High-Dose Chemotherapy and Autologous Hematopoietic Stem Cell Transplantation in Patients With Rheumatoid Arthritis

Results of an Open Study to Assess Feasibility, Safety, and Efficacy

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Objective. To assess the feasibility, safety, and efficacy of high-dose chemotherapy and autologous hematopoietic stem cell transplantation (HSCT) in patients with severe, refractory rheumatoid arthritis (RA).

Methods. Fourteen patients (3 male, 11 female, mean age 43 years, mean disease duration 10 years) with active, destructive, refractory RA entered the study. Autologous hematopoietic stem cells were collected by leukapheresis after mobilization with a single infusion of cyclophosphamide (CYC; 4 gm/m²) and subcutaneous injections of filgrastim (granulocyte colony-stimulating factor). Immunomagnetic selection of CD34+ cells from the leukapheresis products was performed to deplete potentially autoreactive lymphocytes. The conditioning regimen consisted of intravenous administration of high doses of CYC (cumulative dose 200 mg/kg), with subsequent reinfusion of the graft. Patients were monitored for disease activity, disability, adverse effects, and hematopoietic and immunologic reconstitution.

Results. All 14 patients completed the mobilization and leukapheresis procedures successfully, and 12 proceeded to receive conditioning and transplantation. Engraftment occurred in all of these patients, with rapid hematologic recovery. No major unexpected toxicity was observed. Marked improvement of disease activity was recorded in 8 of 12 patients at >50% of the visits, with a followup ranging from 7 months to 21 months. The clinical responders included 2 patients who had previously failed treatment with tumor necrosis factor (TNF) blocking agents.

Conclusion. High-dose chemotherapy followed by autologous HSCT is feasible and safe, and can result in long-term improvement of disease activity in patients whose condition previously did not respond to conventional antirheumatic drugs or TNF blocking agents. The persistence of active disease in some patients may reflect the heterogeneity of the underlying disease process.

A new treatment approach, involving intense immunosuppression and autologous hematopoietic stem cell transplantation (HSCT), has emerged in recent years for the treatment of severe, refractory rheumatoid autoimmune diseases including rheumatoid arthritis (RA) (1–4). The rationale of this strategy is based on the concept of immunoablation by intense immunosuppression, with subsequent regeneration of naive T lymphocytes derived from reinfused hematopoietic progenitor cells (5). In several patients with intractable RA, long-term remissions were observed with this strategy, although failures have been reported as well (6,7). Only small numbers of RA patients have been treated thus far. Although different treatment protocols have been used, high-dose chemotherapy (HDC)
as a means to achieve immunoablation has been invariably used in all studies.

To extend previous findings on selected cases, we conducted an open study to investigate the feasibility, safety, and efficacy of HDC + HSCT in a cohort of patients with therapy-refractory, active, destructive RA. In addition, the extent and duration of immunoablation was assessed, and the relationship between immunologic changes and the clinical responses resulting from a period of intense immunosuppression was examined.

**PATIENTS AND METHODS**

**Patient selection.** This was a multicenter, open-label phase I/II study. The protocol was approved by the ethics committees from the participating institutions. All patients provided written informed consent. Eligibility criteria were as follows: an established diagnosis of RA according to the American College of Rheumatology (ACR; formerly the American Rheumatism Association) criteria (8), progressively erosive disease with large joint involvement, failure to respond to ≥4 second-line drugs including maximal tolerable doses of methotrexate and combination therapy, active disease as defined by ≥6 swollen joints and ≥6 tender joints and ≥1 hour of morning stiffness, and age 18–60 years. Exclusion criteria were as follows: pulmonary impairment that was defined as a total lung capacity, vital lung capacity, or diffusion capacity <70% of predicted values; cardiac impairment that was defined as clinical evidence of heart failure with a left ventricular ejection fraction of <50%; liver disease that was defined as an aspartate aminotransferase (AST) or alanine aminotransferase (ALT) or bilirubin level >2 times the upper limit of normal on 2 repeated tests; renal impairment that was defined as a creatinine clearance rate of <70 ml/minute; a white blood cell count <2.0 × 10^9/liter; a platelet count <100 × 10^9/liter; a hemoglobin level <6.0 mmoles/liter; acute or chronic infection; positive test result for human immunodeficiency virus; concurrent neoplastic disease or evidence of myelodysplasia; uncontrolled systemic hypertension; active peptic ulcer disease; positive pregnancy test result; previous joint arthroplasty; and concomitant therapy with antiplatelet agents.

**Treatment schedule.** Autologous hematopoietic stem cells were mobilized using a single infusion of cyclophosphamide (CYC) at 4 g/m² followed by filgrastim (granulocyte colony-stimulating factor [G-CSF]) at 10 µg/kg/day subcutaneously until leukapheresis. Administration of filgrastim commenced 5 days after the CYC infusion. Patients underwent leukapheresis as soon as the CD34+ blood levels exceeded 20 × 10^6/ml. Leukapheresis was performed on a continuous flow cell separator machine to obtain at least 5 × 10^8 CD34+ cells/kg body weight. Immunomagnetic selection of CD34+ cells from the leukapheresis product was performed using the CliniMACS device (Miltenyi Biotec, Munich, Germany), with the purpose of obtaining a minimum of 2 × 10^8 CD34+ cells/kg and a maximum of 2 × 10^9 CD34+ cells/kg. All disease-modifying antirheumatic drugs (DMARDs) were discontinued before mobilization, and corticosteroids were tapered thereafter when possible. Nonsteroidal antiinflammatory drugs were continued in the lowest dosage needed to control pain and morning stiffness.

The conditioning regimen consisted of CYC at 50 mg/kg/day intravenously for 4 consecutive days (total of 200 mg/kg). Hyperhydration, alkalization of urine, and mesna were given in order to prevent hemorrhagic cystitis. The interval between the last dose of CYC and infusion of the stem cells was at least 48 hours. Following transplantation, patients were nursed in laminar flow rooms. All blood products were irradiated (25 Gy) prior to infusion. Antibiotic decontamination and antiemetic treatment were given according to local practice. The use of corticosteroids as antiemetic therapy was left to the institution’s practice. Patients treated at the Leiden University Medical Center (n = 8) all received methylprednisolone at a dose of 2 mg/kg during conditioning, for 6 consecutive days. In the other institutions (University Medical Center Utrecht and University Medical Center Nijmegen), no steroids were given (n = 4).

**Assessment of toxicity.** Safety was assessed according to the World Health Organization (WHO) toxicity criteria (9). Furthermore, the units of transfused red blood cells and units of transfused platelets, infections, number of days of hospitalization, and rehospitalization records were recorded as well.

**Assessment of efficacy.** The following clinical and laboratory investigations were performed at screening, prior to stem cell mobilization (considered baseline), before conditioning, and every 3 months after transplantation: physical examination, including the swollen joint count (0–66), tender joint count (0–68), and Ritchie articular index (10) (0–78), Health Assessment Questionnaire (HAQ [11]; 0–3), patient’s assessment of pain on a visual analog scale (VAS; 0–10), patient’s assessment of disease activity on a VAS (0–10), and physician’s global assessment of disease activity (0–10). Laboratory measurements were performed at the same time points and included the erythrocyte sedimentation rate, hemoglobin, hematocrit, white blood cell count with differential, platelet count, C-reactive protein level, IgM rheumatoid factor, anti-cyclic citrullinated peptide (anti-CCP), and total serum IgM, IgG, and IgA.

Based on the above-mentioned data, efficacy was determined by the 4-variable Disease Activity Score (DAS) (primary study parameter) (12), the ACR response criteria (13), and the HAQ.

**Flow cytometric detection of cell surface antigens.** Immunophenotyping studies were done on peripheral blood mononuclear cells obtained at baseline (prior to stem cell mobilization), prior to conditioning, and at 3, 6, 9, and 12 months after transplantation. The following combination of markers was used in order to identify different cell types: CD45–fluorescein isothiocyanate (FITC) (Becton Dickinson, San Jose, CA)/CD14–phycoerythrin (PE) (Dako, Glostrup, Denmark), CD3-FITC/CD4-PE (both Becton Dickinson), CD3-FITC/CD8-PE (both Becton Dickinson), CD3-FITC/CD16+CD56-PE (both Becton Dickinson), CD57-PE (both Becton Dickinson), HLA–DR–FITC (Dako)/CD3-PE (Becton Dickinson), CD10-FITC (Dako)/CD20-PE (Becton Dickinson), CD19-FITC (Becton Dickinson)/CD5-PE (Dako), CD45RO-FITC (Dako)/CD4-PE (Becton Dickinson), CD45RA-FITC/CD24-PE (both Becton Dickinson), CD45RA-FITC/CD8-PE (Becton Dickinson), and CD45RA-FITC/CD8-PE (both Becton Dickinson).

**Statistical analysis.** Treatment efficacy was evaluated by testing whether there was a difference in the DAS and HAQ...
scores between baseline and the 3-monthly evaluations after transplantation, using the Wilcoxon signed-rank test. Responders were defined as those patients attaining a good response based on the European League Against Rheumatism (EULAR) response criteria for the DAS (14) at 3 months after transplantation. The Wilcoxon rank-sum (Mann-Whitney U) test was used to determine whether the clinical response was consistent by testing the differences in the HAQ score and the ACR response categories between responders and nonresponders. In order to evaluate the relationship between the extent and duration of immunoablation, the Wilcoxon signed-rank test was used to assess whether there were significant differences in the HAQ score and the ACR response categories between responders and nonresponders. Correlations were calculated by linear regression.

RESULTS

Patient data. Fourteen patients with active, progressively erosive, refractory RA entered the study (mean age 43 years, range 22–55 years, mean disease duration 10 years, range 2–20 years). All patients had received the maximal tolerable dose of methotrexate, and 5 patients had also failed treatment with tumor necrosis factor (TNF) blockade (Table 1). All 14 patients completed the mobilization procedure successfully, and 12 patients proceeded to receive conditioning and transplantation. One patient chose not to proceed to conditioning because of marked improvement of disease activity after mobilization, and another patient was withdrawn from the study when pulmonary embolism was diagnosed before conditioning. Both the median and mean intervals between the first dose of CYC for mobilization and reinfusion of stem cells were 56 days (range 32–90 days).

Baseline parameters of disease activity are summarized in Table 2. All patients had a DAS >3.7 at baseline, which is defined as high disease activity (11).

Graft. Immunomagnetic selection of CD34+ cells from the leukapheresis products was performed to deplete potentially autoreactive cells, using the CliniMACS device (Miltenyi Biotec). After selection, the grafts contained a median of 4.62 $\times 10^6$ CD34+ cells (range 2.77–7.45), corresponding to a median of 6.9 $\times 10^6$ CD34+ cells/kg body weight (range 4.8–11.1). The median number of CD3+ T cells in the graft was <44.0 $\times 10^4$ (range 8–100), corresponding to a log depletion of 3.74 (range 3.0–4.6). The median percentage of CD3+ T cells in the infused product was <0.1% (range 0.03–0.36%).

Toxicity. Nausea, vomiting, and alopecia were observed in all patients. Other treatment-related morbidities occurred in 9 of 12 patients and included thrombosis of the vena subclavia due to an intravenous catheter (1 of 12), hydradenitis (1 of 12), metrorrhagia (1 of 12), and transplantation. One patient chose not to proceed to

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Table 1. Patient characteristics*

<table>
<thead>
<tr>
<th>Patient/sex/age</th>
<th>Disease duration, years</th>
<th>Rheumatoid factor</th>
<th>HLA–DRB1 typing</th>
<th>Previous therapy</th>
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</thead>
<tbody>
<tr>
<td>TX01/F/43</td>
<td>11</td>
<td>+</td>
<td>0301/0301</td>
<td>HCQ, intramuscular gold, SSZ, MTX, AZA, D-Pen, prednisone, CSA, CSA + MTX</td>
</tr>
<tr>
<td>TX02/F/51</td>
<td>7</td>
<td>+</td>
<td>0404/1044</td>
<td>HCQ, oral gold, D-pen, MTX, AZA, SSZ, prednisone, MTX + prednisone</td>
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<tr>
<td>TX03/F/52</td>
<td>15</td>
<td>+</td>
<td>0404/0801</td>
<td>HCQ, CSA, D-pen, intramuscular gold, SSZ, MTX, AZA, HCQ + AZA + prednisone, HCQ + CSA</td>
</tr>
<tr>
<td>TX04/F/52</td>
<td>20</td>
<td>+</td>
<td>0301/0408</td>
<td>HCQ, intramuscular gold, D-pen, prednisone, MTX, SSZ, anti-TNF, MTX + prednisone</td>
</tr>
<tr>
<td>TX05/F/40</td>
<td>17</td>
<td>–</td>
<td>0101/0402</td>
<td>HCQ, SSZ, intramuscular gold, MTX, anti-TNF, AZA, CSA, MTX + SSZ + HCQ</td>
</tr>
<tr>
<td>TX06/F/55</td>
<td>12</td>
<td>+</td>
<td>0404/1101</td>
<td>HCQ, SSZ, prednisone, intramuscular gold, MTX, AZA, CSA, D-Pen, prednisone + MTX + SSZ + HCQ, anti-TNF</td>
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<td>SSZ, prednisone + MTX + intramuscular gold</td>
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<tr>
<td>TX08/F/32</td>
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<td>–</td>
<td>01/0301</td>
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<td>TX09/M/53</td>
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<td>+</td>
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<td>HCQ, gold, MTX, anti-TNF, MTX + CSA</td>
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<tr>
<td>TX10/M/52</td>
<td>4</td>
<td>+</td>
<td>0101/0701</td>
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<td>TX11/F/26</td>
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<td>+</td>
<td>DR4†</td>
<td>SSZ, MTX, intramuscular gold, AZA, anti-TNF, prednisone</td>
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<td>8</td>
<td>+</td>
<td>DR1/DR15†</td>
<td>Oral gold, D-pen, MTX, IL-10, prednisone + HCQ</td>
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<tr>
<td>TX13/F/22</td>
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<td>+</td>
<td>DR1/DR17†</td>
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<tr>
<td>TX14/M/38</td>
<td>5</td>
<td>+</td>
<td>DR1/DR11(5)†‡</td>
<td>MTX, MTX + HCQ</td>
</tr>
</tbody>
</table>

* HCQ = hydroxychloroquine; SSZ = sulfasalazine; MTX = methotrexate; AZA = azathioprine; D-Pen = D-penicillamine; CSA = cyclosporin A; anti-TNF = tumor necrosis factor blocking agents; IL-10 = interleukin-10.
† Serologically determined.
12), herpes zoster (2 of 12), pseudomembranous enterocolitis (1 of 12), pneumothorax (1 of 12), and febrile neutropenia necessitating temporary antibiotic treatment (7 of 12). In 2 of these latter 7 patients, a causative microorganism was isolated. The total duration of hospitalization (including mobilization) was 33 days (range 25–53). The median number of hospitalization days due to fever was 5 (range 5–20) in 8 patients. Three patients experienced WHO grade 3 toxicity, of whom 2 had intolerable diarrhea requiring therapy and 1 had elevations in the levels of bilirubin and AST and ALT. Mortality did not occur.

**Engraftment.** Engraftment occurred in all patients, with rapid hematologic recovery. The median duration of neutropenia (defined as <0.5 × 10^9 neutrophils/liter) was 12 days (range 8–17 days), and the median duration of a platelet count <20 × 10^9/liter was 3 days (range 0–5). The average number of units of transfused red blood cells, including transfusions after mobilization, was 5.3 (range 0–9). The average number of units of platelet transfusions was 2.7 (range 0–5). The duration of neutropenia correlated with the age of the patient (r = 0.65, P = 0.023). The mean amount of CYC used (including mobilization) was 19,896 mg, with a median of 19,350 mg (range 15,520–25,200 mg).

**Clinical efficacy.** Followup of patients ranged from 7 to 21 months. Figure 1 shows the course of the mean DAS in the 12 patients. There was a significant decrease in the DAS after administration of HDC + HSCT at 3 (P = 0.005), 6 (P = 0.003), 9 (P = 0.011), and 12 (P = 0.018) months. At 3 months posttransplantation, 6 patients fulfilled the criteria for good response based on the EULAR response criteria for the DAS. The other patients subsequently had either no response (n = 5) or a moderate response (n = 1).

Patients were divided into responders (good response) and nonresponders (moderate and no response). The former group of patients (n = 6) all had a DAS <2.4 (low disease activity), while the latter group (n = 6) all had a DAS >3.7 (high disease activity) at 3 months after transplantation (Figure 2). There was a clear difference in the DAS between patients who had a subsequent favorable disease course and those who did not. The difference in DAS between the 2 groups was statistically significant at 3 (P = 0.004), 6 (P = 0.029), and 9 (P = 0.014) months. There were no statistically significant differences in the DAS between responders and nonresponders after mobilization (P = 0.144). At the 3-month evaluation, no patient was receiving DMARDs.

These results were similar when the ACR criteria for response were taken to determine clinical efficacy (responders satisfied the ACR 50% response criteria at 3 months and at consecutive months of followup). Clinical efficacy according to the 20%, 50%, and 70% ACR response criteria was found at a frequency of 8 of 12, 6 of

### Table 2. Baseline characteristics of disease activity in the 14 study patients*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swollen joint count (0–66)</td>
<td>24</td>
<td>7–39</td>
</tr>
<tr>
<td>Tender joint count (0–68)</td>
<td>25</td>
<td>11–49</td>
</tr>
<tr>
<td>VAS pain</td>
<td>6.6</td>
<td>2.2–9.7</td>
</tr>
<tr>
<td>VAS disease activity</td>
<td>6.2</td>
<td>2.0–8.4</td>
</tr>
<tr>
<td>HAQ</td>
<td>1.80</td>
<td>1–2.5</td>
</tr>
<tr>
<td>ESR, mm/hour</td>
<td>56</td>
<td>12–100</td>
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<tr>
<td>CRP, mg/dl</td>
<td>59</td>
<td>6–129</td>
</tr>
<tr>
<td>DAS</td>
<td>5.39</td>
<td>3.82–7.24</td>
</tr>
</tbody>
</table>

* VAS = visual analog scale; HAQ = Health Assessment Questionnaire; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; DAS = Disease Activity Score.
12, and 3 of 12 at 3 months, 8 of 12, 7 of 12, and 2 of 12 at 6 months, 5 of 8, 5 of 8, and 2 of 8 at 12 months, and 3 of 4, 3 of 4, and 2 of 4 at 15 months, respectively (Table 3). Two patients fulfilled the DAS criterion for remission (DAS1.6) (15) at 3 months, 3 patients at 6 months, and 1 at 12 months. Responders included 2 patients who had failed treatment with TNF blockade.

In 7 of 12 patients, DMARDs were reinstated (minimum of 3 months after transplantation) because of signs of active disease, resulting in amelioration of disease activity in 3 of the 7 patients (2 patients received leflunomide, 1 patient was given methotrexate). The mean duration of the DMARD-free period after transplantation was 130 days, with a median of 105 days (range 99–204). Of the 7 patients who received DMARDs, 1 patient was a responder at 3 months, whereas 6 patients did not respond. The responder experienced an early relapse of disease activity 1 month after transplantation (DAS 7.41), which spontaneously declined (to 2.38). However, signs of disease activity were still apparent. Upon reinstitution of methotrexate, the DAS decreased to 1.82. Of the 6 nonresponders, 2 responded favorably on reinstatement of DMARDs, from a DAS of 4.83 to 2.82 and from 5.10 to 2.64, respectively. The 4 patients who failed to respond upon reinstatement of DMARDs had a DAS ranging from 4.42 to 6.35 at 6–12 months after transplantation, which was at least 3 months after the start of DMARDs.

The followup of the mean HAQ score showed statistically significant differences between baseline and 6 months (P = 0.005) and 12 months (P = 0.022) (Figure 3). Figure 4 shows the mean HAQ score of responders and nonresponders. A statistically significant difference was found at 3 months (P = 0.005).

**Laboratory measurements.** Differences between the baseline and 3-monthly measurements of laboratory parameters were assessed. An overall decrease in the titer of IgM rheumatoid factor was observed, which was statistically significant at 3 (P = 0.041), 6 (P = 0.011), and 9 (P = 0.046) months. The same was found for the levels of anti-CCP, an antibody directed toward citrullinated peptides and specific for patients with RA (16), at 3 and 6 months (P = 0.028 and P = 0.041), respectively, versus baseline. The relationship between clinical response and immunologic changes was evaluated by testing whether laboratory parameters differed between responders and nonresponders. No correlation was found between either IgM rheumatoid factor or anti-CCP levels and responders or nonresponders at baseline or after transplantation. However, patients with a good clinical response had a significantly higher level of total IgG in peripheral blood than did nonresponders at baseline (mean 15.0 gm/liter versus 9.03 gm/liter, P = 0.004) and after 3 months (mean 11.39 gm/liter versus 7.85 gm/liter, P = 0.025). Furthermore, there was a significant decrease in total IgG in the responder group after 3 months (mean 15.0 gm/liter versus 11.39 gm/liter; P = 0.028).

**Flow cytometry.** Immunophenotyping of peripheral blood mononuclear cells showed prolonged (>6 months) depletion of CD45RA+ T cells after transplan-

### Table 3. Patients fulfilling the American College of Rheumatology criteria for improvement at 3, 6, 12, and 15 months of followup*

<table>
<thead>
<tr>
<th>Criteria</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
<th>15 months</th>
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<tbody>
<tr>
<td>70%</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>50%</td>
<td>6</td>
<td>7</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>20%</td>
<td>8</td>
<td>8</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>No response</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

* Values are the no. of patients.

Figure 3. Mean Health Assessment Questionnaire (HAQ) score (range 0–3). * = significant change from baseline (P < 0.05). Bars show the mean ± SEM.

Figure 4. Mean HAQ score of responders (AVG resp) and nonresponders (AVG non-resp). Responders had a DAS <2.4 and an ACR >50% response, and nonresponders had a DAS >3.7 and an ACR <50% response at 3 months. Bars show the mean ± SEM. * = significant difference between responders and nonresponders (P < 0.05). See Figures 1–3 for definitions.
from baseline (P

stead of myeloablation, and CD34 selection of the graft therapeutic agent, CYC, aimed at lymphoablation opted for a treatment regimen based on a single chemotherapy, whereas levels of CD8 \( ^+ \) cells, CD19 \( ^+ \) cells, CD14 \( ^+ \) cells, and CD3 \( ^- \), CD16 \( ^+ \)/CD56 \( ^+ \) cells quickly recovered. Levels of circulating CD8 \( P = 0.5 \), CD19 \( P = 0.69 \), CD14 \( P = 0.14 \), and CD3 \( ^- \), CD16 \( ^+ \)/CD56 \( ^+ \) \( P = 0.225 \) cells at 1 year were not significantly different statistically from baseline levels. The decreases in levels of circulating CD3 \( ^+ \) cells, naive CD4 \( ^+ \) cells (CD4 \( ^+ \), CD45RA \( ^+ \)), and memory CD4 \( ^+ \) cells (CD4 \( ^+ \), CD45RO \( ^+ \)) were statistically significant at 6, 9 and 12 months \( P \leq 0.043 \), 3, 6, 9, and 12 months \( P \leq 0.028 \), and 3, 6, and 12 months \( P \leq 0.028 \), respectively (Figure 5).

**DISCUSSION**

Fourteen patients with intractable RA were enrolled in an open, phase I/II study on the clinical and immunologic effects of HDC and autologous HSCT. Twelve patients completed the consecutive treatment steps of 1) mobilization of autologous hematopoietic stem cells with a single intravenous dose of CYC (4 gm/m\(^2\)) and subsequent subcutaneous G-CSF injections, 2) leukapheresis with ex vivo manipulation of the graft, 3) conditioning with CYC (200 mg/kg), and 4) autologous HSCT. One patient chose not to proceed to conditioning because of marked improvement of disease activity after mobilization. In another patient, pulmonary embolism was diagnosed when he was admitted to undergo conditioning; because of the absence of a temporal relationship, it was thought to be unrelated to the treatment protocol.

The treatment protocol was designed to combine practicality, safety, and efficacy. For these reasons, we opted for a treatment regimen based on a single chemotherapeutic agent, CYC, aimed at lymphoablation instead of myeloablation, and CD34 selection of the graft to diminish the putative risk of reinfusing autoreactive or pathogeneic lymphocytes. CYC was added to the mobilization not only to enhance the yield of progenitor cells, but also to limit the risk of disease flare following G-CSF administration. We also chose not to add any posttransplant immunosuppressive agent routinely, to avoid interference with the interpretation of clinical sequelae; instead, a wait-and-watch policy was adopted.

From a technical viewpoint, the treatment steps appeared feasible in all patients. The consecutive procedures of the treatment were well tolerated by most patients. No unexpected major toxicity or treatment-related mortality occurred, although in several patients, infectious complications necessitated extra hospital admissions for parenteral antibiotic treatment.

Hematologic recovery was uneventful in all patients, showing an inverse relationship with the age of the patient. Long-lasting lymphopenia was observed, which could mainly be attributed to slow recovery of naive CD4 \( ^+ \) T lymphocytes. With respect to efficacy, mobilization resulted in transient amelioration of disease activity in 5 of 14 patients (defined as an ACR 20\% response before conditioning), which was reinforced by the intensification of conditioning and transplantation procedures. In 8 of 12 patients, clinically meaningful improvements (defined as a good response according to the EULAR response criteria) were recorded in >50\% of the followup visits. In 7 patients, treatment with a DMARD (methotrexate in 2, leflunomide in 5) was reinstituted because of relapse or persistent disease activity. This resulted in subsequent improvement in 3 of the 7 patients. Interestingly, the disease in 2 patients had been refractory to these drugs (even in higher doses) before transplantation, suggesting that some degree of sensitivity to conventional drugs had been regained, which has been observed by others as well (4). Nevertheless, 4 of 12 patients failed to improve.

The individual clinical response at 3 months was found to be predictive of the subsequent disease course. These disease courses displayed a dichotomous pattern, enabling categorization into responders and nonresponders. Nonresponders did not differ from responders with respect to disease or patient-related variables, such as age, disease activity and duration, previous therapy, or presence of rheumatoid factor, although the numbers of patients may have been too low to detect such predictive factors. The observation of clearly divergent disease courses in the patients treated could reflect the heterogeneity of the disease processes. Indeed, the observed differences in total IgG at baseline and after therapy could be indicative of this.

The clinical results of our study are consistent...
with those reported previously from single-center pilot studies in which different regimens were used. Long-term remissions as well as relapses and progressive disease have been reported with the various regimens used, both in RA and in other autoimmune diseases. Our data do not allow definitive conclusions on whether the immunologic effects of the treatment are only quantitive (“debulking” of inflammatory load) or qualitative as well (e.g., tolerization of pathogenic T lymphocytes). From a T cell–centered perspective, it might be inferred from the present study that not all pathogenic T lymphocytes were eradicated or that some had been reinfused with the graft. In fact, such mechanisms clearly can be operative after intense immunosuppression and autologous stem cell transplantation (17). This would imply that remissions can only be achieved by further intensification, e.g., by in vivo T cell depletion. Clearly, this could add to the toxicity. The same would be anticipated when myeloablative regimens in combination with either autologous or allogeneic stem cell transplantation are employed.

From the patient’s and treating physician’s perspective, responses were clinically meaningful in a number of patients, with resultant enhanced quality of life (results not shown). It remains to be proven that any superior efficacy of a more rigorous approach would compensate for increased toxicity in terms of quality-adjusted life expectancy (18). Adequate assessment of risk/benefit requires properly designed and conducted prospective, randomized controlled trials with a long followup. Such studies will need to be multicenter, given the low expected rate of recruitment of patients due to the recent introduction of effective, less toxic antirheumatic therapies such as anti-TNF blocking agents and leflunomide. The key issue to be addressed, in our opinion, is whether a brief period of intense immune suppression aimed at immunoa blation is superior to continuous, moderate immune suppression in terms of safety, tolerability, and efficacy, and whether these effects can be maintained during a longer followup.

ACKNOWLEDGMENTS

We would like to acknowledge E. W. N. Levahrt for measuring titers of IgM rheumatoid factor, H. C. Kluij-Nelenmans for the immunophenotyping of peripheral blood cells, and M. Frolich for measuring levels of immunoglobulin isotypes.

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