Cyclophosphamide plus granulocyte colony stimulating factor (G-CSF) is more effective than G-CSF alone in mobilizing hematopoietic progenitor cells in severe, refractory rheumatoid arthritis.

In seven patients with rheumatoid arthritis, candidates for autologous hematopoietic stem cell transplantation, cyclophosphamide (4 g/m²) plus G-CSF proved to be more effective than G-CSF alone for stem cell mobilization allowing ex vivo manipulations aimed at reducing T-cells in the graft. Furthermore, cyclophosphamide induced a significant improvement in arthritis, lasting 1 to 19+ months.

Autologous hematopoietic stem cell transplantation (AH SCT) has been proposed as a treatment for severe, refractory rheumatoid arthritis (RA).1 Although it needs to be clarified by controlled studies like this one, AH SCT with ex vivo T-cell depletion might be more effective than an unmanipulated graft reducing the reinfusion of autoreactive T-cells.2 Peripheral blood stem cells are now replacing bone marrow cells as a source of hematopoietic progenitors. Release of a large number of hematopoietic stem cells as required for engraftment can be obtained by granulocyte colony-stimulating factor (G-CSF) either alone or in combination with chemotherapy.2,3 In patients with malignancies, mobilization with cyclophosphamide and G-CSF allows the collection of a larger number of stem cells than G-CSF alone.2 This may be useful when T-cell depletion is planned, as ex vivo graft manipulation is associated with a reduction of stem cell content.4 Previous studies have looked at the combination of cyclophosphamide plus G-CSF in RA,1,5 however direct comparisons between combination treatment and G-CSF alone have not been formally undertaken.

After signing informed consent, seven patients with severe RA refractory to conventional therapy were enrolled in a clinical trial approved by the ethical committee of the Maugeri Foundation Institute for Clinical Research. Bone marrow examination was normal in all cases; one patient (#6) had developed stage I non-Hodgkin’s lymphoma (treated with surgery and radiotherapy) 3 years before this study. Three patients (#1-3) received I.V. cyclophosphamide (4 g/m²) followed by lenograstim 10 µg/kg/d starting from day 4 until stem cell collection. Four patients (#4-7), matched for sex, age, disease activity and duration, received lenograstim 10 µg/kg/d until stem cell collection. CD34+ cell count in the peripheral blood was monitored daily by FACSscalibur (Becton Dickinson Immunocytometry Systems, San José, CA, USA). Positive immunoselection was performed using a Isolex 300i cell separator device (Baxter Health Care, Deerfield, IL, USA). Since the number of CD34+ cells required for safe hematopoietic recovery is ≥2.5x10^6/kg and considering that immunoselection may cause a loss of hematopoietic progenitors, this procedure was performed only in case of harvesting ≥5x10^6 CD34+ cells/kg. An aliquot of the CD34+ cells exceeding 5x10^6/kg was stored unselected as a back-up graft.

Stem cell mobilization was highly effective in patients receiving cyclophosphamide plus growth factor as compared to G-CSF alone, so that CD34+ cell immunoselection could be performed only in the former patients (Table 1). No patient experienced flares of the disease; neutropenic fever was observed in patient #3 but no infections or bleeding were documented and no patient required blood transfusions. Patients who received cyclophosphamide had complete hair loss lasting 3 months. The clinical outcomes of RA following mobilization are shown in Table 2 according to the core set criteria of the American College of Rheumatology.6

The number of CD34+ cells in the peripheral blood of RA patients decreased after cyclophosphamide with partial T-cell depletion was performed only in case of harvesting at least 5x10^6 CD34+ cells/kg.

### Table 1. Stem cell mobilization, collection and immunoselection in seven patients with severe, refractory rheumatoid arthritis following stem cell mobilization allowing ex vivo manipulations aimed at reducing T-cells in the graft.

<table>
<thead>
<tr>
<th>Pts.</th>
<th>Sex/ Age (years)</th>
<th>Disease duration (years)</th>
<th>Circulating CD34+ cells (peak value/µL)</th>
<th>CD34+ cells collected (x10^6/kg)</th>
<th>CD34+ cells stored as unselected graft (x10^6/kg)</th>
<th>CD34+ cells stored after selection (x10^6/kg)</th>
<th>T-cells Log of graft manipulation</th>
<th>Assessment by pts.</th>
<th>Assessment by phys.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M/58 (7.2)</td>
<td>2.5</td>
<td>512</td>
<td>28.5</td>
<td>2.0</td>
<td>1.5</td>
<td>3.5</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>2</td>
<td>M/47 (4.5)</td>
<td>1.5</td>
<td>402</td>
<td>21.8</td>
<td>1.5</td>
<td>1.2</td>
<td>3.4</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>M/55 (10)</td>
<td>2.5</td>
<td>354</td>
<td>13.6</td>
<td>1.6</td>
<td>1.2</td>
<td>3.8</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>4</td>
<td>M/49 (7.7)</td>
<td>2.5</td>
<td>74</td>
<td>5.2</td>
<td>1.5</td>
<td>1.2</td>
<td>3.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>5</td>
<td>M/58 (6)</td>
<td>2.5</td>
<td>29</td>
<td>3.3</td>
<td>1.3</td>
<td>1.2</td>
<td>3.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>6</td>
<td>M/56 (5.1)</td>
<td>1.5</td>
<td>11</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td>—</td>
</tr>
<tr>
<td>7</td>
<td>M/53 (3.8)</td>
<td>2.5</td>
<td>37</td>
<td>4.2</td>
<td>3.2</td>
<td>1.2</td>
<td>3.5</td>
<td>2.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

### Table 2. Clinical outcome in patients with severe refractory rheumatoid arthritis following stem cell mobilization. Patients #1-3, who received cyclophosphamide plus G-CSF, experienced a significant clinical improvement lasting 13 months, 19+ months and 1 month, respectively. Patients #4-6, who received G-CSF alone, did not show any change in disease activity. For the assessment questionnaire, CRP= C-reactive protein.
patients after cyclophosphamide + G-CSF mobilization is very high compared with the numbers observed in cancer patients by our group and others. Breban et al recently reported CD34+ cell collections of up to 35 × 10^6/kg in four RA patients treated with 4 g/m^2 cyclophosphamide and 5 µg/kg/d G-CSF. In our study stem cell mobilization was even more marked, probably due to the higher doses of G-CSF.

Mobilization with G-CSF alone, as also reported by Snowden et al., allows the collection of about 3.0-3.5 × 10^6 stem cells/kg hampering any ex vivo graft manipulation. G-CSF appears to be less effective in RA patients than in healthy donors; this is in accordance with the evidence of markedly affected myelopoiesis in advanced RA. Stem cell mobilization by G-CSF alone was unsuccessful in one of our RA patients who had had lymphoma.

At present there is no definite proof that AHSCT may cure RA. High-dose cyclophosphamide is often effective in refractory autoimmune rheumatic diseases, and a recent study has shown that clinical improvement after unmanipulated AHSCT is dependent on the dose of cyclophosphamide used as conditioning regimen. Our results show that 4 g/m^2 cyclophosphamide may induce a sustained clinical improvement in some patients with refractory RA. Recurrence of the disease was found in all 3 cases but in two of them it was successfully controlled by low-dose conventional therapy for 13 and 19+ months.

In conclusion cyclophosphamide plus G-CSF is superior to G-CSF alone for stem cell mobilization in RA. Furthermore, clinical improvement can be obtained in some patients after mobilizing cyclophosphamide therapy, allowing a wait-and-see policy before AHSCT.

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Key words: rheumatoid arthritis, hematopoietic stem cell transplantation, CD34+ cells, cyclophosphamide, granulocyte colony-stimulating factor.

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References

1. Tyndall A, Gratwohl A. Blood and marrow stem cell transplants in autoimmune disease: a consensus report written on behalf of the European League against Rheumatism (EULAR) and the European Group for Blood and Marrow Transplantation (EBMT). Bone Marrow Transplant 1997; 19:643-5.


