Autologous Stem Cell Transplantation in Children With Severe Progressive Systemic or Polyarticular Juvenile Idiopathic Arthritis

Long-Term Followup of a Prospective Clinical Trial

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Objective. To assess the safety and efficacy of intensive immunosuppression followed by T cell–depleted autologous hematopoietic stem cell transplantation (ASCT) for induction of disease remission in children with refractory progressive juvenile idiopathic arthritis (JIA).

Methods. Twenty-two patients with progressive refractory JIA were followed up over a median period of 80 months after pretreatment with intensive immunosuppression followed by ASCT in a multicenter, prospective, phase II clinical trial. Hematopoietic stem cells were harvested from the patients’ bone marrow, depleted of T cells, and kept frozen until used for ASCT. Pretreatment of patients consisted of a combination of antithymocyte globulin, cyclophosphamide, and low-dose total body irradiation. Patients were followed up for ASCT-related complications, recovery of hematologic and immune system parameters, and disease outcomes.

Results. Reconstitution of hematologic values to normal range was rapid. Recovery of immune system parameters, especially normalization of CD4+, CD45RA+ naive T cells, was delayed, occurring at ≥6 months after ASCT. The prolonged period of immune deficiency resulted in a large number of viral infections and may have contributed to the development of macrophage activation syndrome (MAS), leading to death, in 2 patients. After ASCT, 8 of the 20 evaluable patients reached complete clinical remission of their JIA, 7 were partial responders, and 5 experienced a relapse of their disease (occurring 7 years after ASCT in 1 patient). Later during followup, 2 of the patients whose disease relapsed died from infections that developed after restarting immunosuppressive medication.

Conclusion. Intensive immunosuppression followed by ASCT resulted in sustained complete remission or marked improvement in 15 of 22 patients with progressive refractory JIA. The procedure, however, is associated with significant morbidity and risk of mortality due to prolonged and severe depression of T cell immunity. After fatal complications due to MAS were observed in some patients, the protocol was amended in 1999, to ensure less profound depletion of T cells, better control of systemic disease before transplantation, antiviral prophylaxis after transplantation, and slow tapering of corticosteroids. Following these protocol modifications, no additional ASCT-related deaths were observed among the 11 patients who received the modified treatment.

Juvenile idiopathic arthritis (JIA) is a heterogeneous group of pediatric illnesses that share the
common feature of chronic joint inflammation (1). Although the prognosis in the majority of patients has improved because of advances in treatment strategies, including the use of modulators of proinflammatory cytokines and their receptors (2,3), the disease in a considerable number of children, particularly those with polyarticular or systemic JIA, is refractory to therapy and tends to be progressive (4,5). Disease progression often leads to destructive arthritis with severe joint deformities, growth retardation, and disability. The patient’s health is further compromised by the cumulative effects of toxicity of antirheumatic medications (6,7). Whereas disease- and treatment-related mortality in children with JIA now occurs less frequently, in fewer than 1% of patients (4), 65% of the deaths occur among patients with systemic-onset JIA.

This grim prospect warranted a clinical trial of intensive immunosuppression in selected JIA patients, with the aim of terminating the autoimmunologic process. The immunopathogenesis of JIA is yet to be elucidated; however, there is indirect evidence that T cell dysregulation plays a key role in disease onset and is
also likely to be a factor in the perpetuation of the disease (8,9). It is conceivable that in JIA, there is a disruption in the balance between the activated effector CD4+ T lymphocytes (the Th1 subset), which produce proinflammatory cytokines, and the tolerizing, so-called regulatory CD4+ T lymphocytes (known as Tregs), which produce antiinflammatory cytokines (10,11). Intensive immunosuppressive therapy followed by rescue with autologous hematopoietic stem cells from the bone marrow, which are purged of potentially autoreactive mature T lymphocytes, leads to the differentiation and maturation of precursor T lymphocytes in the presence of the hypothetical autoantigen(s). This may redress the balance between functionally different T cell populations and thus restore the nonautoreactive (“normal”) state (12,13).

Data from experimental animal models of adjuvant-induced arthritis have shown that allogeneic as well as T cell–depleted (pseudo)autologous stem cell transplantation (ASCT) is efficacious in achieving remission of arthritis (14). In addition to previous case observations of the reversal of autoimmune disease in patients treated with allogeneic stem cell transplantation for hematologic malignancy, results of ASCT for autoimmune disorders such as multiple sclerosis and rheumatoid arthritis have been reviewed recently (15–17). At present, more than 700 ASCT procedures, of which at least 50 have been in children with JIA, have been described worldwide for the treatment of progressive autoimmune diseases (18,19).

We herein report the results from a long-term followup of patients in a prospective, multicenter, phase II clinical study aimed at the evaluation of the safety and efficacy of T cell–depleted ASCT preceded by a regimen of intensive immunosuppression. In an earlier, preliminary report of the clinical trial results, findings from short-term followup of the first 4 patients were published (19,20). The present report describes the findings in 22 patients with therapy-resistant JIA who were followed up over a median period of 80 months.

**PATIENTS AND METHODS**

**Patients.** Between March 1997 and June 2001, 23 patients with JIA were prospectively enrolled in the clinical trial, which was executed in 3 different centers (Figure 1). One patient chose not to undergo ASCT after harvesting of the bone marrow, while the remaining 22 patients underwent transplantation. Eighteen patients had systemic JIA and 4 patients had polyarticular JIA. There were 8 girls (7 with systemic JIA) and 14 boys (11 with systemic JIA), and the median age of the patients was 8.5 years (range 4–18 years).

The median disease duration prior to transplantation was 70 months (range 13–135 months). Written informed consent was obtained from all patients and/or their parents, and each institution’s board of medical ethics approved the study.

The trial was performed in accordance with the guidelines of the European League Against Rheumatism and the European Group for Blood and Marrow Transplantation (EBMT) for bone marrow transplantation in JIA (21,22). The inclusion criteria for this trial were the presence of active disease that had not responded to conventional medication for at least 1 year according to the Pediatric Rheumatology International Trials Organization (PRINTO) criteria (23), and failure of active disease to respond to treatment with steroids in combination with at least 2 disease-modifying antirheumatic drugs (DMARDs), e.g., high-dose methotrexate (MTX) (1 mg/kg/week) and high-dose cyclosporin A (5 mg/kg/day), or steroid dependency (minimal dosage required to suppress symptoms 0.3 mg/kg/day), or an unacceptable adverse reaction to either DMARDs or steroid therapy. Since tumor necrosis factor α (TNFα) blockade became available in 1999 as a therapeutic option and has since been proven of clinical benefit, especially in patients with polyarticular JIA (2,3), the inclusion criteria from March 1999 thereafter also included failure of disease to respond to anti-TNFα therapy; we included 5 patients refractory to anti-TNFα treatment (24) (Table 1). The exclusion criteria for this trial were cardiopulmonary insufficiency, renal or liver failure, presence of acute or chronic infections, end-stage rheumatic disease, history of poor compliance, and, from 1999 thereafter, uncontrollable active systemic disease, i.e., a persistent spiking fever and rash.

Following the occurrence of fatal macrophage activation syndrome (MAS) in some patients, an international consensus was reached with respect to transplantation procedures and surveillance of patients after ASCT, resulting in an amended protocol in 1999. The revised protocol dictated a less profound T cell depletion of the graft, control of systemic disease before transplantation, antiviral prophylaxis, and slow tapering of corticosteroids after transplantation. Monitoring for early signs of MAS and preemptive treatment with steroids and cyclosporin A were also introduced (19,25).

The patients’ disease characteristics and treatment regimen prior to ASCT as well as outcomes after ASCT are summarized in Table 1. All patients had impaired physical growth and osteoporosis at the time of ASCT. Fourteen patients also had erosions of the joints.

**Bone marrow harvest and T cell depletion.** Aspirates of unprimed bone marrow were the source of the autologous hematopoietic stem cells in all 22 patients. The bone marrow graft was depleted of mature T cells by 2 different methods, using either negative selection of T cells by immunorosette-sedimentation, under good manufacturing practice conditions, with specific monoclonal antibodies (anti-CD2 and anti-CD3) coupled to autologous red blood cells (n = 17), or using CD34+ selection by ClinMACS (Miltenyi Biotec, Munich, Germany) (n = 5), performed at Sanquin Pharmaceutical Services (Sanquin, Amsterdam, The Netherlands) (26). The T cell–depleted graft was cryopreserved until used for ASCT. Following the development of MAS which resulted in death in 2 patients (25), T cell depletion of the graft was restricted to the immunorosette technique, to ensure that the graft would contain a limit of between $1 \times 10^5$ and $5 \times 10^7$ T cells/kg.
which was previously shown in animal experiments to not interfere with the immune-abrogative effect of the pretreatment (27).

Conditioning regimen. The intensive immunosuppressive pretreatment consisted of antithymocyte globulin (rabbit-ATG; Imtix-SangStat, Lyon, France) administered intravenously (IV) on days 9 to 6 (cumulative dose 20 mg/kg), cyclophosphamide (cumulative dose 200 mg/kg) administered IV on days 5 to 2, and total body irradiation (4 Gy, single dose) administered on day 1. This was followed by reinfusion of the T-cell–depleted graft (28) on day 0. DMARDs were stopped prior to this conditioning regimen. Steroids were slowly tapered and cumulatively discontinued in 3 patients at 3 months after ASCT, in 8 patients at 6 months after ASCT, and in 13 patients at 12 months after ASCT.

Posttransplantation supportive care was provided according to standard stem cell transplantation protocols. All patients received selective antimicrobial suppression of the intestinal microflora (29) during the posttransplantation period and had received prophylactic treatment with aerosolized pentamidine for pneumocystis pneumonia. Seventeen of 22 patients received antiviral prophylaxis with (val)aciclovir intravenous immunoglobulin; SSZ = sulfasalazine; Auro = auromyosine; CYC = cyclophosphamide; HCQ = hydroxychloroquine; MAS = macrophage activation syndrome; anti-TNFα = anti–tumor necrosis factor α.
† Prior to ASCT, all patients received nonsteroidal antiinflammatory drugs, steroids, and methotrexate.

Table 1. Characteristics of the patients with juvenile idiopathic arthritis prior to ASCT and outcome after ASCT

<table>
<thead>
<tr>
<th>Patient/sex/JIA subtype</th>
<th>Dx–Tx, months</th>
<th>Age at ASCT, years</th>
<th>Duration of followup, months</th>
<th>Medication prior to ASCT†</th>
<th>Outcome</th>
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<tbody>
<tr>
<td>1/F/systemic</td>
<td>69</td>
<td>6.6</td>
<td>104</td>
<td>CSA, AZA, IVIG</td>
<td>Failure/ongoing disease</td>
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<td>2/F/polyarticular</td>
<td>47</td>
<td>7.9</td>
<td>101</td>
<td>CSA</td>
<td>Remission</td>
</tr>
<tr>
<td>3/M/systemic</td>
<td>108</td>
<td>11.4</td>
<td>97</td>
<td>CSA</td>
<td>Partial responder</td>
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<tr>
<td>4/F/systemic</td>
<td>73</td>
<td>11.2</td>
<td>92</td>
<td>CSA, AZA, IVIG, SSZ</td>
<td>Remission</td>
</tr>
<tr>
<td>5/M/systemic</td>
<td>117</td>
<td>13.8</td>
<td>91</td>
<td>AZA, SSZ, Auro</td>
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</tr>
<tr>
<td>6/M/polyarticular</td>
<td>31</td>
<td>5.6</td>
<td>89</td>
<td>SSZ</td>
<td>Partial responder</td>
</tr>
<tr>
<td>7/M/systemic</td>
<td>71</td>
<td>10.5</td>
<td>88</td>
<td>CSA, AZA, CYC, HCQ, SSZ</td>
<td>Partial responder</td>
</tr>
<tr>
<td>8/M/systemic</td>
<td>55</td>
<td>9.4</td>
<td>84</td>
<td>CSA, AZA, IVIG</td>
<td>Remission</td>
</tr>
<tr>
<td>9/F/systemic</td>
<td>135</td>
<td>14.9</td>
<td>4</td>
<td>CSA, AZA, HCQ</td>
<td>Deceased (MAS)</td>
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<td>10/M/polyarticular</td>
<td>86</td>
<td>12.3</td>
<td>80</td>
<td>CSA, SSZ, HCQ, Auro</td>
<td>Partial responder</td>
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<tr>
<td>11/M/systemic</td>
<td>13</td>
<td>4.2</td>
<td>0.6</td>
<td>CSA</td>
<td>Deceased (MAS)</td>
</tr>
<tr>
<td>12/F/systemic</td>
<td>36</td>
<td>10.6</td>
<td>72</td>
<td>CSA, CYC, anti–TNFα</td>
<td>Failure/deceased</td>
</tr>
<tr>
<td>13/M/systemic</td>
<td>45</td>
<td>4.9</td>
<td>13</td>
<td>CSA</td>
<td>Partial responder</td>
</tr>
<tr>
<td>14/F/systemic</td>
<td>27</td>
<td>5.3</td>
<td>64</td>
<td>CSA</td>
<td>Partial responder</td>
</tr>
<tr>
<td>15/M/polyarticular</td>
<td>75</td>
<td>8.4</td>
<td>64</td>
<td>CSA, SSZ</td>
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<tr>
<td>16/M/systemic</td>
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<td>7.7</td>
<td>62</td>
<td>CSA, anti–TNFα</td>
<td>Remission</td>
</tr>
<tr>
<td>17/M/systemic</td>
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<td>8.1</td>
<td>57</td>
<td>CSA</td>
<td>Failure/ongoing disease</td>
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<td>18/M/systemic</td>
<td>42</td>
<td>5.2</td>
<td>54</td>
<td>CSA, anti–TNFα</td>
<td>Failure/ongoing disease</td>
</tr>
<tr>
<td>19/M/systemic</td>
<td>106</td>
<td>11.6</td>
<td>52</td>
<td>CSA, anti–TNFα</td>
<td>Remission</td>
</tr>
<tr>
<td>20/F/systemic</td>
<td>33</td>
<td>4.8</td>
<td>72</td>
<td>CSA</td>
<td>Partial responder</td>
</tr>
<tr>
<td>21/F/systemic</td>
<td>71</td>
<td>8.4</td>
<td>16</td>
<td>CSA, anti–TNFα</td>
<td>Failure/deceased</td>
</tr>
<tr>
<td>22/M/systemic</td>
<td>97</td>
<td>18.2</td>
<td>80</td>
<td>CSA, CYC, IVIG</td>
<td>Partial responder</td>
</tr>
</tbody>
</table>

* ASCT = autologous stem cell transplantation; Dx–Tx = time from diagnosis to ASCT; CSA = cyclosporin A; AZA = azathioprine; IVIG = intravenous immunoglobulin; SSZ = sulfasalazine; Auro = auromyosine; CYC = cyclophosphamide; HCQ = hydroxychloroquine; MAS = macrophage activation syndrome; anti–TNFα = anti–tumor necrosis factor α.
† Prior to ASCT, all patients received nonsteroidal antiinflammatory drugs, steroids, and methotrexate.

Assessment of outcome. We used the core set of outcome variables for clinical trials and the preliminary definition of improvement in JIA as described by Giannini et al and the PRINTO (23). Outcome measures included the following: 1) physician’s global assessment of overall disease activity as measured on a 10-cm visual analog scale (VAS), 2) parent’s/patient’s global assessment of overall well-being as measured on a 10-cm VAS, 3) functional ability as assessed on the Childhood Health Assessment Questionnaire (30), 4) the number of joints with active disease as measured by the Fuchs Swelling Index (31), 5) the number of joints with limitation of motion as measured on the Pediatric Escola Paulista de Medicina Range of Motion scale (32), and 6) the erythrocyte sedimentation rate. The criteria for improvement were defined as at least 30% improvement from baseline in 3 of 6 core set variables, with no more than 1 of the remaining variables worsening by >30% (23).

The core set variables were assessed prior to ASCT and at regular intervals after ASCT, i.e., at 3, 6, and 12 months post-ASCT and yearly thereafter. Patients were also evaluated for 50% and 70% improvement of disease activity, according to the American College of Rheumatology (ACR) response criteria (33). Since no exact definition of complete remission is available for JIA, we adopted the criteria for remission of JIA as described by Wallace et al (34). We defined the condition of clinical remission without medication as absence of signs of disease activity (joints with active disease and/or abnormal laboratory test results) after a period of at least 12 months without antirheumatic therapy, including local corticosteroid injections and nonsteroidal antiinflammatory drugs. Improvement or partial response was defined as improvement of disease activity according to the outcomes of the core set.
variables, after reinstitution of low-dose DMARDs or steroids following a relapse.

**Immunoreconstitution.** Peripheral blood lymphocyte (PBL) subsets were determined before ASCT and at regular intervals following ASCT, i.e., at 3, 6, and 12 months post-ASCT and yearly thereafter, using 4-color flow cytometry (fluorescence-activated cell sorter analysis; Becton Dickinson, San Jose, CA) and appropriate monoclonal antibodies. The dynamics of reconstitution of different lymphoid subsets, including CD3+ T lymphocytes, CD4+ and CD8+ T lymphocyte subsets, memory CD45RO+ T cells, naive CD45RA+ T cells, CD19+ B lymphocytes, and CD3−,CD16+/CD56+ natural killer (NK) cells, were determined, with results expressed as the absolute cell counts per microliter of peripheral blood. Normalization of these cell counts was defined as levels reaching the 5th percentile of age-matched reference values (35). For normalization of CD45RO+ and CD45RA+ T cells, we used the 5th percentile of our internal reference data for age-matched healthy controls.

**Statistical analysis.** The date of cutoff for longitudinal evaluation was October 1, 2005. Treatment efficacy was evaluated by testing whether there was a difference in the core set criteria scores between the baseline evaluation and each regular evaluation after transplantation, using Wilcoxon’s matched pairs signed rank test (23,36). The levels of lymphocyte subsets (in absolute number/µl) after ASCT were compared with baseline levels prior to transplantation, using Wilcoxon’s matched pairs signed rank test. Kaplan-Meier survival analysis was used to assess overall survival and disease-free survival. P values less than 0.05 were considered significant. Patients were censored from the study if there was reinstitution of (second-line) antirheumatic therapy post-ASCT or if the patient had died post-ASCT. Statistical analyses were carried out using SPSS 12.0.1 (Chicago, IL).

**RESULTS**

**ASCT procedure and overall JIA outcome.** The graft contained a median of $1.5 \times 10^6$ CD34+ cells/kg body weight (range 0.4–6.0 $\times 10^6$). After T cell depletion, a median of $2.8 \times 10^6$ CD3+ cells/kg body weight (range 0.5–35.4 $\times 10^6$) (n = 17) remained in the graft following immunomodulation, and a median of $1.0 \times 10^4$ CD3+ cells/kg body weight (range 0.2–2.5 $\times 10^4$) (n = 5) remained in the graft following CD34+ selection. The conditioning regimen was well tolerated in all patients.

During the first pretreatment infusion of ATG, all patients developed an adverse reaction, characterized by rash and fever, which responded to treatment with IV steroids; in 1 patient, 3 doses of ATG, instead of 4, were administered because of severe adverse reactions, namely hypotension and circulatory insufficiency. Of the 22 patients, 2 died after developing MAS early during the followup, and 2 other patients died later during the followup following reinstitution of immunosuppressive treatment after prior treatment failure (Table 1 and Figure 1). The post-ASCT followup period for the surviving children (n = 18) ranged from 52 months to 104 months (median 80 months). At the time point of evaluation for the present study, October 1, 2005, 15 (83%) of 18 children had completed a followup of ≥5 years.

The probability of overall survival of the whole population of 22 patients with JIA at 5 years was 82% (Figure 2), and the probability of disease-free survival, censored for relapse and death as events, was 36% (Figure 2). Eight patients (36%), including 2 who failed to respond to anti-TNFα therapy prior to ASCT, reached complete remission and, after a median followup of 80 months, remained disease-free without antirheumatic medication. Yearly radiographic examinations of the joints of these patients continue to indicate that the progression of joint destruction has been arrested (results not shown).

Two children died from MAS, which occurred at 18 days post-ASCT in 1 patient and 4 months post-ASCT in the other patient, and the deaths were associated with *Staphylococcus epidermidis* bacteremia and Epstein-Barr virus (EBV) reactivation (25); these patients were therefore not evaluable for the study of disease course after ASCT. Of the 20 evaluable patients, 12 experienced a relapse of disease, i.e., 11 had a relapse between 2 months and 16 months after ASCT and 1 had...
a very late relapse, at 7 years after ASCT (Figure 1). Second-line antirheumatic therapy (low-dose corticosteroids or DMARDs [MTX, cyclosporin A, etanercept]) was reinstituted in all patients whose disease relapsed, resulting in a positive treatment response in 7 patients, who showed 50% improvement (n = 1) or 70% improvement (n = 6) in disease activity according to the ACR response criteria; these patients were categorized as partial responders.

Five of the patients who experienced relapse had progressive disease despite receiving antirheumatic treatment; these patients were categorized as having failed treatment. In 4 of these patients, the relapse occurred within the first year after ASCT and was preceded by a viral infection. The fifth patient who had treatment-resistant progressive disease after relapse had remained in complete drug-free remission for 7 years after undergoing ASCT; at the age of 14 years, during
pubertal changes, she developed a relapse that was nonresponsive to conventional treatment.

Two of the patients whose disease relapsed died, at 13 months post-ASCT in 1 patient and at 16 months post-ASCT in the other patient, as a result of complications of EBV and varicella zoster virus (VZV) infections that developed while being treated with intensive immunosuppression for the relapse (Table 1), as has been described elsewhere (20). The other 3 patients who experienced a relapse continue to have ongoing progressive disease. One of these patients requires the use of a wheelchair because of progressive destruction of the joints. The ongoing disease in these 3 patients is as refractory to DMARDs and other antirheumatic drugs as it was before ASCT. Allogeneic stem cell transplantation is planned as a treatment option for 1 of these children.

The core set of outcome parameters before and after ASCT are shown in Figure 3. No correlation was found between either the numbers of T cells reinfused or the numbers of circulating T cells at 3 months after ASCT and any of the clinical outcomes (results not shown).

Recovery of hematologic and immune system parameters. The absolute neutrophil counts in peripheral blood exceeded 500/µl between 12 days and 30 days after ASCT (median 20 days), and the absolute platelet counts exceeded 20,000/µl without transfusions between 3 days and 28 days after ASCT (median 19 days). Hemoglobin levels normalized within 3 months after transplantation. A median of 2.5 (range 0–6) red blood cell transfusions and 2.7 (range 0–7) platelet transfusions per patient were administered during the aplastic phase. Substitution with IV immunoglobulins (150 mg/kg per week) was provided to prevent infections in 5 patients during the first 3–6 months post-ASCT.

Figure 4 shows the individual counts and the median numbers of T lymphocytes, B lymphocytes, and T cell subsets following ASCT. The absolute numbers of CD3+ lymphocytes normalized (>800/µl) between 3 months and 24 months post-ASCT, and, after a median of 6 months (range 4–12 months), the in vitro proliferative responsiveness of PBLs returned to normal levels following stimulation with phytohemagglutinin (results not shown). The median absolute numbers of CD3−,CD16+/CD56+ NK cells had already reached normal range (>100/µl) at 3 months post-ASCT and remained at normal levels during followup (results not shown). The numbers of CD19+ B cells reached the 5th percentile of age-matched reference values (>200/µl) within 3–6 months after ASCT. The absolute numbers of CD8+ lymphocytes expanded between 3 months and 12 months after ASCT. The CD4+:CD8+ ratios remained inverted post-ASCT up to 12 months in 6 of 19 patients.

Long-term followup of the changes in T cell subsets showed a prolonged and profound lymphopenia of naive CD4+,CD45RA− cells (Figure 5). Almost all of the CD4+ and CD8+ cells detectable during the first 6 months post-ASCT were of the CD45RO+/CD45RA− memory type. After ASCT, from 12 months onward, the absolute numbers of CD3+ cells (P = 0.11), CD8+ cells (P = 0.64), CD3−,CD16+/CD56+ cells
(\(P = 0.20\)), CD4⁺,CD45RO⁺ cells (\(P = 0.23\)), and CD8⁺,CD45RA⁺ cells (\(P = 0.08\)) were not significantly different from the absolute numbers prior to ASCT. In contrast, the absolute numbers of CD3⁺,CD4⁺ cells (\(P = 0.05\)), CD4⁺,CD45RA⁺ cells (\(P = 0.01\)), CD8⁺,CD45RO⁺ cells (\(P = 0.04\)), and CD19⁺ cells (\(P = 0.03\)) were still lower at the 12-month post-ASCT time point than the absolute numbers prior to ASCT.

**ASCT-related complications.** All children who developed fever and malaise following ATG infusion showed improvement after treatment with steroids. Nausea, vomiting, and alopecia occurred in almost all of the patients during the conditioning regimen and early after ASCT. One patient developed phlebitis and thrombosis of the vena subclavia, which was related to a deep-indwelling IV catheter. Morbidity, for the most part, was due to infections, which mostly occurred early after ASCT, but in some patients occurred later after ASCT (Table 2).

<table>
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<tr>
<th>Patient</th>
<th>Infection</th>
<th>Months after ASCT</th>
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<tbody>
<tr>
<td>1</td>
<td>VZV, skin</td>
<td>&lt;3</td>
</tr>
<tr>
<td>2</td>
<td>CNS, blood; VZV, skin</td>
<td>&lt;3</td>
</tr>
<tr>
<td>3</td>
<td>Xanthomonas maltophilia, stool; HSV, hepatitis</td>
<td>&lt;3</td>
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<tr>
<td>4</td>
<td>Streptococcus species, blood; VZV, skin</td>
<td>&lt;3</td>
</tr>
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<td>5</td>
<td>VZV, skin; CMV reactivation</td>
<td>&lt;3</td>
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<td>6</td>
<td>Streptococcus mitis, blood; generalized VZV; primo CMV; Candida esophagitis</td>
<td>&lt;3</td>
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<td>7</td>
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<td>8</td>
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<td>CMV reactivation; EBV (MAS); Candida esophagitis</td>
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<td>VZV, skin; hepatitis A; adenovirus type 3, feces</td>
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<td>12</td>
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<tr>
<td>22</td>
<td>None</td>
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* ASCT = autologous stem cell transplantation; VZV = varicella zoster virus; CNS = coagulase-negative staphylococcus; HSV = herpes simplex virus; CMV = cytomegalovirus; EBV = Epstein-Barr virus; MAS = macrophage activation syndrome.
observed among the patients. Nine patients developed VZV infection between 1 month and 12 months after ASCT, 4 patients experienced cytomegalovirus (CMV) reactivation, and 1 patient had primo CMV infection. All infections responded well to IV antiviral medication. In addition, some unusual pathogens were identified as the cause of infection, i.e., 1 patient had a cutaneous infection with Mycobacterium szulgai at 6 months after ASCT. As described earlier, 2 patients died after developing MAS, which was preceded in 1 patient by an EBV infection and in the other patient by a bacterial infection (19,25).

**DISCUSSION**

Direct evidence of a pivotal role of T cells in the immunopathogenesis of JIA is lacking. An exogenous eliciting antigen is not known, and an association with the major histocompatibility complex either has not been demonstrated (in systemic JIA) or has been observed to be weak (in polyarticular JIA) (37). There is, however, recent indirect evidence to suggest an imbalance between activated CD4+ Th1 cells and (suppressive) regulatory CD4+ Treg cells, which may provoke and maintain an autoinflammatory process, mainly targeting the synovium with the help of macrophages and fibroblasts; the synovial CD4+ T cells express activation markers, secrete Th1-type cytokines, and have a restricted heterogeneity of their T cell receptors (8,11). Moreover, the observation that, in cases of uncontrollable progressive JIA the disease is terminated by extinction in a crippled state, indicates that a mimicry autoantigen is involved in the autoimmune process.

The aim of this phase II intervention study was, therefore, to eradicate the dominating autoaggressive T cells and to allow the stem cells to repopulate the T cell compartment in a normal autotolerant way. This working hypothesis is supported by the finding that in normal ontogeny, the development of regulatory T cells predominates over that of activated effector T cells (38–40). In a study by de Kleer et al, investigators showed that ASCT in JIA patients resulted in the predominance of tolerizing autoreactive T cells and restoration of the CD4+CD25+ immunoregulatory network in JIA (13).

Results from (pseudo)ASCT in animal models of experimental autoimmune encephalomyelitis and adjuvant-induced arthritis (14,41) have suggested that, under certain conditions, similar outcomes for these autoimmune diseases may be obtained after ASCT in humans. The major prerequisite conditions were sufficient immunosuppressive pretreatment, ablation of (autoreactive) T cell memory in the host, and elimination to a certain threshold of mature T cells in the graft, in order to avoid reinfusion of a large number of autoreactive T cells (9,42).

In the present study, we have demonstrated that by translating these conditions of ASCT as a treatment approach in children with JIA, a sustained complete remission or marked improvement was achieved in 15 (68%) of the 22 patients. A stable clinical condition was obtained within 3–12 months after ASCT and the clinical parameters remained stable during followup of at least 4 years (from 52 months to 104 months). Only 1 patient (patient 1 in Table 1) had a very late relapse, beginning at the onset of puberty, indicating that the restored balance between autoaggression and autotolerance may be disturbed in favor of the former, via a change in the levels of sex steroids (43) in genetically susceptible individuals. Of the 5 children who experienced a relapse and had progressive disease, 2 died at a later date (at 13 months and 16 months post-ASCT) following reinitiation of immunomodulating treatment, and the other 3 patients have ongoing progressive disease that remains refractory to DMARDs and other antirheumatic drugs, similar to their condition before ASCT.

As can be seen in Figures 4 and 5, pretreatment of the patients resulted in a severe and prolonged suppression of PBL populations, especially of CD4+CD45RA+ naive T cells. Numerically, the pattern and duration of depression of T cell subsets were not different from that seen after ASCT in patients with cancer (44,45). However, functionally, the immunocompromised condition in patients with JIA was severe. The large number of proven infections in the early post-ASCT period made this manifest (Table 2).

Immunoaablation was clearly the purpose of the pretreatment, in order to eradicate autoimmunologic memory. Whether this goal was achieved can only be investigated indirectly, by testing the immune response to vaccine antigens administered before harvesting of the graft and conditioning. This was done in a separate study, using the T cell–dependent rabies vaccine as a neoantigen and tetanus toxoid as a recall antigen, and by investigating the B cell response as the outcome. It could be shown that the expected memory response to a post-ASCT booster injection was abrogated (46). Unfortunately, the period of severe (T cell) immunologic amnesia, as demonstrated by reactivation of herpes viruses and loss of memory for vaccination antigens, was also connected with fatal MAS, which was observed among the patients. Nine patients developed VZV infection between 1 month and 12 months after ASCT, 4 patients experienced cytomegalovirus (CMV) reactivation, and 1 patient had primo CMV infection. All infections responded well to IV antiviral medication. In addition, some unusual pathogens were identified as the cause of infection, i.e., 1 patient had a cutaneous infection with Mycobacterium szulgai at 6 months after ASCT. As described earlier, 2 patients died after developing MAS, which was preceded in 1 patient by an EBV infection and in the other patient by a bacterial infection (19,25).

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Development of MAS leading to death has previously been observed by us (19,25) and by other investigators (47). However, this outcome has been observed only in patients with JIA whose ASCT involved a >3-log T cell–depleted graft, and has only been observed in association with an infection. As a consequence of these observations, the following precautions were taken in implementing this protocol from 1999 and thereafter: postponement of the pretreatment in those patients with systemic JIA who showed systemic features of active disease, preservation of at least $1 \times 10^7$/kg T cells in the graft, and virologic monitoring with reverse transcription–polymerase chain reaction methods starting at the time of admission until sufficient recovery of specific immunity. Prophylaxis with (val)aciclovir in each patient may be considered, but this approach confers only a restricted antiviral coverage. From 1999 and thereafter, 11 children (10 with systemic JIA) were treated with ASCT according to the amended protocol, with the result that no further cases of MAS occurred.

In further analyses in the present study, no correlation was found between the numbers of T cells reinfused and the clinical outcome. To date, contradictory results have been reported regarding the clinical outcome after ASCT in relation to the degree of T cell depletion (48–50). There have been various discussions regarding the optimal conditioning regimen for children with JIA. It is a matter of debate whether to include low-dose total body irradiation in the pretreatment strategy. The arguments for doing so are the potential for significantly less relapses, as was observed after (pseudo)ASCT in an adjuvant-induced arthritis model in animals (14), and our former observations of improved outcomes after use of exactly the same conditioning regimen in transfusion-sensitized children with severe aplastic anemia (28), in whom the aim was to increase immunosuppression for the prevention of allograft rejection. The known long-term sequela associated with this regimen, most notably growth dysfunction, was not observed in our cohort of patients with severe aplastic anemia, although the numbers of such patients were small. Of the 5 patients with severe aplastic anemia who received conditioning with total body irradiation and who had reached their final height, 4 had no impaired growth, while 1 showed a decrease in the standard deviation score (SDS) of the final height (1.3 SDS) after ASCT but the final length remained within the target height (TH) range (−1.1 SDS under TH) (Bakker B: personal communication). No secondary malignancy was observed in a total of 9 (of 10) patients who were long-term survivors and who were pretreated with the same conditioning regimen for allogeneic stem cell transplantation before 2000 (median followup 13 years).

In contrast to the findings in our patients, stem cell transplantation pretreatment with only cyclophosphamide was associated with an increasing frequency of secondary malignancies in patients over a long-term followup (51,52). Preliminary results with alternative pretreatment regimens for ASCT in JIA patients have, however, been disappointing. In prospective studies from the EBMT, all 3 patients with JIA who have been described in the literature thus far had an early relapse after conditioning with cyclophosphamide and ATG, and among patients with JIA who were pretreated with fludarabine, cyclophosphamide, and ATG, 3 developed MAS shortly after undergoing ASCT (53,54). The combination of fludarabine and cyclophosphamide together with high-dose interleukin-2 (IL-2) for adoptive cell transfer therapy to treat refractory metastatic melanoma has also resulted in autoimmune phenomena such as vitiligo and uveitis (55), as well as many opportunistic infections, illustrating the potential for severe dysregulation and suppression of T cell immunity with such a pretreatment regimen.

In view of the recent progress in the development of new drugs, such as anti–IL-1 and anti–IL-6, for the treatment of JIA, a more restricted selection of candidate patients for ASCT may be expected. This study was started prior to the availability of biologic agents. Biologic therapy has thus far been established as a safe and effective approach to treat refractory JIA (2,56). However, there will remain a number of treatment failures, and therefore the potential for use of ASCT will now be limited to patients in whom prior treatment has failed. The main challenge that remains is the need for identification of patients whose disease is refractory to conventional treatment at an early stage of disease, before tissue destruction has taken place, since these patients would benefit the most from ASCT. When such an indication is present, the type of ASCT procedure that we have described herein is justified. As we have shown, this approach offers a fair chance of long-lasting improvement in patients with severe progressive disease.

**ACKNOWLEDGMENT**

We thank Professor D. W. van Bekkum for his expert advice.
**AUTHOR CONTRIBUTIONS**

Dr. Brinkman had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study design.** Brinkman, de Kleer, ten Cate, van Rossum, Kuis, Wulffraat, Vossen.

**Acquisition of data.** Brinkman, de Kleer, ten Cate, van Rossum, Bekkering, Fasth, van Tol, Kuis, Wulffraat, Vossen.

**Analysis and interpretation of data.** Brinkman, ten Cate, van Tol, Wulffraat, Vossen.

**Manuscript preparation.** Brinkman, ten Cate, van Tol, Vossen.

**Statistical analysis.** Brinkman.

**REFERENCES**


