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High-dose chemotherapy supported by peripheral blood progenitor cells in poor prognosis metastatic breast cancer – a phase I/II study

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Summary Current treatments for metastatic breast cancer are not associated with significant survival benefits despite response rates of over 50%. High-dose therapy with autologous bone marrow transplantation (ABMT) has been investigated, particularly in North America, and prolonged survival in up to 25% of women has been reported, but with a significant treatment-related mortality. However, in patients with haematological malignancies undergoing autologous transplantation, haematopoietic reconstruction is significantly quicker and mortality lower than with ABMT, when peripheral blood progenitor cells (PBPCs) are used. In 32 women with metastatic breast cancer, we investigated the feasibility of PBPC mobilisation with high-dose cyclophosphamide and granulocyte colony-stimulating factor (G-CSF) after 12 weeks' infusional induction chemotherapy and the subsequent efficacy of the haematopoietic reconstitution after conditioning with melphalan and either etoposide or thiopeta. PBPC mobilisation was successful in 28/32 (88%) patients, and there was a rapid post-transplantation haematopoietic recovery: median time to neutrophils $>0.5 \times 10^9 \, \text{L}^{-1}$ was 14 days and to platelets $>20 \times 10^9 \, \text{L}^{-1}$ was 10 days. There was no procedure-related mortality, and the major morbidity was mucositis (WHO grade 3–4) in 18/32 patients (56%). In a patient group of which the majority had very poor prognostic features, the median survival from start of induction chemotherapy was 15 months. Thus, PBPC mobilisation and support of high-dose chemotherapy is feasible after infusional induction chemotherapy for patients with metastatic breast cancer, although the optimum drug combination has not yet been determined.

Keywords: breast cancer; poor prognosis; metastatic; high-dose chemotherapy; peripheral blood progenitor cells

Metastatic breast cancer is usually considered incurable, and treatment aims to relieve symptoms. The presence of visceral involvement carries a particularly poor prognosis with a median survival of less than 6 months (Gregory et al., 1993), and conventional chemotherapy has little or no impact on survival beyond 1–2 years. This may, in part, be because the dose of drug that can be delivered is limited by toxicity; there is certainly some evidence for a dose response curve within the range of doses that are conventionally given (Hryniuk and Bush, 1984), particularly when their mode of action is alkylation (Frei et al., 1989). Early attempts at circumventing dose-limitation because of haematological toxicity, by using autologous bone marrow rescue, were associated with poor results and significant treatment-related mortality (Tannir et al., 1984; Vincent et al., 1988); however, more recent series report a much lower mortality of around 5% (Antman et al., 1992). The discovery that, following myeloablative chemotherapy, previously mobilised peripheral blood progenitor cells were able to reconstitute haematopoiesis more rapidly than those from bone marrow (Schmitz et al., 1994) offered the opportunity to reduce some of the toxicity associated with high-dose chemotherapy.

We therefore investigated the feasibility of performing myeloablative chemotherapy for younger women whose sites of metastatic breast cancer carried a particularly poor prognosis. In view of the data suggesting that this approach held more promise in chemo-sensitive breast cancer (Antman et al., 1992) together with the need to improve the performance status of some patients presenting with stage IV disease, we opted for a 12 week induction chemotherapy regimen to precede the high-dose cycles. The initial high-dose regimen consisted of melphalan and etoposide, which has a low toxicity in the management of lymphoma (Jackson et al., 1994) and which would be anticipated to have activity in breast cancer. However, although we found this regimen to be active, the data on single agent etoposide in breast cancer suggested that this may not be the most effective drug to employ, and thus after ten patients the intention was to conduct a phase I study was melphalan and thiopeta; the latter drug being chosen because of its efficacy in breast cancer (Antman, 1992) as well as its superior \textit{in vitro} activity after dose escalation (Lazarus et al., 1987).

Patients and methods

Between July 1992 and April 1995, we entered 32 women (median age 39, range 27–55 years) with metastatic breast cancer into a phase I/II study of myeloablative chemotherapy supported by peripheral blood progenitor cells. Patients had to have histologically confirmed breast cancer, with definite evidence of relapsed or metastatic disease (see Table 1). The presence of metastases in bone only was insufficient for entry into this study. Thirty patients had an induction regimen of infusional chemotherapy (AcF) consisting of weekly adriamycin (20–30 mg m\textsuperscript{-2}) and continuous 5-fluorouracil (5-FU) 200 mg m\textsuperscript{-2} day\textsuperscript{-1} delivered using an electronic pump (CADD, Pharmacia; or Walkmed, Medex) through a Hickman line for up to 12 weeks. The responses and toxicities of this regimen have been separately reported (Gabra et al., 1996) and will not be discussed further. Patients progressing on this regimen were switched to ECF (epirubicin 50 mg m\textsuperscript{-2}, cisplatin 60 mg m\textsuperscript{-2}, 5-FU 200 mg m\textsuperscript{-2} day\textsuperscript{-1}) (Jones et al., 1994); one woman was given only ECF as induction, and one was referred after treatment with 3 weekly FEC (5-fluorouracil, epirubicin, cyclophosphamide). Patients objectively responding to the induction regimen (and with adequate cardiac function) were then eligible for this study, which was approved by the Lothian ethics committee. Informed consent was obtained from each patient.

Peripheral blood progenitor cells (PBPC) were mobilised using cyclophosphamide 2.5 g m\textsuperscript{-2} (n = 5) or 4 g m\textsuperscript{-2} (n = 27) and 300 µG G-CSF(Amgen) administered from day +1 until harvesting was complete (Craig et al., 1993). PBPC harvesting
was performed on 3 consecutive days using the Baxter CS3000 plus or Cobe Spectra cell separators commencing after the total white blood cell count was greater than 1 x 10^9 l^-1. The product was diluted 1:1 with autologous plasma containing 20% dimethyl sulphoxide (DMSO), cryopreserved using a controlled rate freezer and stored in the vapour phase of liquid nitrogen. Progenitor cells were assessed using the CFU-GM assay in methyl cellulose, as previously described (Craig et al., 1992), with a locally determined safe transplantation threshold of 10 x 10^6 CFU-GM kg^-1 (Craig et al., 1993). Conditioning was commenced once the CFU-GM assays were available and, if the harvest was inadequate, a bone marrow harvest was carried out under general anaesthesia. Initially ten patients were given etoposide 1600 mg m^-2 over 48 h, followed by melphalan 140 mg m^-2. Subsequently, 22 patients had thiopeta 500 mg m^-2 over 4 days in place of etoposide. Twenty-four hours after melphalan the cryopreserved PBPC were rapidly thawed at the bedside in a 37°C water bath and reinfused via the Hickman line. The patients were then allowed home, but readmitted daily for blood counts. Prophylactic antimicrobials (ciprofloxacin 500 mg b.d., fluconazole 50 mg o.d. and acyclovir 200 mg g.d.s.) were commenced on readmission to hospital when the neutrophil count fell below 0.5 x 10^9 cells l^-1. Thereafter, patients were reverse-barrier nursed until the neutrophil count rose above 0.5 x 10^9 l^-1. Other supportive measures were employed as necessary, and febrile episodes were initially treated with gentamicin and cefazidime. All blood products were irradiated, and patients who were seronegative for cytomegalovirus (CMV) received CMV-negative products. Platelets were administered prophylactically to maintain platelets > 5 x 10^11 l^-1.

Following high-dose chemotherapy, patients were treated with adjuvant hormone therapy irrespective of their oestrogen receptor (ER) status. Those who had previously received tamoxifen (11/32) were advised to take megestrol acetate 160 mg daily; the remainder (21/32), tamoxifen 20 mg daily. One month following recovery of the bone marrow, patients were restaged to assess their response to the therapy. Thereafter, follow-up was 3 monthly until relapse, when patients were treated as was considered appropriate. Isolated bone metastases were irradiated and, if systemic therapy was required, chemotherapy was used (routinely CMF, i.e. cyclophosphamide 600 mg m^-2, methotrexate 50 mg m^-2, 5-fluorouracil 600 mg m^-2).

Statistical analysis
All survival analyses have been done using the Kaplan–Meier method, and statistical comparisons were made using the log-rank test. These were performed using the “Sureal” programme (W Gregory, personal communication) running under MS-DOS 6.2 (Microsoft). All other statistical calculations were carried out in Minitab version 5.1.1 (Minitab, State College, PA, USA), also running under MS-DOS 6.2. The confidence interval for the mortality was estimated using the binomial distribution function in Minitab.

Results
Response
Thirty women underwent initial induction with AcF, of whom four progressed and were therefore treated with ECF, one received ECF alone and one was referred in complete response (CR) following four cycles of FEC. There were 8/32 (25%) complete responses to the induction chemotherapy, as assessed before the administration of the high-dose cyclophosphamide, and an overall response rate of 97%. After PBPC transplantation, this rose to 17/32 complete responses (53%) with an overall response rate of 100% (see Table II).

### Table I Summary of patients’ data

<table>
<thead>
<tr>
<th>At first presentation</th>
<th>Stage I</th>
<th>(9%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage II</td>
<td>18</td>
<td>(56%)</td>
</tr>
<tr>
<td>Stage III (including three with unknown size)</td>
<td>8</td>
<td>(25%)</td>
</tr>
<tr>
<td>ER positive (&gt; 20 fmol mg^-1 protein)</td>
<td>12</td>
<td>(38%)</td>
</tr>
<tr>
<td>ER unknown</td>
<td>7</td>
<td>(22%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Before induction therapy</th>
<th>Median age 39 years (range 27–55)</th>
<th>Prior adjuvant chemotherapy 13 (41%)</th>
<th>Prior tamoxifen 11 (35%)</th>
<th>Median disease-free interval 22 months (range 0–60)</th>
<th>Median performance status 2 (range 0–4)</th>
<th>Metastatic sites</th>
<th>Red cell concentrates transfused 2 (0–6 units)</th>
<th>Platelet transfusions (in units of 5) 1 (0–5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>23 (72%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>9 (28%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNS</td>
<td>2 (6%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locoregional</td>
<td>14 (44%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visceral (liver/lung/CNS)</td>
<td>25 (78%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Numbers with percentages in parentheses refer to number of patients.

### Table II Response to induction and high-dose therapy

<table>
<thead>
<tr>
<th>CR After induction therapy</th>
<th>8 (25%)</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR After induction therapy</td>
<td>23 (72%)</td>
<td>14</td>
</tr>
<tr>
<td>SD After induction therapy</td>
<td>1 (3%)</td>
<td>1</td>
</tr>
<tr>
<td>Overall</td>
<td>31 (97%)</td>
<td>32</td>
</tr>
</tbody>
</table>

| CR, complete response; PR, partial response; SD, static disease. |

### Table III High-dose toxicities

<table>
<thead>
<tr>
<th>Toxocities</th>
<th>CR</th>
<th>PR</th>
<th>SD</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematopoietic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days to platelets &gt; 2 x 10^9 l^-1</td>
<td>10</td>
<td>13</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Days to platelets &gt; 3 x 10^9 l^-1</td>
<td>8-17</td>
<td>8-27</td>
<td>8-23</td>
<td></td>
</tr>
</tbody>
</table>

| Other                                  |    |    |    |         |
|----------------------------------------|    |    |    |         |
| Days of fever                          | 4  | 4  | 4  |         |
| Mucositis WHO grade 3 or 4             | 4  | 4  | 4  |         |
| Thiopeta/VP16                          | 4  | 4  | 4  |         |
| Thiopeta/melphalan                     | 14 | 14 | 14 |         |
| Diarrhoea WHO grade 3 or 4             | 14 | 14 | 14 |         |
| Thiopeta/VP16                          | 0  | 0  | 0  |         |
| Thiopeta/melphalan                     | 14 | 14 | 14 |         |

| Late toxicities                        |    |    |    |         |
|----------------------------------------|    |    |    |         |
| Shingles                               | 5  | 5  | 5  |         |
| Prolonged low white count (< 2 x 10^9 l^-1) | 2  | 2  | 2  |         |
Toxicities

The high-dose cyclophosphamide was well-tolerated. There was no evidence of cardiac decompensation. Feverle
neutropenia requiring admission occurred in 10/32 (31%) patients.

Adequate PBPCs were collected from 28/32 (88%) patients, with a median CFU-GM collected per mobilisation of
48.5×10^6 kg^-1 (range 9–191). The four patients with inadequate PBPC yields had harvested bone marrow
reinfused together with the PBPC. The high-dose regimen
was well-tolerated (see Table III for details). Haematopoietic
reconstitution in those transplanted with only PBPC was
rapid, with a median time to neutrophils > 0.5×10^9 l^-1 of 14
days and platelets > 20×10^11 l^-1 of 10 days (see Table III).
(The difference between platelet and neutrophil recovery
times was significant; \(W=387.5, P<0.05\).) Recovery was
slower in those four patients with inadequate PBPC
recruitment, who therefore also received bone marrow; the
longest time to platelets > 20×10^11 l^-1 was 17 days and to
neutrophils > 0.5×10^9 l^-1 was 23 days.

The only major non-haematological toxicity was mucositis,
with 18/32 (56%) experiencing WHO grade 3 or 4 mucositis.
There were no treatment-related deaths. The 95% confidence
interval for this observed mortality of 0% is 0–9%. Furthermore, no patient required parenteral nutrition or
artificial ventilation. Following therapy, two patients experi-
enced persistent low blood counts (leucocytes < 2×10^9 l^-1),
but without any infective problems. Five patients (16%) had
shingles, two had rashes which were not biopsied and two
had self-limiting culture-negative diarrhoea.

Survival

Progression-free survival from the time of high-dose therapy
is shown in Figure 1. To date, 27 patients have relapsed,
giving a median relapse-free survival of 7.5 months overall
and of 7 months for those with visceral metastases. Six out of
twenty-seven (22%) patients relapsing did so with CNS
involvement. A further two (6%) initially relapsed in bone,
never having before had evidence of bone metastases. All
other patients, including those who had bone marrow
reinfused, relapsed at previous sites of disease. However
four of the five patients remaining disease-free at 1 year are
still in complete remission at 14, 17, 25 and 31 months.
Figure 2 shows the overall survival, with a median value of
12 months from high-dose treatment and 15 months from the
start of induction treatment. Actuarial survival is 35% at 2
years, and 12 patients are still alive with progression-free
survivals of 9, 9+, 14+, 17+, 18, 19, 21, 23, 25, 25+, and
31 + months. There was no difference in progression-free
or overall survival between the patients treated with the two
different high-dose regimens \(\chi^2=1.8\) and \(\chi^2=0.4\) respec-
tively). Furthermore, the survival of the small group of seven
women who did not have visceral involvement was not
significantly better than those who did \(\chi^2=0.67\), although
four remain disease-free, including the two longest survivors.

At trial entry, the majority of patients had more than one
site of disease relapse, but there was no difference in outcome
either by the site(s) involved or by the number of different
sites. Furthermore, neither the disease-free interval nor the
original ER status of the tumour nor the administration of
prior adjuvant chemotherapy had any bearing on survival in
this small group of patients. The four patients who had an
autologous bone marrow transplant because of a low PBPC
yield had a poorer survival \(\chi^2=3.7, P=0.054\) despite two
being in CR after induction and one more converting to CR
after ABMT. Three of these patients had visceral metastases,
and three had also had prior adjuvant CMF, proportions
that were not significantly different from the group as a
whole.

Despite the improved response following the PBPC
transplant, there was no difference in survival depending on
whether or not patients had had a complete response to
either the induction or high-dose regimen \(\chi^2=0.3\) and
\(\chi^2=0.1\) respectively).

Discussion

We have shown that PBPC transplantation can be safely
performed in patients with visceral metastatic breast cancer.
The toxicity of this approach, even after 12 weeks’ infusional
induction chemotherapy, was acceptable, with the only major
non-haematological toxicity being self-limiting mucositis.
The lack of any treatment-related deaths is consistent with a
mortality of up to 9%, well within the range seen in many of
the reported larger and more recent North American series
using autologous bone marrow (Antman et al., 1992;
Livingston, 1994).

There has, however, been less experience with PBPCs.
They have been used in other series because of overt bone
marrow involvement, with the reported haematopoietic
recovery and patient survival times being similar to those
seen here (Somlo et al., 1994; Myers et al., 1994; Hester and
Wallerstein, 1993; Kritz et al., 1993). In contrast, the more
prolonged recovery for patients receiving ABMT is well
recognised; and although we did not employ G-CSF after
PBPC, this can further hasten neutrophil recovery (van der
Wall et al., 1995), but with no significant effect on antibiotic
usage, febrile days or platelet counts. One study in the high-
risk adjuvant setting reported that the median recovery was
9 days for 18 patients who received G-CSF after return of
the PBPC and 16 days for the ten patients who did not (van
der Wall et al., 1995). Another study with G-CSF given
after PBPC reinfusion also reported a median recovery time
of 9–10 days (Somlo et al., 1994), whereas a small study of 12 patients given PBPC alone after high-dose therapy reported a neutrophil recovery time of 14 days, with platelet recovery occurring within 12 days (Elías et al., 1991). Thus, although our observation that the platelets recovered significantly earlier than the neutrophils has not to our knowledge been previously noted, this may be a consequence of the use of G-CSF in some of the other reports. PBPC transplantation does therefore have the twin advantages of reducing the period of neutropenia and thrombocytopenia as well as obviating the need for a general anaesthetic to harvest the bone marrow.

It is unclear as to which are the best drugs to be employed in a myeloablative transplant regimen for breast cancer. Laboratory studies show that melphalan, thiotepa and cis-platinum demonstrate steep, almost linear dose-response curves in MCF-7 cells (Frei et al., 1989). Fewer data are available for etoposide, but poor responses were seen when it was used as a single high-dose agent in refractory breast cancer (Antman, 1992). Having established the safety of melphalan and etoposide with PBPC support in pretreated metastatic breast cancer, we then administered thiotepa in place of the etoposide, intending to dose escalate both the thiotepa and melphalan. This was done initially used there was a significant increase in mucositis, with 68% (15/22) patients experiencing grade III or IV mucositis, most of whom needed intravenous diamorphine for 3–5 days. This was in contrast to the experience with etoposide, when only 40% (4/10) had a similar degree of mucositis. The lack of difference in survival between the two regimens employed does not necessarily imply that they are equivalent, as they were employed over different periods and thus there may be a selection bias. Indeed, all ten patients given melphalan and etoposide were classified as having a complete response following high-dose therapy; but none of them had stable or progressive disease during the initial AcF induction therapy, so that they may have represented a ‘better’ group. Although the in vitro data suggest that prior treatment with etoposide can have a synergistic enhancement of the cytotoxicity of alkylators such as BCNU or cyclophosphamide (Tanaka et al., 1991), one cannot draw any conclusions from this study about the relative efficacies of etoposide and thiotepa administered in conjunction with melphalan.

In the South African randomised trial (Bezwoda et al., 1995), following all chemotherapy, all patients classified as responding were given tamoxifen, with 95% of those in the high-dose arm and only 53% in the conventional arm being thus treated. Whether this contributed to the observed survival difference is uncertain, as there were more patients in the high-dose arm who were known to be ER-negative. In contrast in this study, all women were given further ‘adjuvant’ hormone therapy, with megace being given to those who had relapsed whilst on tamoxifen, as even a cytotoxic effect would provide symptomatic if not survival benefit for patients in good remission.

The long-term benefit from high-dose chemotherapy remains uncertain, with many series reporting a 5 year survival of the order of 25% (Livingston, 1994), not very different from advanced high grade NHL. The criteria for offering high-dose chemotherapy in metastatic breast cancer varies and in this series, as with many others, most of the patients have a particularly poor prognosis with conventional chemotherapy. Indeed, the presence of liver metastases predicts for a poor prognosis with both conventional (Gregory 1993) and high-dose chemotherapy (Duphny et al., 1994). Thus, this failure to cure a significant number of women should not be seen as an indictment of the approach as, even in this study, the median survival is more than twice what would be anticipated for patients with liver metastases. Whether this is acceptable will depend on the toxicities observed. To this degree, an extended survival can be obtained for the majority, then it is essential that the regimen is well-tolerated. It is not at present clear what the optimum regimen is for myeloablative therapy in breast cancer, but the combination of melphalan and thiotepa or etoposide can be delivered with a lower mortality and morbidity than one of the most widely used regimens piloted in North America, i.e. cyclophosphamide/cisplatin/BCNU (Peters et al., 1988). Another widely used treatment, the so-called STAMP 5 regimen consisting of carboplatin, cyclophosphamide and thiotepa (Antman et al., 1992) is associated with a similarly low mortality but with more toxicity including 20% transient congestive cardiac failure. The long-term survival that we report for a smaller group of women is very similar to that seen with these more toxic regimens, and there is no apparent loss of response from using only two drugs, nor from using PBPC harvested after 3 months infusional chemotherapy.

What remains unproved is the strategy. What is the true benefit of this approach in stage IV breast cancer? A multicentre randomised trial comparing PBPC-supported myeloablative therapy with the best conventional nonablative therapy is required to determine whether dose intensification can improve the appalling prognosis for women with visceral metastatic breast cancer.

References


