Brief report

Nonmyeloablative conditioning followed by hematopoietic cell allografting and donor lymphocyte infusions for patients with metastatic renal and breast cancer

Marco Bregni, Anna Dodero, Jacopo Peccatori, Alessandra Pescarollo, Massimo Bernardi, Isabella Sassi, Claudia Voena, Alberto Zaniboni, Claudio Bordignon, and Paolo Corradini

The feasibility and toxicity of allogeneic stem cell transplantation after nonmyeloablative conditioning including thiopeta, fludarabine, and cyclophosphamide have been investigated in 6 patients with breast cancer and 7 patients with renal cell cancer. The program included the use of escalating doses of donor lymphocyte infusions (DLI) and/or interferon alpha (IFNα) for patients showing no tumor response and no graft-versus-host disease (GVHD). Patients were at high risk of transplant-related mortality (TRM) because of age, advanced stage, and previous treatments. We observed a partial remission in 4 renal cancer and in 2 breast cancer patients (one at the molecular level in the bone marrow), occurring after cyclosporine withdrawal or after DLI and/or IFNα. All the responses were accompanied by the occurrence of acute GVHD. We conclude that reduced-intensity allogeneic stem cell transplantation is a feasible procedure in renal and breast cancer, and that the exploitation of graft-versus-tumor effect after DLI is a promising finding. (Blood. 2002;99: 4234-4236)

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Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) with myeloablative conditioning has been used in few patients with metastatic solid tumors: a graft-versus-tumor (GVT) effect has been postulated for breast cancer (BC)1,2 and ovarian cancer.3 Very recently, a nonmyeloablative regimen was employed by Childs et al4 in malignant melanoma and renal cell cancer (RCC). The rationale was based on the concept that HSCT can be considered a form of adoptive immunotherapy in chemotherapy resistant tumors. The results in RCC were encouraging, showing a disease regression in 53% of the patients.4 It is noteworthy that the response was associated with graft-versus-host disease (GVHD) occurrence and full donor T-cell engraftment. It has been shown that nonmyeloablative regimens have a lower treatment-related mortality (TRM), and thus can be used also in old and heavily pretreated patients. Donor lymphocyte infusions (DLI) are a well-established treatment for patients with hematologic malignancies relapsing after allogeneic transplantation,5,6 but their potential role in programs of allogeneic transplantation for advanced solid tumors is currently unknown.

We have recently shown that a reduced-intensity conditioning with thiopeta, fludarabine, and cyclophosphamide can provide a successful engraftment with a rather low TRM.5 Here, we report the preliminary results of a pilot study exploring the role of nonmyeloablative conditioning followed by allogeneic HSCT in metastatic BC and RCC. To further enhance the GVT effect, we planned escalating doses of DLI for patients not responding, without any sign of acute GVHD.

Study design

There were 6 patients with advanced BC and 7 patients with RCC, ages 18 to 60 years, with an HLA-identical sibling donor, enrolled in the study from December 1998 to May 2001 (Table 1). Patients had metastatic tumors refractory to standard treatment, evaluable disease, and a life expectancy of more than 6 months. Patients with BC had received a median number of 3 previous chemotherapy lines, including autologous transplantation, and 3 patients had bone marrow involvement at trephine biopsy. All RCC patients had undergone nephrectomy and had previously been treated with cytokine-based therapy.

The preparative regimen consisted of 10 mg/kg thiopeta on day −6 (5 mg/kg was used for RCC); 30 mg/kg cyclophosphamide followed by 30 mg/msq fludarabine on days −4 and −3. Patients received lenogestram-mobilized peripheral blood stem cells from their sibling donor on day 0 (median dose, 5.33 × 10^6 CD34+ cells/kg recipient’s body weight; range, 1.89-7.22).

Cyclosporine A (CSA; target blood levels, 150 ng/mL-300 ng/mL) and short-course methotrexate (MTX; 10 mg/msq day 1; 8 mg/msq days 3 and 6) were used for acute GVHD prophylaxis. CSA was administered at full dose through day +70 and, if GVHD was absent, the dose was tapered by 20% every week. Donor-recipient chimerism analysis was carried out on bone marrow mononuclear cells and on CD3+ and CD13/CD33- cell subsets obtained by cell sorting from peripheral blood mononuclear cells using polymerase chain reaction (PCR) of informative minisatellite regions.6 In patients with mixed donor/recipient T-lymphocyte chimerism or progressive disease on day +60, CSA was tapered off over a 2-week period. Patients who had stable or progressive disease after the withdrawal of CSA and had no evidence of GVHD were eligible to receive up to 3 infusions of DLI, given monthly in escalating doses (1-2 × 10^7 and 1 × 10^8 CD3+ cells/kg recipient’s weight). Patients who had no response to DLI and no GVHD were eligible to receive low-dose subcutaneous interferon alpha (IFNα; 3 × 10^6 IU × 3 per week).

All patients were evaluated by bone marrow biopsy, total-body computed tomographic (CT) scan, and other examinations depending on particular sites of disease, before transplantation and monthly thereafter. Reverse transcriptase (RT)-PCR analysis of maspin and mammaglobin expression was carried out on bone marrow cells, as originally described.10,11

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Results and discussion

All patients had a sustained myeloid and platelet engraftment (neutrophils ≥ 500/mL, median day 12, range 10-14; platelets ≥ 20 000/mL, median day 12, range 8-16 days). On day ± 60, bone marrow chimera was more than or equal to 80% in 12 of 13 patients; peripheral blood (PB) myeloid engraftment was more than or equal to 80% in 10 of 11 patients, and PB lymphoid chimera was more than or equal to 80% in 9 of 11 evaluable patients.

No early TRM was observed during the first 100 days; one patient died of late TRM for lung and brain aspergillus infection. Acute GVHD grades II-III developed in 8 patients (3 after DLI) a median of 95 days after transplantation (range, 12-78). We observed 2 partial responses (patients no. 3 and no. 6): patient no. 3 had an extensive skin and lymph node involvement before allografting that partially regressed along with a grade II acute GVHD developing after DLI. Patient no. 6 was eventually responsive again to DLIs plus IFNα after CSA withdrawal; patient no. 7 relapsed but responded after CSA withdrawal. DLIs were given to 7 patients progressing after allografting, and 3 achieved a partial remission. All responses occurred after the development of acute GVHD, and with full donor T-cell chimera.

There were 3 of 7 RCC patients who progressed soon after the transplantation (median time, 61 days). There were 4 patients who responded after CSA withdrawal; patient no. 7 relapsed but eventually responded again to DLIs plus IFNα (Figure 1A). The other 2 RCC patients had stable disease, and patient no. 11 (papillary RCC) died of progressive disease 10 days after the first DLI. In all BC patients, disease progressed at a median of 69 days after the transplantation (range, 41-232 days). In order to induce a GVTh effect, all patients (except patient no. 5 who died of progressive disease at day +109) received escalating doses of DLIs, at a median of 51 days after disease progression (range, 41-232 days). In order to induce a GVTh effect, all patients (except patient no. 5 who died of progressive disease at day +109) received escalating doses of DLIs, at a median of 51 days after disease progression (range, 41-232 days). In order to induce a GVTh effect, all patients (except patient no. 5 who died of progressive disease at day +109) received escalating doses of DLIs, at a median of 51 days after disease progression (range, 41-232 days). In order to induce a GVTh effect, all patients (except patient no. 5 who died of progressive disease at day +109) received escalating doses of DLIs, at a median of 51 days after disease progression (range, 41-232 days). In order to induce a GVTh effect, all patients (except patient no. 5 who died of progressive disease at day +109) received escalating doses of DLIs, at a median of 51 days after disease progression (range, 41-232 days).

Table 1. Patient characteristics and outcome of treatment

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age, y/sex</th>
<th>Diagnosis/ stage/histo</th>
<th>Site(s) of disease</th>
<th>Status before Tx</th>
<th>PD after Tx (day)</th>
<th>Days to DLI</th>
<th>DLI dose (CD3+ cells/kg)</th>
<th>IFNα</th>
<th>aGVHD grade</th>
<th>cGVHD</th>
<th>Response</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40/F</td>
<td>BC/II B</td>
<td>lung, pleura, skin</td>
<td>PD</td>
<td>+ 232</td>
<td>+ 258</td>
<td>0.9 × 10⁷/kg</td>
<td>–</td>
<td>I (+14)</td>
<td>–</td>
<td>–</td>
<td>1003 + SD</td>
</tr>
<tr>
<td>2</td>
<td>52/F</td>
<td>BC/II A</td>
<td>liver, lung, pleura, bone, BM</td>
<td>PD</td>
<td>+ 70</td>
<td>+ 148</td>
<td>1 × 10⁷/kg</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>464 death, PD</td>
</tr>
<tr>
<td>3</td>
<td>46/F</td>
<td>BC/III B</td>
<td>nodes, skin</td>
<td>PD</td>
<td>+ 60</td>
<td>+ 111</td>
<td>2 × 10⁷/kg</td>
<td>–</td>
<td>II (+122)</td>
<td>extensive</td>
<td>PR (+225)</td>
<td>490 death, PR</td>
</tr>
<tr>
<td>4</td>
<td>40/F</td>
<td>BC/III B</td>
<td>bone, BM</td>
<td>SD</td>
<td>+ 120</td>
<td>+ 132</td>
<td>1 × 10⁷/kg</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>437 + PD</td>
</tr>
<tr>
<td>5</td>
<td>36/F</td>
<td>BC/II A</td>
<td>lung, bone, nodes</td>
<td>SD</td>
<td>+ 41</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>109 death, PD</td>
</tr>
<tr>
<td>6</td>
<td>44/F</td>
<td>BC/II A</td>
<td>bone, BM, pleura</td>
<td>PD</td>
<td>+ 69</td>
<td>+ 131</td>
<td>2 × 10⁷/kg</td>
<td>–</td>
<td>II (+206)</td>
<td>extensive</td>
<td>PR (+227)</td>
<td>417 + PR</td>
</tr>
<tr>
<td>7</td>
<td>60/F</td>
<td>RCC/III B</td>
<td>lung, CNS</td>
<td>PD</td>
<td>+ 70</td>
<td>+ 367</td>
<td>1.2 × 10⁷/kg</td>
<td>–</td>
<td>II (+102 and 559)</td>
<td>extensive</td>
<td>PR (+117)</td>
<td>820 death, PD</td>
</tr>
<tr>
<td>8</td>
<td>54/M</td>
<td>RCC/III B</td>
<td>lung</td>
<td>PD</td>
<td>+ 61</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>II (+81)</td>
<td>limited</td>
<td>PR (+116)</td>
<td>498 + PD</td>
</tr>
<tr>
<td>9</td>
<td>44/M</td>
<td>RCC/IV B</td>
<td>lung</td>
<td>PD</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>III (+89)</td>
<td>extensive</td>
<td>PR (+104)</td>
<td>263 death, PR</td>
</tr>
<tr>
<td>10</td>
<td>48/F</td>
<td>RCC/IV B</td>
<td>lung, CNS</td>
<td>PD</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>II (+12)</td>
<td>SD</td>
<td>235 + SD</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>18/M</td>
<td>RCC/III B</td>
<td>lung, bone, nodes, CNS</td>
<td>PD</td>
<td>+ 30</td>
<td>+ 130</td>
<td>7 × 10⁷/kg</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>140 death, PD</td>
</tr>
<tr>
<td>12</td>
<td>59/M</td>
<td>RCC/II B</td>
<td>lung</td>
<td>SD</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>II (+130)</td>
<td>limited</td>
<td>PR (+189)</td>
<td>208 + PR</td>
</tr>
<tr>
<td>13</td>
<td>47/M</td>
<td>RCC/IV B</td>
<td>lung</td>
<td>SD</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>II (+107)</td>
<td>extensive</td>
<td>SD</td>
<td>194 + SD</td>
</tr>
</tbody>
</table>

Tx indicates transplantation; histo, histology; PD, progressive disease; SD, stable disease; BM, bone marrow; PR, partial remission; DLI, donor lymphocyte infusion; IFNα, interferon alpha; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; BC, breast cancer; RCC, renal cell cancer; CNS, central nervous system.
We and others have previously shown that RT-PCR for maspin and mammaglobin is a sensitive and specific assay for detecting occult BC cells.\textsuperscript{13} This finding suggests a GVT effect at the marrow level.

Our data confirm the existence of a graft-versus-RCC effect demonstrated by Childs et al.\textsuperscript{4} There were 4 of 6 patients with renal clear cell carcinoma and a sufficient follow-up who achieved a PR after withdrawal of CSA or after DLI plus IFN.\textsubscript{\textalpha} Clinical evidence of graft-versus-BCC effect has been reported in a limited number of patients (2/10) by Ueno et al,\textsuperscript{2} and in one anecdotal case by Eibl et al.\textsuperscript{1} However, the study by Ueno et al was different from ours in that it included patients without progressive disease, adopted a myeloablative conditioning regimen with demonstrated antitumor activity, and performed DLI in only one case without response. The dose of T cells to induce a clinical response may vary in different malignancies: Lokhorst et al\textsuperscript{14} have shown that effective DLI doses for relapsed multiple myeloma are higher than those used for chronic myeloid leukemia. Whether this principle applies to solid tumors, and in particular to BC, is still uncertain. Further, the utilization of specific T-cell subsets, the optimal time interval from allograft to DLI, and the schedule of IFN\textsubscript{\textalpha} administration to enhance a GVT effect remain to be determined.

References


Figure 1. Responses to allografting in renal cell cancer and breast cancer. (A) CT thorax scan of patient no. 7 before (i) and after (ii) DLI plus IFN. (B) RT-PCR analysis of mammaglobin and maspin expression in bone marrow cells of patient no. 6 before and after DLI. Bone marrow mononuclear cells were analyzed for mammaglobin and maspin expression pre-allograft (lane 1), before DLI (lane 2), and after DLI (lane 3), respectively. Lane 4: negative control; lane 5: no DNA. MW indicates molecular weight marker.
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