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

In seven patients with rheumatoid arthritis, candidates for autologous hemopietic stem cell transplantation, cyclophosphamide (4 g/m$^2$) 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 to19+ months.

Autologous hematopoietic stem cell transplantation (AHSCT) has been proposed as a treatment for severe, refractory rheumatoid arthritis (RA).[1] Although it needs to be clarified by controlled studies in this setting, AHSCT 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.[1,3] In patients with malignancies, mobilization with cyclophosphamide and G-CSF allows the collection of a larger number of stem cells than G-CSF alone.[3] 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/m$^2$) 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 Facscalibur (Becton Dickinson Immunocytometry Systems, San José, CA, USA); when CD34$^+$ cells exceeded 20/µL, stem cell collection was performed by a Cobe Spectra cell separator (Cobe, Denver, CO, 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 $\geq 2.5\times10^6$/kg and considering that immunoselection may cause a loss of hematopoietic progenitors, this procedure was performed only in case of harvesting $\geq 5\times10^6$ CD34$^+$ cells/kg. An aliquot of the CD34$^+$ cells exceeding $5\times10^6$/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

**Table 1. Stem cell mobilization, collection and immunoselection in seven patients with severe, refractory rheumatoid arthritis after cyclophosphamide plus G-CSF (patients #1-3) or G-CSF alone (patients #4-7). CD34$^+$ cell positive selection to obtain partial T-cell depletion was performed only in case of harvesting at least 5x10$^6$ CD34$^+$ cells/kg.**

| Pts. | Sex/ age (years) | Disease duration (years) | Circulating CD34$^+$ cells (peak value/µL) | CD34+ cells collected (x10$^6$/kg) | CD34+ cells stored as unselected graft (x10$^6$/kg) | CD34+ cells stored after selection (x10$^6$/kg) | T-cells after selection (x10$^4$/kg) | Log of T-cell depletion |
|---|---|---|---|---|---|---|---|---|
| 1 | F/ 58 | 7.2 | 512 | 53.5 | 20.1 | 23.5 | 1.38 | 3.5 |
| 2 | F/ 47 | 4.5 | 402 | 51.8 | 17.4 | 15.3 | 1.7 | 3.34 |
| 3 | F/ 55 | 10 | 334 | 31.6 | 11.6 | 14.5 | 0.82 | 3.82 |
| 4 | F/ 49 | 7.7 | 24 | 2.56 | 2.56 | – | – | – |
| 5 | F/ 58 | 8 | 29 | 3.13 | 3.13 | – | – | – |
| 6 | F/ 56 | 5.1 | 11 | – | – | – | – | – |
| 7 | F/ 53 | 3.8 | 37 | 4.22 | 4.22 | – | – | – |

**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.**

| Pt. | Time of assessment | Current therapy | Swollen/tender joint count | HAQ | CRP mg/L | Pain | Assessment by pts. | Assessment by phys. |
|---|---|---|---|---|---|---|---|---|
| 1 | mobilization | Mtx, HCQ, P 15 mg | 10/28 | 2.0 | 140 | 8.7 | 10 | 8.5 |
| | 1 month | HCQ, P 5 mg | 0/2 | 1.5 | 19 | 2.1 | 2.0 | 4.5 |
| | 13 months | HCQ, P 5 mg | 0/2 | 1.5 | 23 | 2.2 | 2.2 | 4.6 |
| | 19 months | Mtx, HCQ, P 10 mg | 10/22 | 1.8 | 98 | 8.9 | 8.5 | 7.5 |
| 2 | mobilization | Mtx, HCQ, CyA 5mg/kg, P10mg | 20/38 | 1.8 | 133 | 7.2 | 9.1 | 6.9 |
| | 1 month | None | 0/7 | 0.6 | 11 | 1.5 | 1.7 | 0.5 |
| | 19 months | CyA 3mg/Kg, P 5mg | 0/7 | 0.6 | 14 | 2.3 | 1.5 | 1.0 |
| 3 | mobilization | Mtx,HCQ,CyA,P10mg | 13/29 | 1.5 | 30 | 4.5 | 5.3 | 5.7 |
| | 1 month | P 5mg | 0/0 | 0 | 2 | 0.7 | 1.1 | 0 |
| | 3 months | CyA, P5mg,IA steroids | 4/6 | 0.6 | 9 | 3.9 | 4.8 | 2.9 |
| | 5 months | Mtx, CyA, P 5mg | 4/14 | 0.8 | 11 | 4.3 | 4.5 | 3.0 |
| | 15 months | Mtx,HCQ,CyA,P10mg | 8/26 | 1.1 | 48 | 6.5 | 7.1 | 4.9 |
| 4 | mobilization | Mtx,HCQ,CyA,P10mg | 24/34 | 2.1 | 172 | 8.1 | 8.2 | 6.8 |
| | 1 month | Mtx,HCQ,CyA,P10mg | 20/29 | 2.0 | 114 | 7.7 | 8.1 | 6.7 |
| 5 | mobilization | Mtx, HCQ, CyA, P 10 mg | 14/23 | 1.8 | 131 | 5.3 | 6.4 | 6.1 |
| | 1 month | Mtx, HCQ, SSZ, P 10 mg | 14/24 | 1.8 | 135 | 6.1 | 6.0 | 6.3 |
| 6 | mobilization | HCQ, SSZ, P 15 mg | 12/25 | 1.6 | 38 | 5.5 | 4.9 | 5.9 |
| | 1 month | HCQ, SSZ, P 15 mg | 12/18 | 1.5 | 44 | 3.8 | 4.5 | 5.5 |
| 7 | mobilization | Mtx, HCQ, CyA, P 10 mg | 15/28 | 1.3 | 147 | 6.6 | 7.5 | 6.0 |
| | 1 month | Mtx, HCQ, CyA, P 10 mg | 16/26 | 1.3 | 113 | 6.3 | 7.5 | 6.2 |

*Pts.= patients; phys.= physicians; Mtx= methotrexate; HCQ= hydroxychloroquine; P=prednisone; CyA=cyclosporin A; SSZ=sulphasalazine; IA=intra-articular; HAQ=health 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[7] and others.[3] Breban et al.[5] recently reported CD34+ cell collections of up to $35\times10^6$/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.,[8] allows the collection of about $3.0\text{-}3.5\times10^6$ stem cells /kg hampering any ex vivo graft manipulation. G-CSF appears to be less effective in RA patients than in healthy donors;[7] this is in accordance with the evidence of markedly affected myelopoiesis in advanced RA.[9] 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.[10] 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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