horse-ATG group due to the difference in the era of the treatment. One patient in the horse-ATG group and two patients in the rabbit-ATG group had an abnormal karyotype (Table 1). Information on human leukocyte antigens (HLA) was available for 63 patients; HLA-DR15 was negative in 51 and positive in 12 (Table 1).

Comparison of response to horse or rabbit antithymocyte globulin

The response rate to IST with horse-ATG in 46 patients and rabbit-ATG in 49 patients was as follows: 59% (complete response, 9%; partial response, 50%) and 47% (complete, 2%; partial, 45%), respectively, at 4 months (*P*=0.25) and 74% (complete, 9%; partial, 65%) and 53% (complete, 2%; partial, 51%), respectively, at 6 months (*P*=0.04). A late response (>6 months after starting IST) was observed in six patients in the horse-ATG group and one patient in the rabbit-ATG group. At the time of the last follow-up, 26 patients (57%) in the horse-ATG group and 22 patients (45%) in the rabbit-ATG group had achieved a complete or partial response without treatment failure (*P*=ns), while 15 patients (33%) in the horse-ATG group and nine patients (18%) in the rabbit-ATG group had achieved a complete response (*P*=0.10). Detailed information on treatment failures in the remaining 47 patients is shown in *Online Supplementary Figure S1*.

Evaluation of factors other than type of antithymocyte globulin that were related to the response to immunosuppressive therapy

Previous studies on IST in adult patients with MDS identified the following factors that favored a good response: younger age, refractory anemia, lower blast count, hypocellular bone marrow, shorter interval between diagnosis and IST, low platelet count, expression of HLA-DR15, and presence of a paroxysmal nocturnal hemaglobinuria clone.[9-13,33] As shown in Table 2, there were no significant differences in age, sex, HLA-DR15 expression, and blood counts at diagnosis between responders (complete/partial) and non-responders at 6 months. Univariate analysis revealed that the median

interval between diagnosis and starting IST was significantly shorter in the non-responders than in the responders. However, this observation was not confirmed in multivariate analysis. The use of horse-ATG remained the only factor related to the response to therapy. All three patients with an abnormal karyotype (Table 1) before IST responded at 6 months, but one of them relapsed with the same karyotype [46,XY,del(13)(q13q21)]. The patient with 47,XY,–2,+2 mar experienced cytogenetic remission after IST.

Relapse and clonal evolution after immunosuppressive therapy

Relapse of cytopenia was observed in seven of the 60 responders (complete/partial) at 6 months and occurred at a median of 20 months (7–24 months) after IST initiation (horse-ATG, n=5; rabbit-ATG, n=2). The cumulative incidence of relapse at 4 years in responders was 15% (7%–33%) in the horse-ATG group and 9% (2%–35%) in the rabbit-ATG group (*P*=ns). All six patients with relapse who received transplants from an alternative donor are

Table 1. Clinical characteristics of the 95 patients with refractory cytopenia of childhood who received immunosuppressive therapy with either horse or rabbit antithymocyte globulin.

	Horse-ATG (n = 46)	Rabbit-ATG (n = 49)	*P* value
Median age at IST (years, range)	10.1 (1.4-17.4)	10.1 (1.4-18.5)	ns
Sex: male/female	30/16	29/20	ns
Median ANC ($\times 10^9$/L, range)	0.4 (0.03-1.4)	0.4 (0.02-3.8)	ns
Median Hb (g/dL, range), n = 93	8.1 (2.4-12.8)	7.7 (3.1-12.8)	ns
MCV (normal/elevated for age), n = 93	14/31	23/25	0.10
HbF (normal/elevated for age), n = 34	1/10	4/19	ns
Median platelet count ($\times 10^9$/L, range), n = 93	14 (1-126)	12 (0-94)	ns
Karyotype: normal/abnormal/no result of metaphase cytogenetics	23/1*/22	35/2**/12	ns
HLA-DR15: negative/positive, n=63	16/3	35/9	ns
Median interval between diagnosis and IST (days, range)	64 (1-304)	53 (1-330)	ns
Median follow-up after IST (days, range)	2213 (14-3959)	749 (179-1636)	<0.001
Median follow-up after IST for survivors (days, range)	2250 (625-3959)	760 (179-1636)	<0.001

*ATG: antithymocyte globulin, horse-ATG (Lymphoglobulin®), rabbit-ATG (Thymoglobulin®), IST: immunosuppressive therapy, ANC: absolute neutrophil count, Hb: hemoglobin, MCV: mean corpuscular volume, HbF: fetal hemoglobin, HLA: human leukocyte antigen. All blood values given are prior to transfusion. *47,XY,-2,+2mar, **46,XY,del(13)(q13q21) (n = 1); constitutional 46,XY,inv(9)(p11q12) (n = 1).*

Table 2. Clinical characteristics prior to immunosuppressive therapy in responders and non-responders: univariate and multivariate analysis.

Univariate analysis Variables	Responders n = 60	Non-responders n = 35	*P* value
Median age at IST (years, range)	10.6 (1.4-18.5)	8.8 (1.9-17.9)	ns
Sex: male/female	40/20	19/16	ns
Median ANC ($\times 10^9$/L, range)	0.5 (0.03-38.3)	0.3 (0.02-12.8)	0.10
Median Hb (g/dl, range), n = 93	8.0 (3.0-12.8)	7.8 (2.4-12.1)	ns
MCV (normal/ elevated for age), n = 93	20/40	17/16	0.09
HbF (normal/ elevated for age), n=34	3/20	2/9	ns.
Median platelet count ($\times 10^9$/L, range), n = 93	14 (0-126)	12 (1-94)	ns
HLA-DR15: negative/positive, n = 63	27/7	24/5	ns
Median interval between diagnosis and IST (days, range)	70 (7-330)	37 (1-304)	0.004
ATG: horse/rabbit	34/26	12/23	0.03

Multivariate analysis Variables	Risk group	Reference group	Odds Ratio	95%CI	*P* value
Absolute neutrophil count	≥0.4×10^9/L	<0.4 ×10^9/L	1.90	0.72-5.02	ns
Mean corpuscular volume	elevated	normal	1.21	0.45-3.24	ns
Interval between diagnosis and IST	≥50 days	<50 days	2.36	0.92-6.05	0.07
ATG	horse	rabbit	2.67	1.05-6.83	0.04

IST: immunosuppressive therapy, Hb: hemoglobin, HbF: fetal hemoglobin, HLA: human leukocyte antigen, ATG: antithymocyte globulin All blood values given are prior to transfusion. Responders are defined as patients who achieved complete or partial remission at 6 months. Patients who received HSCT (n=4) for no response or who died before 6 months were included in non-responders for the purpose of this statistical analysis.

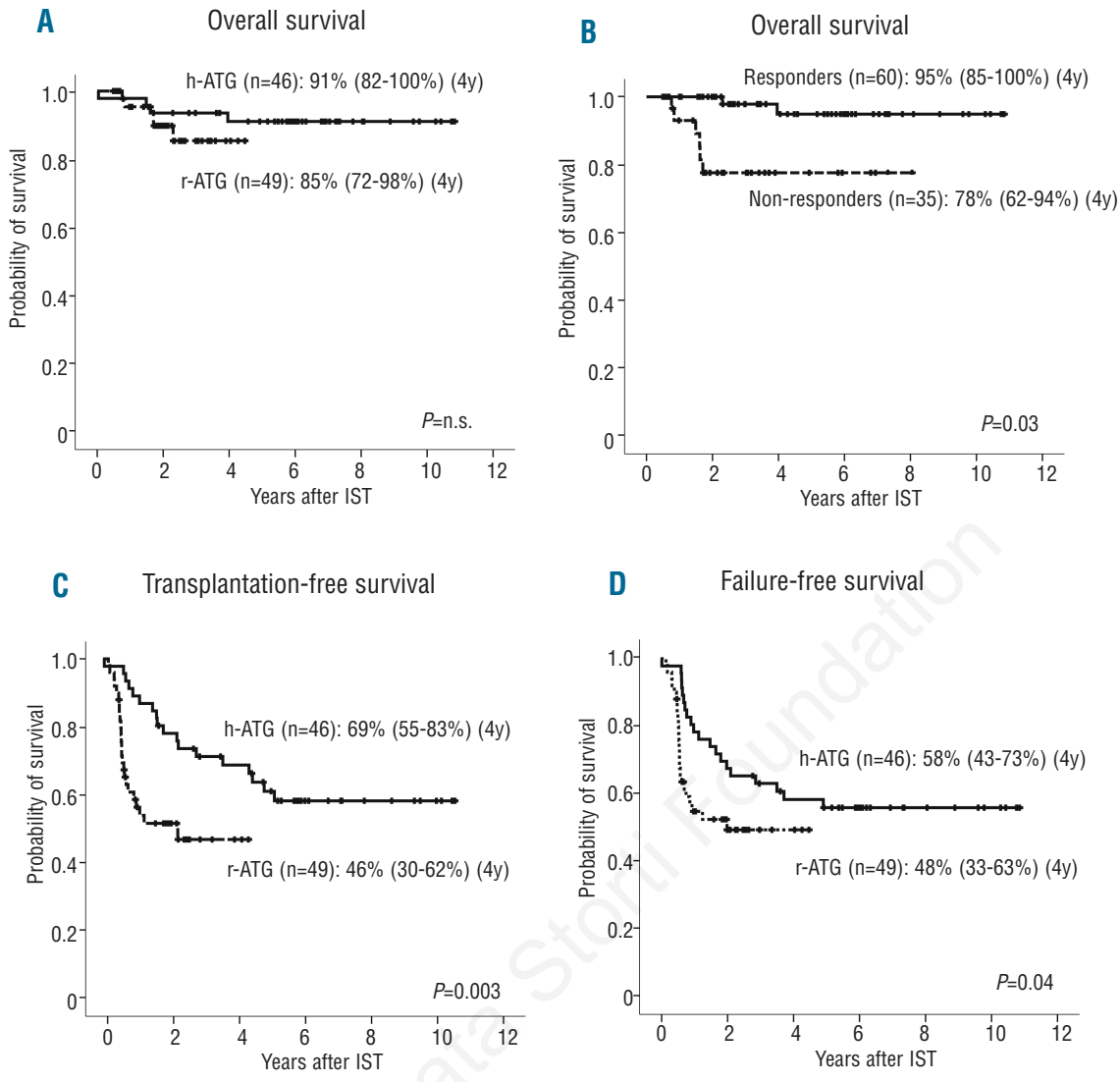

Figure 2. Overall (A-B), transplantation-free (C), and failure-free (D) survival rates after immunosuppressive therapy (IST) in children with refractory cytopenia of childhood treated with either horse antithymocyte globulin (horse-ATG) or rabbit-ATG. To estimate failure-free survival, death, clonal evolution, a second course of IST, requirement of hematopoietic stem cell transplantation, and relapse were considered to indicate treatment failure (D).

alive and disease-free, while one patient died of infection before HSCT could be performed.

Clonal evolution was observed in eight patients at a median time of 14 months (6–64 months) after IST initiation (horse-ATG, n=6; rabbit-ATG, n=2); six of these patients had responded to IST (*Online Supplementary Table S2*). The cumulative incidence of clonal evolution at 4 years was 14% (7%–30%) in the horse-ATG group and 4% (1%–17%) in the rabbit-ATG group (*P*=ns). Six patients developed an abnormal karyotype; five developed an aberration of chromosome 7 and one exhibited del(16)(q12q23). One patient developed clinical paroxysmal nocturnal hemoglobinuria with hemolysis, two showed disease progression to advanced MDS with an increase in blasts, and five had no morphological progression. All eight patients with clonal evolution underwent HSCT: five are alive and disease-free and three died from transplantation-related complications (sepsis, n=1; adenovirus infection, n=1; and graft failure, n=1).

Second-line therapy

Five patients received a second course of IST because of no response (n=3) or partial response (n=2) at 7–25 months after the first course of IST. Both patients in partial remission at the start of the second course showed hematologic improvement after the therapy, although one develoed a relapse of cytopenia 42 months after the second course. None of the patients with no reponse after the first course of IST responded to the second course of IST.

A total of 40 patients, including three who received a second course of IST, underwent HSCT as second-line therapy from either a unrelated donor (n=35), matched sibling donor (n=3), or mismatched family donor (n=2) because of no response (n=24), partial response (n=1), relapse (n=7), or clonal evolution (n=8). In four patients in the rabbit-ATG group, HSCT was performed within 6 months after starting IST. Although 33 patients are alive and disease-free after HSCT, seven died from transplantation-related complications. No patient exhibited disease

relapse after HSCT. The overall survival rate of the 40 patients after second-line HSCT was 80% (66%–96%) at 4 years, which was similar to that of the 84 patients given first-line HSCT [90% (81%-95%), *P*=ns.].

Survival following immunosuppressive therapy

As shown in Figure 2A, a total of nine patients died during the follow-up period, resulting in an overall survival probability at 4 years of 88% (80%–96%) for the total cohort, 91% (82%–100%) for the horse-ATG group, and 85% (72%–98%) for the rabbit-ATG group (*P*=ns). One patient died from intracranial bleeding on day 14 while another died from infection following a relapse at 27 months after IST initiation. The remaining seven patients suffered fatal transplantation-related complications. Responders (complete/partial) at 6 months had a significantly better overall survival rate (95%; 85%–100%) than that of non-responders (78%; 62%–94%; *P*=0.03; Figure 2B). At 4 years, the transplantation-free survival rates were 69% (55%–83%) and 46% (30%–62%; *P*=0.003) while the failure-free survival rates were 58% (43%–73%) and 48% (33%–63%; *P*=0.04) in the horse-ATG and rabbit-ATG groups, respectively (Figure 2C,D).

Discussion

This study investigated the outcome of IST in a large cohort of 95 children with RCC and compared the efficacy of horse-ATG and rabbit-ATG. Sixty-four percent of the patients responded to IST with an overall survival rate of 88%, suggesting that IST is an effective therapy for selected patients with RCC. The response and overall survival rates in this study were higher than 20%–40% and 50%–70%, respectively, achieved in adults.[9-13] This difference can be explained, in part, by the careful selection of pediatric subjects for IST. In fact, previous studies in adults included patients with different MDS subtypes such as refractory anemia, refractory anemia with ringed sideroblasts, and refractory anemia with excess of blasts.[9-13] In addition, age, bone marrow cellularity, and karyotypes varied greatly in these adult studies. In a broad heterogeneous cohort of patients with MDS, the effects of IST are generally modest. Appropriate subsets of patients must be selected to optimize the results of IST. In the EWOG-MDS studies, only children with RCC, a hypocellular bone marrow, and a karyotype other than monosomy 7/7q- or three or more chromosomal aberrations were eligible for IST because these variables were known to be associated with a favorable response to IST and a low risk of disease progression.[11-13,26] Seven patients with RCC and a normocellular bone marrow who received IST were excluded from this analysis because they did not meet the eligibility criteria. Indeed, only one of these patients responded to IST, supporting our recommendation to use IST only in children with a hypocellular bone marrow. Nevertheless, the main reason for the favorable survival observed in this study is the fact that the majority of children who failed IST were rescued by second-line HSCT.

In addition to bone marrow hypocellularity, several other factors have been reported to be associated with a favorable response to IST, including a younger age, the presence of HLA-DR15, and a short disease duration before IST.[11,12,33] Not surprisingly, age had no impact on the response to IST in the pediatric population in the current study. The expression of HLA-DR15 also showed no relationship with response. In contrast to previous reports, univariate analysis showed that the median duration of disease before starting IST was significantly shorter in non-responders than in responders (37 and 70 days, respectively), although this was not confirmed in the multivariate analysis.[11,12] However, these results should be interpreted carefully because the median disease duration in this study was considerably shorter than the 8–19 months found in previous studies of adults.[11,12]

Two preparations of horse-ATG have been used widely for IST in patients with aplastic anemia and MDS: ATGAM®, which has been used almost exclusively in the United States, and Lymphoglobulin®, which was used in Europe and Asia. Lymphoglobulin® was withdrawn from the market in 2007 and replaced by rabbit-ATG (Thymoglobulin®) in most European and Asian countries. Biological studies indicate that, compared to horse-ATG, rabbit-ATG depletes lymphocytes more efficiently and causes more prolonged lymphocytopenia.[34] In addition, rabbit-ATG has been reported to be an effective second-line treatment in patients with aplastic anemia who have previously failed IST with horse-ATG.[35,36] However, several recent studies on aplastic anemia showed an inferior response and/or decreased survival after rabbit-ATG compared with those after horse-ATG,[16-19] whereas other studies described comparable results.[20-22] Notably, none of the reports indicated a superior efficacy of rabbit-ATG over horse-ATG. The majority of previous reports in adults with MDS have involved horse-ATG, and there are limited data on the efficacy of rabbit-ATG. Stadler *et al.* reported a randomized controlled trial comparing horse-ATG (n=20) and rabbit-ATG (n=15) in adults with MDS (refractory anemia, n=24; refractory anemia with excess of blasts, n=10; and chronic myelomonocytic leukemia, n=1)[10] and showed a similar response rate for both therapies (horse-ATG, 30%; rabbit-ATG, 27%; *P*=ns). Subgroup analysis of patients with refractory anemia revealed identical response rates (42%) for those treated with horse-ATG (n=15) or rabbit-ATG (n=15). For patients treated with rabbit-ATG in combination with cyclosporine A, Kadia *et al.* reported a response rate of 24% in patients with low- to intermediate-risk MDS,[23] while Broliden *et al.* showed that 30% of patients with low-risk MDS responded to therapy.[24]

Our study on the efficacy of IST in RCC patients was not a randomized controlled trial, which represents a relevant limitation of the investigation. Nevertheless, the study comprised a homogeneous cohort of pediatric patients with RCC with hypoplastic bone marrow and a favorable karyotype, thereby providing a reasonable basis for comparing the efficacy of rabbit-ATG and horse-ATG. The response rate at 6 months in the rabbit-ATG group was significantly inferior to that in the horse-ATG group, resulting in lower transplantation-free and failure-free survival rates in the rabbit-ATG group. However, because of successful second-line HSCT, the overall survival rate was comparable between patients in the rabbit-ATG and horse-ATG groups.

Some previous studies showed increased risks of severe infectious complications following IST with rabbit-ATG.[16,18] Detailed information about infections was not available in this study. However, there was only one death due to an infectious complication and no Epstein-Barr virus-associated lymphoproliferative disorder fol-

lowing IST was observed in this study. Given these results and previous reports of IST in patients with aplastic anemia,[16-19] we conclude that IST for children with RCC should include horse-ATG, currently available as ATGAM®.

This study also demonstrated some disadvantages of IST for RCC. Patients who received IST remained at risk of clonal evolution and relapse. The failure-free survival rate after IST was approximately 50%, and it remains to be seen whether a plateau can be reached 5 years after IST initiation (Figure 2D). Notably, most clonal evolutions were observed in responders in this study. Because most non-responders received HSCT in this study, they had a reduced risk of clonal evolution. Although it was not statistically significantly different, the incidence of clonal evolutions was higher in the horse-ATG group than in the rabbit-ATG group, due to the significantly shorter median follow-up for patients treated with rabbit-ATG than that for patients treated with horse-ATG. Clonal evolution was frequently associated with the occurrence of monosomy 7. Most patients with clonal evolution could be cured by HSCT if the procedure was performed before progression to advanced MDS. It is, therefore, crucial to monitor blood counts and repeat bone marrow examinations with cytogenetic analysis for early detection of clonal evolution, and if indicated, second-line HSCT should be performed immediately. HSCT also rescues the majority of patients with a relapse of cytopenia.

Recently, the outcome of unrelated donor-HSCT in patients with MDS has improved because of more precise HLA typing, advances in supportive care, better strategies for graft-*versus*-host disease prophylaxis, and introduction of reduced intensive conditioning regimens that decrease the risk of transplant-related mortality and late complications. The preliminary results of the EWOG-MDS study showed a cure rate of over 90% in children with RCC after unrelated donor-HSCT using a reduced intensive conditioning regimen.[37] Compared with unrelated donor-HSCT, the advantage of IST is that therapy can be started immediately, as illustrated by the fact that the median time between diagnosis and initiation of therapy was 60 days in the group that received IST and 134 days in the group that received unrelated donor-HSCT as primary therapy (*Online Supplementary Table S1*). When choosing between IST and upfront unrelated donor-HSCT for a child with RCC, the risk of severe complications of HSCT, such as chronic graft-*versus*-host disease, needs to be considered. Upfront unrelated donor-HSCT is a suitable therapy if a 9/10 or 10/10 HLA-compatible donor can be found in a short time period after diagnosis. HSCT is indicated for all non-responders after 6 months of IST, while early HSCT needs to be considered in patients with prolonged and very severe neutropenia (absolute neutrophil count $<0.2\times10^9$/L). There was no difference in survival rates between patients who underwent HSCT as first-line or second-line treatment in this study.

In conclusion, IST is an effective treatment option in selected patients with RCC. However, patients treated with IST remain at risk of relapse and clonal evolution. As in studies on aplastic anemia, we observed that rabbit-ATG was less effective than horse-ATG. Future studies should pursue the objective of identifying relevant and reliable biomarkers for the selection of children with RCC who may benefit from IST. Finally, evaluation of the long-term outcome of IST and the comparison of this outcome with that of HSCT is important to establish the most appropriate treatment strategy for children with RCC.

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