Patient selection for high-dose chemotherapy in stage II and IV breast cancer
Schrama, J.G.

Citation for published version (APA):
Chapter 1.

Introduction
Introduction

Breast cancer is a common disease and a major cause of death all over the world. During 2000, in the Netherlands alone, 10,000 patients were diagnosed with breast cancer. In Europe the number of new breast cancer patients is approximately 345,000 per year and in the USA it is 202,000 [1]. Breast cancer continues to be a frequently fatal disease and despite major advances in treatment, about 40% of the patients will ultimately die of the disease. Local treatment (surgery and radiotherapy) is quite effective and locoregional recurrences are relatively rare (below 10%). Most patients who die through treatment failure, die of secondary metastases. Consequently, treatment efforts are focused on either prevention or treatment of metastases. This has resulted in hormonal adjuvant treatment of hormone sensitive tumours (primarily tamoxifen) to reduce the risk of recurrence. Present studies are focusing on improvement of the hormonal treatment and especially on the role of the new agents such as aromatase-inhibitors and ovarian ablation in premenopausal patients. Furthermore, many patients and even more after the recent Oxford overview [2], are treated with adjuvant chemotherapy. Despite these improvements in adjuvant therapy, many patients relapse. Metastatic breast cancer continues to be essentially incurable, even after an initial response to hormonal or chemotherapeutical treatment. Therefore, several strategies are directed to overcome the drug resistance of metastases. One of these strategies is high-dose chemotherapy with stem cell or bone marrow transplantation.

Rationale for high-dose chemotherapy (HD-CT)

The goal of this treatment should be, as in leukaemia and lymphomas, the achievement of long-term, disease-free survival or cure in a significant number of the patients. The principles of autologous stem-cell or bone marrow transplantation have been derived from the principles of allogeneic transplantation in haematological malignancies [3]. Autologous transplantation can overcome bone marrow suppression, but is lacking a possible contribution of graft versus host disease as anti-tumour-effect. Bone marrow or stem cell transplantation in the autologous setting allows higher therapeutic doses of myelosuppressive agents which should result in a better tumour response. The most suitable chemotherapeutic agents for this procedure are alkylating agents. Alkylators have myelosuppression as the dose-limiting factor and have a broad spectrum of anti-tumour activity. Preclinical studies have shown the existence of a steep dose-response relation and a linear increase in log-cell kill with increasing doses of alkylating agents. This linear dose-response relation is maintained through multiple logs of cell reduction [3,4]. The combination of different chemotherapeutic agents is based on the differences in mechanisms of action and resistance. The biological mechanism of the diverse alkylating agents differs, so they lack cross resistance [3,5] and even synergism has been postulated. Another prerequisite for the combination regimen is that the drugs have a non-overlapping toxicity profile. For instance, if myelotoxicity is overcome, the dose-limiting toxicity for BCNU (carmustine) is pulmonary and hepatic toxicity and for carboplatin nephro-and neurotoxicity [6,7].
The hope that high-dose chemotherapy is able to induce long-term disease-free survival in a subgroup of high-risk breast cancer patients and even in metastatic disease is based on results of a large number of phase II studies (reviewed by Peters et al. [8]). Peters et al. performed a study of HD-CT as initial and only treatment in metastatic breast cancer patients and reported an impressive progression-free survival in favour of high-dose chemotherapy. When compared with historical data, 3 of the 22 patients achieved long-term survival [9]. Other evidence that it is possible to achieve long-term survival with HD-CT in metastatic disease came from the Transplant Registries in Europe [10] and North America [11], which showed that high-dose chemotherapy could induce long-term survival in 10 to 20% of the stage IV patients. These initial encouraging results led to a somewhat uncritical acceptance of HD-CT in breast cancer and many patients were treated off protocol.

**High-dose chemotherapy in the adjuvant treatment of breast cancer**

The most cited study is the study by Peters et al. [12]. In this study 85 patients with high-risk breast cancer were treated with alkylating HD-CT and the results suggested a dramatic increase in progression-free survival for high-dose chemotherapy over historical controls treated with conventional therapy. This and other encouraging results of small and uncontrolled studies led to a marked increase of the popularity of high-dose chemotherapy and many patients were treated in and off-protocol. Subsequently, several events have caused the conversion of the initial enthusiasm into criticism and even rejection. One of these factors was the discovery of scientific fraud in a South African study [13]. In addition, several small randomized studies did not show the expected survival benefit and the results of the study of Peters et al. [12] could not be replicated. This was probably due to a stricter patient selection in the first high-dose studies than in the conventional studies [14,15] and an improvement in the general prognosis of breast cancer as result of better adjuvant treatment.

Until 2002, 12 randomized studies of high-dose chemotherapy in primary high-risk breast cancer have been reported (five of them in peer-reviewed journals), including a total of 4000 patients (Table 1).

Three of these 12 studies where small and included less than 100 patients. The first study was a study of our institution [16,17] in which 81 patients with breast cancer and extensive lymph node involvement were randomized after up-front chemotherapy and surgery for additional conventional therapy or HD-CT with peripheral blood stem cell (PBSC) transplantation. The final results of this study are described in this thesis. The study did not show a benefit for the high-dose arm, but it was underpowered and there was a long interval between the induction chemotherapy and the high-dose treatment, theoretically allowing regrowth of micrometastatic tumour cells. In another small single-institution study from the M.D. Anderson Cancer Centre [18], 78 patients with high-risk breast cancer were randomized after eight cycles adjuvant therapy to either two courses HD-CT (cisplatin, cyclophosphamide and etoposide) or no further therapy. This study did not show an advantage for progression-free survival (PFS) and
overall survival (OS), but this study was also underpowered. Moreover, the dosage of the chemotherapeutic agents was not truly high-dose.

<table>
<thead>
<tr>
<th>Author</th>
<th>Study</th>
<th>Selection</th>
<th>No of randomized patients</th>
<th>Induction therapy</th>
<th>Conventional arm</th>
<th>HD-arm</th>
<th>RFS-analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche [19]</td>
<td>PEGASE 01</td>
<td>7N</td>
<td>314</td>
<td>4xFEC</td>
<td>Observation</td>
<td>CMA</td>
<td>HD better</td>
</tr>
<tr>
<td>Nitz [20]</td>
<td>West German</td>
<td>9N</td>
<td>403</td>
<td>4xEC→</td>
<td>2xEC</td>
<td>ECF</td>
<td>RFS and OS</td>
</tr>
<tr>
<td>Tallman [21]</td>
<td>INT0121</td>
<td>10N</td>
<td>540</td>
<td>6xCAF</td>
<td>Observation</td>
<td>CT</td>
<td>HD Better</td>
</tr>
<tr>
<td>Peters [22]</td>
<td>American</td>
<td>10N</td>
<td>783</td>
<td>4xCF</td>
<td>Intermediate</td>
<td>CPB</td>
<td>No difference</td>
</tr>
<tr>
<td>Rodenhuis [16]</td>
<td>Dutch pilot</td>
<td></td>
<td>81</td>
<td>4xFEC</td>
<td>1xFEC</td>
<td>CTC</td>
<td>HD less relapses</td>
</tr>
<tr>
<td>Schrama [17]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>Hortobagyi [18]</td>
<td>M.D. Anderson</td>
<td>4N-10N</td>
<td>78</td>
<td>8xCF</td>
<td>Observation</td>
<td>CEC</td>
<td>No difference</td>
</tr>
<tr>
<td>Bergh [23]</td>
<td>Scandinavian</td>
<td>8N</td>
<td>525</td>
<td>9xHDFEC</td>
<td>3x LD FEC</td>
<td>CTCb</td>
<td>9xHDFEC better</td>
</tr>
<tr>
<td>Zander [26]</td>
<td>German</td>
<td>10N</td>
<td>307</td>
<td>6xCF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tokuda [27]</td>
<td>Japanese</td>
<td>10N</td>
<td>97</td>
<td>3xEC</td>
<td>3xCF</td>
<td>CTM</td>
<td>HD better, ns</td>
</tr>
<tr>
<td>Rodenhuis [28]</td>
<td>Dutch National Intergroup Study</td>
<td>4N</td>
<td>885</td>
<td>4xFEC</td>
<td>1xFEC</td>
<td>CTC</td>
<td>HD better</td>
</tr>
</tbody>
</table>

Table 1. Randomized studies of high-dose vs. conventional dose chemotherapy in primary high-risk breast cancer

The CALGB/Intergroup study [22] compared high and intermediate dose cyclophosphamide, BCNU and cisplatin (CBP) after four cycles cyclophosphamide, adriamycin, 5-fluoro-uracil (CAF). A point of criticism towards this study is that intermediate CBP is not a standard treatment. In this study fewer relapses were seen in the high dose arm, not resulting in a better

1 N=lymphnodes, 7N means 7 tumor-positive lymphnodes
2 CMA=cyclophosphamide 120 mg/kg, mitoxantrone 45 mg/m2, melfalan 140 mg/m2
3 EC-T=tandem epirubicine 90 mg/m2, cyclophosphamide 3000mg/m2-Thiotepa 400 m/m²
4 CAF=cyclophosphamide, adriamycin and 5-fluoro-uracil, different dosages
5 intermediate CPB=cyclophosphamide 900 mg/m², cisplatin 90 mg/m², carmustin 90 mg/m² with G-CSF
6 CPB=cyclophosphamide 5625 mg/m², cisplatin 165 mg/m², carmustin 600 mg/m²
7 CEC=cyclophosphamide, etoposide, cisplatin
8 HD FEC = epirubicin 50 mg/m2, HD FEC= high-dose epirubicin tailored to patients tolerance
9 AFM=doxorubicin 25 mg/m2/day, 3, 5-fluoro-uracil (500 or 750 mg/m2/day) and methotrexate 250 mg/m²
10 CPB=cyclophosphamide 5625 mg/m², cisplatin 165 mg/m², carmustin 600 mg/m²
11 1xFEC= cyclophosphamide 7 g/m², 1x methotrexate 8 g/m², 2x epirubicin 120 mg/m², 1x thiotepa 600 mg/m² plus melphalan 160-180 mg/m²
12 CTM=cyclophosphamide 1500 mg/m², thiotepa 150 mg/m², mitoxantrone 10 mg/m² on 4 consecutive days
13 CT=cyclophosphamide 6 g/m², thiotepa 600 mg/m² (Tokuda), Thiotepa 800 mg/m² (Tallman)
14 FEC=5-fluoro-uracil, epirubicin, cyclophosphamide
15 *=significant, ns=non-significant
16 updated results after 74 months of follow-up (personal communication)
overall survival, presumably due to the high early toxic mortality-rate of 7.4 % mostly due to BCNU-related pulmonary toxicity.

The Scandinavian study of Bergh et al. [23] (525 patients) compared three cycles of induction FEC (5-fluoro-uracil, epirubicin, cyclophosphamide) followed by CTCb (cyclophosphamide, thiotepa, carboplatin) or nine cycles of FEC with the dose tailored to individual tolerance. These authors reported an improved PFS for the tailored FEC arm at a follow-up time of three years. In this study, six patients developed leukaemia and another three myelodysplasia, all in the conventional arm. The major comment on this study is that there was undertreatment in the high-dose arm as the cumulative dose of epirubicin (150 mg/m$^2$) in the high-dose arm was low compared to that in the conventional arm. Moreover, in this study probably patients with metastatic disease were included, as patients with bone-marrow involvement and abnormal bone scans were not excluded.

The large Anglo-Celtic study [24] randomized between four times adriamycin (75 mg/m$^2$) followed by either 8 times CMF (cyclophosphamide, methotrexate, 5-fluoro-uracil) or HD-CT (cyclophosphamide and thiotepa). The disease-free survival at a median follow-up of 4 years was not significantly different between the two treatment arms (51 and 54%).

The Milan study of Gianni et al. [25] showed no difference of PFS and OS for the whole patient group, but subgroup analyses showed a trend to a better PFS in the group of patients which were younger than 36 years and with 4-9 tumour-positive lymph nodes.

The French Pegase 01 study [19] found a significant advantage in PFS for HD-CT at 3 years (50 vs. 70.8%, p<0.003) without a difference in OS, but this study might be confounded by the fact that tamoxifen was only administered in post-menopausal women. The German [26] and the Japanese study [27] all showed a trend for an increase in PFS without (yet) an increase in overall survival.

The study of Tallman et al. [21] used a regimen with six courses of CAF, (cyclophosphamide 100 mg/m$^2$ orally day 1-14, adriamycin 30 mg/m$^2$ intravenously (iv) day 1 and 8 and 5-fluorouracil 500 mg/m$^2$ iv day 1 and 8, every 28 days) followed by observation or high dose chemotherapy (carboplatin 6 g/m$^2$ and thiotepa 800 mg/m$^2$). They found no difference in overall or disease-free survival, but there was a high incidence of acute leukaemia and myelodysplasia (1.6% of the whole patient group), all in the high-dose treatment arm.

The Dutch National Study [28] is the largest randomized trial with 885 patients included. In this study patients were randomized for 4 times FEC with either an additional FEC-course or CTC with PBSC transplantation, followed by hormonal therapy and radiotherapy if indicated. An updated analysis of the data after 74 months of follow-up showed a significantly better relapse-free survival (RFS) for the whole patient group (p=0.025, two sided). This RFS advantage was limited to patients with HER-2 negative disease. This subgroup also had a significant OS advantage after HD-CT.

In summary, three of the twelve studies showed no benefit for high-dose chemotherapy and in contrast, one study reported an advantage for the conventional arm. Another study had less relapses, but this was balanced by a higher mortality rate. Nevertheless, four preliminary abstracts reported a better PFS without an increase in survival. In the large Dutch National
Chapter 1

Study, RFS-curves show a difference in favour of HD-CT, and even an impressive benefit in the patient group with HER-2 negative tumours. It is therefore important to analyse the role of the HER-2 status in the other randomized studies.

High-dose chemotherapy in advanced breast cancer

Several studies have reported prognostic and predictive factors for benefit of HD-CT in metastatic breast cancer patients. Most studies agree that limited disease, good clinical condition and responsiveness to conventional-dose chemotherapy are favourable factors. An uncontrolled but prospective Dutch study [29] showed that patients in first complete remission following conventional-dose chemotherapy had a long-term PFS of 43%, and similar data from the European bone marrow registry showed a 35% PFS for 3+ years in patients transplanted in first remission of advanced breast cancer [10]. These and many other reports confirm the early study of Peters et al. [9] which showed that a single course of HD-CT without any other treatment could induce long-term PFS in 3 of the 22 patients. This data indicates that HD-CT could induce long-term PFS in a subgroup of patients, but the question remains whether this subgroup would also achieve long-term survival after conventional-dose therapy. It is very plausible that the uncontrolled high-dose studies have had a selection bias. Not many randomized studies have been performed in metastatic breast cancer (Table 2).

### Table 2. Randomized studies of high-dose vs. conventional dose chemotherapy in advanced breast cancer

<table>
<thead>
<tr>
<th>Author</th>
<th>Study</th>
<th>Selection</th>
<th>randomized patients (x1)</th>
<th>Induction therapy</th>
<th>Conventional arm</th>
<th>High-dose arm</th>
<th>RFS-analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stadtmauer[31]</td>
<td>Philadelphia</td>
<td>metastatic</td>
<td>166 (553)</td>
<td>4-6×CAF or CMF²</td>
<td>8 CMF</td>
<td>CTCb</td>
<td>No difference</td>
</tr>
<tr>
<td>Peters [30]</td>
<td>Duke</td>
<td>metastatic</td>
<td>98 (423)</td>
<td>2-4×AFM³</td>
<td>No further treatment</td>
<td>CPB³</td>
<td>Immediate HD better</td>
</tr>
<tr>
<td>Madan [34]</td>
<td>Duke</td>
<td>metastatic</td>
<td>69 (85)</td>
<td>2-4×AFM</td>
<td>No further treatment</td>
<td>CPB</td>
<td>Immediate HD better</td>
</tr>
<tr>
<td>Lotz [35]</td>
<td>Pegase 04</td>
<td>metastatic</td>
<td>61</td>
<td>Conventional therapy</td>
<td>2-4 cycles</td>
<td>CMM³</td>
<td>HD better, ns</td>
</tr>
<tr>
<td>Biron [36]</td>
<td>Pegase 03</td>
<td>metastatic</td>
<td>180 (308)</td>
<td>4xFEC²</td>
<td>No further treatment</td>
<td>CT³</td>
<td>HD better, ns</td>
</tr>
<tr>
<td>Crump [37]</td>
<td>NCIC</td>
<td>metastatic</td>
<td>219 (386)</td>
<td>4×A or T³</td>
<td>2-4 A or T</td>
<td>CMC¹⁰</td>
<td>HD better, ns</td>
</tr>
</tbody>
</table>

¹ Number of patients entered in the study
² CAF=cyclophosphamide, Adriamycin, 5-fluoro-uracil or CMF=cyclophosphamide, methotrexate, 5-fluoro-uracil, depending on the adjuvant treatment
³ CTCb=cyclophosphamide 1500 mg/m²/d, thiotapec 125 mg/m²/d and carboplatin 200 mg/m²/d as continuous infusion for 4 days
⁴ AFM=doxorubicin 25 mg/m²x3, 5-fluoro-uracil (500 or 750 mg/m²x5 and methotrexate 250 mg/m²
⁵ CPB=cyclophosphamide 5625 mg/m², cisplatin 165 mg/m², carmustine 600 mg/m²
⁶ CMM=cyclophosphamide, mitoxantrone, melphalan (mitoxantrone 45 mg/m², cyclophosphamide 120 mg/kg, melphalan 140 mg/m²), FEC=5-fluoro-uracil, epirubicin, cyclophosphamide
⁷ CT=cyclophosphamide 6 g/m², thiotapec 800 mg/m²
⁸ Anthracyclins or T=taxanes depending on the previous adjuvant therapy
⁹ CMC=cyclophosphamide 1.5 g/m², mitoxantrone 17.5 mg/m², carboplatin 450 mg/m² daily for 4 days
Introduction

The only randomized study published in a peer-reviewed journal is the study of Stadtmauer et al. (Philadelphia study) [31]. This large study showed an improvement of PFS in the high-dose group and no difference in long-term disease-free survival between the treatment groups (both lower than 10%). This was, of course a discouraging result, but this study has several limitations. In the first place, the number of actual randomized patients was low (199 chemoresponsive patients of the 553 included patients were treated with HD-CT).

Secondly, the patients in the conventional group received a higher cumulative dose of cyclophosphamide in comparison to the high-dose chemotherapy group.

Furthermore, the CTCb regimen was employed, which differs from our CTC regimen in the fact that the cyclophosphamide and thiotepa are given as continuous infusions. Recent studies have shown that thiotepa reduces the bioactivation of cyclophosphamide and therefore reduces the area under the curve of the active metabolites [32, 33].

In addition, there was a long interval between the last dose of conventional therapy and transplantation. This was because of the logistics and the time required for bone marrow or stem cell mobilization and harvesting. In theory, this could have resulted in tumour-regrowth during the treatment-free interval. Two other randomized studies of HD-CT in advanced breast cancer have compared immediate versus delayed use of HD-CT. One study was in 98 hormone-refractory patients with metastatic disease in complete remission after induction chemotherapy [22] and the other was in 69 patients with chemo-sensitive hormone refractory metastatic breast cancer, which was confined to the bones [34]. Both studies found a statistically significant improvement of PFS in the immediate high-dose group (0.9 vs. 0.3 years/ p=0.04) without an overall survival advantage. Two multicentre French studies randomized 61 and 180 patients, which were responding to standard-dose chemotherapy. In the first study (Pegas 04) [35], patients were randomized to either receive two to four additional courses of conventional chemotherapy or high-dose chemotherapy (mitoxantrone 45 mg/m², cyclophosphamide 120 mg/kg, melphalan 140 mg/m² (CMA)). In the second study (Pegas 03) [36], patients were randomized after four times FEC to no further treatment or HD-CT (thiotepa 800 mg/m² and cyclophosphamide 6000 mg/m²). Both studies showed an insignificant trend for improved PFS. The Canadian study [37] randomized after four induction courses with taxanes or anthracyclins between 2-4 additional cycles of standard therapy or 1-2 cycles of high-dose treatment (cyclophosphamide, mitoxantrone, carboplatin) and found a benefit in PFS but not in OS.

Many efforts have been made to identify the characteristics associated with long-term progression-free survival following high-dose chemotherapy in metastatic breast cancer. Most studies agree that patients who are likely to benefit from HD-CT are younger patients (< 50 years) with oligometastatic disease, or with otherwise limited metastatic disease, that responds well to standard-dose chemotherapy. Oligometastatic disease is defined as a small tumour burden with the possibility of local therapy (surgery or radiotherapy) of the detectable tumour mass after HD-CT. Oligometastatic disease has been retrospectively identified as a favourable prognostic factor for outcome, independent of chemotherapy sensitivity, after both conventional-dose [29,38] and high-dose chemotherapy [37,39,40,41,42]. Schneeweis et al.
[43] found a positive oestrogen receptor or positivity of both hormone receptors and complete response after HD-CT as independent prognostic factors in a multivariate analysis. Nieto et al. [44] performed a retrospective analysis of potential prognostic variables in 60 patients with minimal metastatic disease treated with HD-CT (Cisplatin, Carmustin, Cyclophosphamide). This group found that HER-2 overexpression, the number of sites and the primary axillary nodal ratio were independent predictors for outcome. HER-2 has shown to be an unfavourable predictive factor for response to HD-CT treatment in seven other studies [45,46,47,48,49,50,51].

Different treatment strategies have been employed to improve the results of high-dose chemotherapy in metastatic breast cancer. One strategy is to introduce new agents in the treatment schedules and another one is the repeated administration of courses of high-dose chemotherapy. Our group has selected the latter strategy. A series of three consecutive full dose courses of the previously reported CTC schedule, containing cyclophosphamide 6000 mg/m$^2$, thiotepa 480 mg/m$^2$ and carboplatin 1600 mg/m$^2$, has shown to be associated with unacceptable major toxicities [52] such as haemorrhagic cystitis, veno-occlusive disease of the liver and haemolytic uremic syndrome. We reduced the dose of each course with one third and preliminary results have shown that such a regimen is feasible [53]. In this thesis, we describe the results of a phase II study applying this regimen in patients with metastatic breast cancer [54].

**Patient selection for high-dose chemotherapy in breast cancer**

There are strong indications that high-dose chemotherapy is beneficial for a subset of patients and much work has been done to identify this patient group more precisely. HER-2 is considered as an important prognostic factor. Patients with HER-2 positive breast tumours have been shown to have a poor prognosis despite conventional adjuvant therapy [55] and therefore many of these patients have participated in dose-intensive therapy regimens [56,57]. But retrospective analysis of uncontrolled high-dose studies in metastatic and adjuvant setting have shown that HER-2 positive tumours respond very poorly to HD-CT [45,46,47,48,49,50,51]. The preferential selection of HER-2 positive tumours for treatment regimens incorporating high-dose therapy may thus have resulted in a negative patient inclusion bias [58]. Retrospective studies have shown that HER-2 overexpression in tumours is associated with sensitivity to anthracyclin based regimens [59] and it is thought that HER-2 positive tumours are relatively resistant to alkylators and thus to an alkylating high-dose regimen. The fact that HER-2 tumours are more sensitive to anthracyclins can be explained by the finding that expression of HER-2 is often correlated with overexpression of topo-isomerase II alpha (Topo2A). Topo-isomerase is the target enzyme for topo-isomerase II inhibitors such as anthracyclins and in vitro data have shown a relation between the intranuclear topo-isomerase II alpha levels and sensitivity to anthracyclins [60]. In addition, it has been reported that Topo2A gene amplification, deletion or both is found in 50-80% of the breast tumours with HER-2 amplification. No Topo2A gene aberrations were found in
tumours without HER-2 overexpression [61]. In the large Dutch randomized study (N4+ study) 885 patients were randomized to either high-dose or conventional therapy. An unplanned subgroup analysis of 620 patients with no or low HER-2 expressing tumours showed a remarkably significant increase in PFS (hazard ratio 0.66, p=0.002), lower relapse rate after HD-CT (28% in the high-dose versus 41% percent in the conventional group) and a overall survival benefit for the high-dose therapy arm. This means that patients with HER-2 positive tumours had no benefit of HD-CT.

In the Dutch National study the younger patients (< 40 years) were more sensitive to HD-CT. Surprisingly, the effect of high-dose chemotherapy appears to be stronger in patients with tumours having a low grade of malignancy. These findings are different from what we assumed about sensitivity of tumours and this knowledge has consequences for the selection of patients for the HD-CT. These findings have to be verified in the other large randomized studies. In the near future it will probably be possible to identify more exactly the patients with tumours responsive to HD-CT by the new promising micro-array techniques [62].

Post high-dose chemotherapy treatment

Despite the curative intent of high-dose chemotherapy, most patients will ultimately relapse. New strategies have to be developed to maintain the PFS and achieve a cure. Initially, hormone sensitive tumours have been treated with an endocrine therapy post-HD-CT. Montemurro [63] studied 109 patients with hormone-sensitive tumours who were non-randomly assigned to maintenance endocrine treatment after HD-CT and found an association between hormonal treatment for adjuvant or metastatic disease and progression-free survival. In many trials of HD-CT the treatment regimen is followed by tamoxifen in hormone-receptor positive patients. The role of aromatase-inhibitors in this setting has not yet been evaluated.

One strategy, to attack (micro)metastatic disease is immunotherapy. Immunotherapy may not have the capacity to control a large tumour mass. From this point of view, the post-transplantation period with a maximally reduced tumour burden could be the optimal timing for immunotherapy. Subsets of cancer cells can (re)express molecules on their surface which are not typically present on the surface of the normal cells. Unfortunately, breast cancer is not a very immunogenic tumour. What is more, post transplantation T-cell function is reduced after HD-CT [64,65]. A cancer vaccine treatment strategy is based on the assumption that the immune system could react with an immune-response to the antigens on the cancer cells. This immune response may, however, be inadequate due to low immunogenicity of the antigen, down regulation of MHC molecules, lack of adequate co-stimulators or the presence of inhibitory cytokines. In our institution, De Gast et al. [66] have performed a study with interleukine-2 (IL-2), granulocyte/macrophage-colony stimulating factor (GM-CSF) and donor-lymphocyte reinfusion to induce a rapid recovery of the T-cells and thus obtain an anti-tumour effect early after transplantation. They found a rapid recovery of CD8+ T cells with granulocyte-colony stimulating factor (G-CSF) alone while the recovery of CD4+ cells required lymphocyte reinfusion and GM-CSF. It is yet unclear whether this will result in delay
of relapse or survival benefit. Porrata et al. [67] found a relation between the early absolute lymphocyte count (ALC) at day 15 post-autologous stem cell transplantation in metastatic breast cancer and the disease-free survival in these patients. In this thesis, the relationship between ALC on day 15 and survival in a similar patient group was studied and found no significant relationship (chapter 5). Cytokines such as interleukins and hormones such as prolactin [68] have been extensively studied as immunotherapy. Recently, IL-2 has been used also in breast cancer. Sosman et al. [69] described a pilot-study of mobilisation with IL-2 and G-CSF and found enhanced numbers of anti-tumour effector cells, and fewer but sufficient CD34+ cells in the mobilized graft. Moreover, post-stem-cell immune-reconstitution of anti-tumour immune effector cells was increased. Several other studies have looked in to the feasibility of IL-2 in breast cancer patients [70,71]. Some investigators have combined IL-2 with interferon [72] with or without natural killer (NK) cells [73,74] to upregulate natural killer cell function and therefore anti-tumour activity.

A range of different vaccines have been tested with inactivated and irradiated cancer cells or genetically modified cancer cells. A few pilot vaccination studies in breast cancer have been published: Wood et al. [75] immunized 20 patients in a pilot study with a vaccine made of irradiated breast cells and combined this with G-CSF. Peripheral blood mononuclear cells were collected by leukocytapheresis and expanded by IL-2 and anti-CD3. After HD-CT, lymphocytes were reinfused to eliminate minimal residual disease. Holmberg et al. [76] administered five times a cancer vaccine from day 30 after transplantation. In this non-randomized study with 40 patients they found a trend for a decrease in relapse and death with vaccination. Dendritic cell-based vaccines infusion could possibly improve the presentation of the antigen to the immune system. Only preclinical data is available [77].

The role of trastuzumab (monoclonal antibody directed to HER-2) in treatment of minimal residual disease after HD-CT in patients with HER-2 positive breast cancer is very interesting. But according to the available data, HD-CT is not of benefit in the patients with HER-2 positive tumours.

Pilot studies of other antibodies are under development (monoclonal antibody 17-1a [78]). Administration of the immunosuppressive drug cyclosporine A following autologous stem cell transplantation paradoxically elicits a systemic autoimmune syndrome resembling graft-versus host disease (GVHD) and is associated with auto reactive CD8+ T cells that recognize the major histocompatibility complex. Van der Wall et al. [79] investigated the administration of cyclosporine A and interferon gamma and found GVHD in 36 % of the patients and autotoxicity against their own lymphoblasts in vitro, but no correlation of GVHD with PFS and OS. In summary, not many clinical trials have yet been performed, and in pilot studies general immunoactivation has been seen without tumour-regression.

Another policy to control minimal residual disease is chemotherapy. Some cancer cells can escape the damaging alkylating agents in the HD-CT, for instance by DNA repair mechanisms. Patients after HD-CT are usually not treated with chemotherapy immediately after HD-CT, because the concern is that the stem cell graft is very sensitive to myelosuppression and might be irreversible damaged. Previous studies studied have shown
that taxane administration immediately after HD-CT was safe without delayed toxicities [80,81,82]. In our institute, we are now performing a study in which patients with metastatic breast cancer are treated with three courses of tiny CTC followed by weekly oral paclitaxel for 6 months. The data of this study are still immature. An additional interesting point of paclitaxel is that it has shown to have anti-angiogenic properties [83]. Other anti-angiogenic agents are not freely available for trials in this setting and their activity still has to be proven in humans.

**Aim and outline of the thesis**

High-dose chemotherapy in breast cancer appears to be an effective treatment modality with the potential to improve the adjuvant therapy for a subgroup of patients and to achieve long-term (disease-free) survival in a subgroup of patients with oligometastatic disease. Despite a complete remission after HD-CT, many patients will ultimately relapse. Apparently, HD-CT induces a state of minimal residual disease and future strategies have to be developed to eradicate these tumour cells after the HD-CT. The therapy is now acceptably safe and the aplastic phase has been shortened by the introduction of growth factors and peripheral stem cell mobilisation rather than bone marrow harvesting. The treatment regimen is loosing its unpredictable organ toxicity by the strategy of pharmacokinetic dose-monitoring. Long-term toxicity seems limited, but the follow-up time of the large randomized studies is still insufficient. The recent findings suggest that although high-dose chemotherapy is certainly not indicated for all breast cancer patients, it may become part of the standard treatment of breast cancer in a selected group of patients. It is even still possible that long-term evaluation of the patients will elucidate a survival advantage for HD-CT as the follow-up of some trials is very short.

This thesis describes the experience with high-dose chemotherapy in patients with stage II and stage IV breast cancer. It has become clear that high-dose chemotherapy is effective in only a subgroup of patients, and in these studies we focused on prognostic factors for long-term survival after high-dose chemotherapy. We also studied the efficacy of multiple courses HD-CT in stage IV breast cancer. As the studies of HD-CT have matured, it is possible to analyse both the short and long term toxicity.

In chapter 2 an overview of the literature on dose intensive chemotherapy in locally advanced breast cancer is given. In chapter 3 the final analysis of a randomized study in patients with operable breast cancer with extensive lymphnode involvement is described. In this study, we also analysed the prognostic factors for long-term survival.

Dose intensification can be done in different ways. In our institution we chose the policy of multiple courses of high-dose chemotherapy. Chapter 4 describes the results of a phase II study of multiple courses HD-CT (three courses of each two-thirds of the full course HD-CT).

Anti-tumour immunity may play a role in controlling minimal residual disease after high-dose chemotherapy. In theory, protracted recovery of the immunity could lead to defective control of minimal residual disease. In chapter 5 we looked at lymphocyte recovery after HD-CT.
Although toxicity of HD-CT has diminished by the use of growth factors and a better supportive care, it is still a toxic treatment. In chapter 6 we analysed the short and long-term toxicity in the first 100 HD-CT courses (the CTC regimen) administered in the Antoni van Leeuwenhoek Hospital.

Many patients do relapse after HD-CT and most of them receive hormonal treatment or chemotherapy. In chapter 7 we studied efficacy and feasibility of palliative chemotherapy after HD-CT.

HER-2 has shown to be an important negative predictive factor for response to HD-CT. In chapter 8 we studied HER-2 and the response to HD-CT in metastatic breast cancer. We used immunohistochemic staining, CISH and tissue microarray on the pathologic specimens of tumour tissue. We also studied the role of topoisomerase II alpha in relation to HER-2 and as predictive factor for response to HD-CT. A summary of this thesis is given in chapter 9 (summary) and 10 (summary in dutch).
Introduction


24 Crown JP, Lind M, Gould A, et al. High-dose chemotherapy (HDC) with autograft (PBP) support is not superior to cyclophosphamide (CPA), methotrexate and 5-FU (CMF) following doxorubicin (D) induction in patients (pts) with breast cancer (BC)
and 4 or more involved axillary lymphnodes (4+LN): the Anglo-Celtic I study. Proc Am Soc Oncol 2002; 21: abstr 166

25 Gianni A and Bonnadonna G. Five year result of the randomized clinical trial comparing standard versus high-dose myeloablative chemotherapy in the adjuvant treatment of breast cancer with >3 positive nodes (LN+). Proc Am Soc Oncol 2001; 20: abstr 180


34 Madan B, Broadwater G, Rubin P, et al. Improved survival with consolidation high-dose cyclophosphamide, cisplatin and BCNU (HD-CPB) compared with observation in women with metastatic breast cancer (MBC) and only bone metastases treated with


36 Biron P, Durand M, Roche H, et al. High dose thiopeta, cyclophosphamide (CPM) and stem cell transplantation after 4 FEC100 compared with 4 FEC alone allowed a better disease free survival but the same overall survival in first line chemotherapy for metastatic breast cancer. Results of the Pegase 03 French protocol. Proc Am Soc. Am 2002; 21: abstr 670


Introduction


47 Bewick M, Conlon M, Gerard S, et al. HER-2 expression is a prognostic factor in patients with metastatic breast cancer treated with a combination of high-dose cyclophosphamide, mitoxantrone, paclitaxel and autologous blood stem cell support. Bone Marrow Transpl 2001; 27: 847-853


Armstrong DK, Davidson NE. Dose intensity for breast cancer. Oncology (Huntington) 2001; 15: 701-708


Cayeux S, Meuer S, Pezzutto A, et al. T-cell ontogeny after autologous bone marrow transplantation: failure to synthesize interleukin-2 (IL-2) and lack of CD2- and CD3-mediated proliferation by both CD4- and CD8+ cells even in the presence of exogenous IL-2. Blood 1989; 74: 2270-2277


Holmberg LA, Oparin DV, Gooley T, et al. Clinical outcome of breast and ovarian cancer patients treated with high-dose chemotherapy, autologous stem cell rescue and
Chapter 1

THERATOPE STn-KLH cancer vaccine. Bone marrow transplant 2000; 25: 1233-1241


78 Hempel. Combination of high-dose chemotherapy and monoclonal antibody in breast-cancer patients: a pilot trial to monitor treatment effects on disseminated tumor cells. Cytotherapy 2000; 2: 287-296


