Individually Tailored
Toxicity-based Chemotherapy

Studies on Patients with Primary
and Metastatic Breast Cancer

BY

HENRIK LINDMAN
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Abstract
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Standard dosing of chemotherapy based on body surface area (BSA) results in large individual differences in toxicity due to a large inter-patient variability in pharmacokinetics (PK) and pharmacodynamics (PD). This results in under-dosing in certain patients with a potentially weaker antitumoral effect.

Three clinical studies of individually tailored dosing of chemotherapy, based on haematological toxicity were conducted. In the first study, 26 women with metastatic breast cancer were treated with tailored and dose-escalated 5-fluorouracil, epirubicin and cyclophosphamide, supported by G-CSF (dFEC). In the second study 525 patients with high-risk primary breast cancer were randomised between dFEC and high-dose chemotherapy with autologous bone-marrow transplantation. The feasibility of a FEC regimen with doubled cyclophosphamide dose to mobilise peripheral stem cells was investigated. In the third study, 44 metastatic patients were treated with tailored epirubicin and docetaxel (ET). PK and PD were also investigated in these patients. The potential effects of G-CSF on MRI tumour evaluation were studied in 18 patients with skeletal metastases.

Toxicity-based dosing entailed an evenly distributed two- to three-fold range of tolerated doses in all three studies. Efficacy and toxicity were not correlated to tolerated dose-levels.

Tailored dFEC resulted in a response rate of 81% and the same regimen resulted in fewer breast cancer relapses compared with standard FEC followed by high-dose therapy. Toxicity was manageable except for an increased rate of secondary leukaemia. The modified FEC could safely mobilise sufficient numbers of stem-cells. Tailored ET resulted in a response rate of 63%. The inter-individual variability in drug clearance was larger than the inter-occasion variability and a semi-physiological model of PK and PD could predict leukocyte nadir and duration. An increased diffuse MR signal in the long TE IR-TSE sequence was observed in normal bone-marrow during G-CSF treatment; this could be mistaken as disseminated metastatic disease and could obscure focal metastases.

In conclusion, the concept of individually tailored toxicity-based dosage of chemotherapy was equally feasible in primary and metastatic breast cancer, in two different chemotherapy regimens and in treatment with or without G-CSF support and may provide a pragmatic way of overcoming the shortcomings of standard BSA-based dosing.

Keywords: breast cancer, tailored chemotherapy, toxicity-based dosing, high-dose therapy, MR imaging, G-CSF, epirubicin, docetaxel, cyclophosphamide, 5-fluorouracil

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Dedicated to the memory of Astrid, our beloved daughter
List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.


V. Sandström, M., Lindman, H., Nygren, P., Lidbrink, E., Bergh, J., Karlsson, M. A model describing the relations between pharmacokinetics and hematological toxicity of the epirubicin-docetaxel regimen in breast cancer. (submitted)


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Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>5-Fu</td>
<td>5-fluorouracil</td>
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<tr>
<td>ABMT</td>
<td>autologous bone-marrow transplantation</td>
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<tr>
<td>AUC</td>
<td>area under the curve</td>
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<tr>
<td>BSA</td>
<td>body surface area</td>
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<tr>
<td>CI</td>
<td>95% confidence interval</td>
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<tr>
<td>CMF</td>
<td>cyclophosphamide-methotrexate-5-fluorouracil</td>
</tr>
<tr>
<td>CR</td>
<td>complete response</td>
</tr>
<tr>
<td>CTCb</td>
<td>cyclophosphamide-thiotepa-carboplatin high-dose treatment</td>
</tr>
<tr>
<td>CV</td>
<td>coefficient of variance</td>
</tr>
<tr>
<td>Cyclo</td>
<td>cyclophosphamide</td>
</tr>
<tr>
<td>dFEC</td>
<td>tailored and dose-escalated 5-fluorouracil-epirubicin-cyclophosphamide</td>
</tr>
<tr>
<td>Doxo</td>
<td>doxorubicin</td>
</tr>
<tr>
<td>Dtax</td>
<td>docetaxel</td>
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<tr>
<td>Epi</td>
<td>epirubicin</td>
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<tr>
<td>ER</td>
<td>oestrogen receptor</td>
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<tr>
<td>ET</td>
<td>epirubicin-docetaxel</td>
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<tr>
<td>FAC</td>
<td>5-fluorouracil-doxorubicin-cyclophosphamide</td>
</tr>
<tr>
<td>FEC</td>
<td>5-fluorouracil-epirubicin-cyclophosphamide</td>
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<tr>
<td>G-CSF</td>
<td>granulocyte-colony stimulating factor</td>
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<tr>
<td>GFR</td>
<td>glomerular filtration rate</td>
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<tr>
<td>Hb</td>
<td>haemoglobin</td>
</tr>
<tr>
<td>MR</td>
<td>magnetic resonance</td>
</tr>
<tr>
<td>NS</td>
<td>not significant</td>
</tr>
<tr>
<td>ORR</td>
<td>overall response rate</td>
</tr>
<tr>
<td>OS</td>
<td>overall survival</td>
</tr>
<tr>
<td>PD</td>
<td>progressive disease</td>
</tr>
<tr>
<td>Pd</td>
<td>pharmacodynamic</td>
</tr>
<tr>
<td>PgR</td>
<td>progesterone receptor</td>
</tr>
<tr>
<td>Pk</td>
<td>pharmacokinetic</td>
</tr>
<tr>
<td>PR</td>
<td>partial response</td>
</tr>
<tr>
<td>Ptax</td>
<td>paclitaxel</td>
</tr>
<tr>
<td>SD</td>
<td>stable disease</td>
</tr>
<tr>
<td>TDL</td>
<td>tailored dose level</td>
</tr>
<tr>
<td>TDM</td>
<td>therapeutic drug monitoring</td>
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<tr>
<td>TE IR-TSE</td>
<td>echo-time inversion-recovery turbo-spin-echo</td>
</tr>
<tr>
<td>TTP</td>
<td>time-to progression</td>
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<tr>
<td>WBC</td>
<td>white blood cell count</td>
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</table>
1 Introduction

1.1 Basic aspects of breast cancer
Breast cancer is the most common malignancy in women and is the main cause of death in young and middle-aged women (15–64 years) in Sweden. Primary breast cancer originates from the mammary glands and is, at the time of detection, usually considered curable. However, the prognosis of the disease, mainly influenced by possible regional lymph nodal metastases, is still hard to predict and the risk of developing distant metastases exist for 20 years or more. When distant metastases develop (i.e. metastatic breast cancer), the disease is generally considered fatal. During the past decades, we have experienced a considerable increase in our knowledge of the disease and in treatment efficacy. Today we are aware that we face a heterogeneous group of tumours with unique genetic characteristics in a heterogeneous group of patients in whom individualised treatment adjustments need to be further developed.

Epidemiology
Breast cancer incidence has approximately doubled compared with 1960. The number of new breast cancer cases in Sweden in the year 2001 was 6,569, of whom 33 were men, resulting in an age-adjusted incidence of 138 per 100,000 women. The number of deaths from breast cancer was 1,487, giving a mortality rate of 30 per 100,000. Breast cancer represented 29% of all new cancers in women and, for a Swedish woman, the lifetime risk of developing breast cancer is now above 12%. Most new breast cancers are detected in women aged 55–59 years (Figure 1).

The incidence of and mortality from breast cancer is markedly higher in Western countries than in Asian or African countries. Furthermore, there is 50% increased risk of developing breast cancer in urban, when compared with rural areas. The knowledge that breast cancer mortality rates among immigrants in an area tends to move towards the rate in native-born women in the same area, indicates that the relative contribution of environmental
factors is more important than that of genetic factors. The increasing incidence further implies this to be the case.

The average annual age-standardised breast cancer rate increase in Sweden has been 1.8% during the last decade, but the mortality figures have slowly decreased and are, despite the high incidence, one of the lowest in Europe. The annually increasing cure rate, since the late 1980s also observed in the United States and United Kingdom, could have many explanations, but earlier diagnosis and more efficient adjuvant therapies, are cornerstones that cannot be neglected.

![Figure 1. Histogram of the incidence (absolute number) of breast cancer in different age groups in Sweden 2001.](image)

**Aetiology**

Breast cancer was one of the first tumour types to be subjected to epidemiological research because it was easily accessible and could be correctly diagnosed. In 1743, Ramazzini from Padua in Italy reported that breast cancer was more frequent among nuns than other women. The observation that the absence of childbirth is associated with an increased risk of breast cancer has consistently been observed, and the relative risk compared with parous women has been estimated to 1.3–1.7. The knowledge that normal breast epithelial cells respond markedly to changes in hormonal concentrations made it possible to see a role of hormones as promoters of breast cancer cell growth. An early onset of menarche has a weak association to an increased breast cancer risk and the same circumstance, but better defined, has been seen for a late onset of
An interesting and confirming finding of the association between ovarian function and breast cancer evolution is the protective effect of bilateral oophorectomy. If the intervention is performed prior to 35 years of age, the relative risk is 0.36, after which it increases with age and approaches 1 at 50 years of age. The protective benefit of childbirth is more complex – both age at first birth and parity seem to play a role. An age of 20 years at first full-term pregnancy is associated with a relative risk of about 0.5 compared with nulliparous women, then slowly increasing to be >1 for women with first birth after 35 years of age.

The possible hazard of exogenous hormones, i.e. oestrogen or progesterone, used as contraceptives or as replacement therapy after menopause, has been acknowledged and resulted in a number of studies. A recent report shows clearly an increased breast cancer risk in women who undergo prolonged hormone-replacement therapy, especially with oestrogen-progestagen combinations. Other factors that influence endogenous oestrogen levels and may correlate to an increased breast cancer risk, are weight gain and high fat and alcohol intake.

Experiences from the nuclear explosion in Hiroshima and therapeutic irradiation to the breast after Mb Hodgkin has shown that radiation can initiate breast cancer, if the irradiation occurred at a younger age. Hereditary breast cancer is estimated to represent 5–10% of new cases and is mostly connected to mutations in the BRCA1 or BRCA2 genes. A lifetime risk of breast cancer of between 50–85% has been estimated for female mutation carriers.

**Diagnosis and screening of primary breast cancer**

Before the screening era, the patient herself detected 98% of all breast cancers. The primary tumour initially most often presents as a painless lump, which later can affect the skin or retract the nipple, and in more advanced cases, shows signs of fixation to the chest wall, pain, ulceration and the appearance of enlarged axillary lymph nodes. A proper investigation of a breast tumour should include, besides physical examination, mammography followed by percutaneous fine-needle aspiration for cytology or core-needle biopsy for histopathological examination (“triple diagnosis”). Ultrasonography is frequently performed as it provides additional information concerning the axillary node status, differential diagnosis and facilitates the biopsy of the lesion. Difficulties arise when, e.g. axillary metastases are discovered without any sign of a primary tumour in the breast. Additional investigations with sophisticated modalities such as MR imaging, sestamibi scintimammography or positron emission tomography.
have been reported efficient in the search for such an unknown breast
tumour.\textsuperscript{34-36}

Breast cancer fulfils the criteria of a disease suitable for screening and
seven studies of screening mammography have been reported showing a
decreased mortality of about 23\% in the group invited in the screening
programme.\textsuperscript{37} An additional, often neglected positive effect of earlier
detection is the decreased morbidity and costs because fewer patients will
require mastectomy or adjuvant chemotherapy. Criticisers of screening
mammography have pointed out the problem with over-diagnosis followed
by possible psychological distress. The number of false positive cases with
or without subsequent excision are, however, today low (about 0.1\% and
1.2-1.4\%, respectively) according to the annual reports 2000-2002 from the
Department of Radiology, Uppsala University Hospital. Future aspects of
mammography screening may concern optimal interval depending on
estimated risk.\textsuperscript{37}

\textbf{Classification}

Anatomic staging of breast cancer follows the TNM guidelines worked out
by the UICC in the mid 1970s and classifies the disease according to
prognosis.\textsuperscript{38} Progress in tumour biology and the introduction of screening
have made the TNM system less interesting as a source of prognostic
information. Nevertheless, the number of lymph node metastases is still
considered to be the strongest prognostic factor\textsuperscript{39, 40} and a larger size of the
primary tumour is correlated to a poorer prognosis. An important revision of
the TNM guidelines was recently made and was applied from January 1\textsuperscript{st},
2003.\textsuperscript{41, 42} There are important differences compared with earlier versions:
metastases in supraclavicular lymph node have been reclassified as N3 rather
than M1, micrometastases are distinguished from isolated tumour cells and
the classification of lymph node status considers the number of involved
axillary lymph nodes. Based on TNM, patients are divided into prognostic
stage groups of which stage I represent patients with early breast cancer with
the best prognosis and stage IV patients with metastatic breast cancer (Table
1).
Definition of TNM (revised version):

**Primary tumour (T):**
- T0, no evidence of primary tumour;
- T1, tumour 2 cm or less;
- T2, tumour more than 2 cm but not more than 5 cm;
- T3, tumour more than 5 cm;
- T4, tumour with direct extension to chest wall or skin, or inflammatory carcinoma.

**Regional lymph nodes (N):**
- N0, no node metastases;
- N1, metastases in 1–3 axillary nodes and/or internal mammary nodes with microscopic disease;
- N2, metastases in 4–9 axillary nodes or in clinically apparent internal mammary nodes;
- N3, metastases in 10 or more axillary nodes, or in infra- or supraclavicular nodes, or in both axillary and internal mammary nodes.

**Distant metastasis (M):**
- M0, no distant metastases;
- M1, presence of distant metastases.

Table 1. Stage grouping of breast cancer according to the revised version 2003

<table>
<thead>
<tr>
<th>Stage</th>
<th>T</th>
<th>N</th>
<th>M</th>
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<tbody>
<tr>
<td>Stage I</td>
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<td>Stage IIA</td>
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<td>Stage IIB</td>
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<td>Stage IIIA</td>
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<td>Stage IIIB</td>
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<td>Stage IIIC</td>
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<td>Stage IV</td>
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Morphological subtypes – biological prognostic and predictive factors

The histopathological characterisation of the tumour provides important information. Of several morphological subtypes, the ductal carcinoma is most common (75–84%) followed by the lobular carcinoma (12–14%).43, 44

The grade of differentiation, i.e., tumour grade, is based on three morphological characteristics: tubule formation, nuclear pleomorphism and mitotic index, each scored 1–3.45, 46 The added scores are thus in the range 3–9, and a higher score is associated with a poorer prognosis.46, 47
The determination of the S-phase reflects the proportion of replicating tumour cells at a given moment, and higher rates are related to a poorer prognosis.48-50 Patients with breast cancers characterised by a high S-phase derived greater benefit from chemotherapy while patients with low S-phases tended to derive more benefit from radiotherapy.51 This indicates that S-phase may be a useful predictive factor.

The distinct growth response in normal breast cells is mediated by oestrogen and progesterone receptors (ER and PgR), which are expressed in tumour cells in varying proportions. Less expression of ER or PgR, is observed in less differentiated tumours (i.e. high tumour grade) and are independently connected to worse prognosis.40, 52, 53 Moreover ER and PgR are the best established predictive markers of therapy outcome. Patients with tumours without, or with very low receptor levels, do not benefit from hormonal therapy while receptor-positive patients are most likely to respond.54, 55

Lately, another receptor has been the object of major attention, the tyrosine-kinase growth factor receptor HER2 (HER-2/neu, c-erbB-2, ERBB2) in the epidermal growth factor receptor family. An over-expression of HER2 predicts a possible response to therapy with a monoclonal antibody (trastuzumab, Herceptin®) targeted to the receptor.56, 57 However, less than 20% of breast cancers are estimated to have the necessary over-expression (3+) of the HER2 gene (15% are HER2 3+ according to a world-wide study).44

The newly established microarray techniques make it possible to get “finger prints” from every single cancer with information of the RNA expression of thousands of genes. Promising preliminary data have been reported5-8 and we are likely to have a new instrument for a more precise prediction of prognosis and therapy outcome on an individual level.

1.2 Treatment principles for primary breast cancer

The treatment of primary breast cancer is based on surgery, radiotherapy and systemic medical therapy. Including the diagnostic work-up, the collaboration from physicians of at least four specialties (i.e. radiology, pathology, surgery and oncology) is required in the management of every breast cancer patient. Regular multidisciplinary therapy conferences are thus desirable and are indeed mandatory at most cancer centres.
Surgery

William Halsted, the father of the era of modern breast cancer surgery, presented the radical mastectomy in the year 1894. He also introduced the previously predominant mechanistic principle of the step-wise metastatic progression of breast cancer locally or particularly via the lymphatic system (Halsted’s principle). The subsequent evolution towards more extended radical surgery failed, however, to increase survival and led to increased morbidity. A new principle of tumour progression explained treatment failure by a systemic dissemination of cancer cells from the primary breast tumour before surgery rather than insufficient loco-regional tumour cell eradication. The development of breast-conserving treatment combined with radiotherapy made it possible to reach the same cure rate with less morbidity.

Sector resection with axillary node dissection is today the first choice in small or medium-sized primary tumours, while larger tumours or multicentric lesions are preferably dealt with by modified radical mastectomy. Axillary dissection is generally considered to be a diagnostic intervention and because of the morbidity of the procedure, less extensive alternatives have been introduced. Sentinel node biopsy of the first lymph node in the drainage chain could possibly identify N0 patients who do not need to undergo a subsequent nodal dissection. Lower axillary sampling is a less extensive operation compared with axillary dissection and seems to provide sufficient diagnostic information.

Radiotherapy

In 1895, Wilhelm K Röntgen discovered X-rays, a new form of radiation that was able to penetrate various materials. A few months afterwards, the first breast cancer patient was treated with the new modality. It was early realised that large single exposures result in grave adverse effects in normal tissue and treatment schedules with smaller daily doses were established resulting in a better therapeutic index. Development of equipment and radiation physics led to more precise radiotherapy dosing and during the last decades the rapid evolution of computers has led to computerised dose-planning.

Apart from the treatment of symptomatic lesions, particularly skeletal metastases, the most important role for radiotherapy in breast cancer treatment is the postoperative local irradiation of the site of the primary tumour and the irradiation of the regional lymph nodes. Breast-conserving surgery alone leads to a local relapse risk of about 30% that is reduced by 2/3 with local adjuvant radiotherapy delivered to the breast only, to a total dose of about 50 Gy in daily 2 Gy fractions.
The treatment of regional lymph nodes is a controversial topic in oncology. During the past 50 years a large number of studies have reported a reduction of loco-regional recurrences from 30% to 10% after 20 years follow-up.\(^70\) In old studies, a clear reduction in distant relapse and breast cancer deaths were counterbalanced by an increased risk from other causes, mainly cardiovascular disease.\(^71\) However, a meta-analysis of 18 studies including more than 6,000 node-positive patients examined the benefit of loco-regional radiotherapy in conjunction with definitive surgery and adjuvant systemic treatment.\(^72\) The analysis showed a yearly reduction in overall mortality by 17%, which strongly supports the fact that a reduction in loco-regional recurrence may prevent secondary systemic spread from regional sites. This is in agreement with a hypothesis that both the old mechanistic Halstedian principle and the newer principle of breast cancer as a systemic disease, are valid. The three largest modern studies,\(^73-75\) have been analysed carefully and, interestingly, the relative reduction in loco-regional relapse is greater in patients with less extensive disease (1–3 positive nodes).\(^76-78\) Besides that, no excess cardiac mortality or morbidity have yet been observed in these studies after 15 years follow-up.\(^77\)

Further efforts to minimise radiation doses to normal tissue with optimised doses to treatment targets are desirable\(^79,\ 80\) and hopefully in the future we may be able to individualise doses and treatment, based on tumour and patient biology in a more advanced way.

**Hormonal therapy**

Since the steroid hormones oestrogen and progesterone play a major role in the aetiology of breast cancer, it is easy to understand their fundamental role in the treatment of breast cancer. The first report of endocrine therapy was published in 1896,\(^81\) where an antitumoral effect was related to surgical oophorectomy. Next step towards modern breast cancer therapy was taken when the anti-oestrogen agent tamoxifen was introduced in late 1960s. It was the first targeted therapy for breast cancer patients and tamoxifen could, in contrast to ovarian ablation, be applied in the treatment of postmenopausal women.

Another step forward was taken when measurements of hormone receptors (ER and PgR) were available. Since exclusively receptor-positive patients (about 75% of all patients) respond to hormonal therapy,\(^54,\ 55\) the efficacy of tamoxifen and ovarian ablation increased markedly when only these patients were selected for therapy. The introduction of selective aromatase inhibitors in 1990s is the latest large step in the evolution of hormone therapy for breast cancer. By inhibiting of a peripheral aromatase
inhibitor, the total body aromatisation in postmenopausal women is reduced by 97–100%\textsuperscript{82, 83} with subsequent drop in oestrogen levels.

The effect of adjuvant tamoxifen has been quantified in the Oxford overview of 37,000 randomised patients and the proportional mortality reduction was 26% when adjuvant tamoxifen was given for 5 years.\textsuperscript{54} Therefore, and because adjuvant tamoxifen therapy is associated with low toxicity, it is widely used in receptor-positive primary breast cancer patients. The effect of adjuvant ovarian ablation is well recognised, but because of the establishment of adjuvant tamoxifen therapy in premenopausal patients, it is now rarely indicated. However, adjuvant chemotherapy has, at least in elderly premenopausal patients, an ovarian ablative effect, which may partly explain the positive effect in receptor-positive women,\textsuperscript{84, 85} but an additive effect to tamoxifen has not yet revealed.

Early reports of an adjuvant study of selective aromatase inhibitor therapy (anastrozole) indicates a superior outcome compared with tamoxifen in postmenopausal women.\textsuperscript{86} As more data on this subject will soon be available, it is not unlikely that we will face a shift with the new aromatase inhibitors gradually replacing tamoxifen, a shift we have already faced in the treatment of metastatic breast cancer.

**Adjuvant chemotherapy**

The use of cytotoxic drugs to eradicate disseminated micrometastases has been tested since 1958.\textsuperscript{87-89} Modern adjuvant polychemotherapy is based on the early experiences with 12 months of cyclophosphamide, methotrexate, 5-Fu (CMF) therapy in Milan.\textsuperscript{90, 91} In 1985, the first Oxford meta-analysis was presented, and after decades of conflicting results, the benefit of adjuvant polychemotherapy was clearly demonstrated compared with no chemotherapy or single-agent chemotherapy.\textsuperscript{92}

Present recommendations for adjuvant chemotherapy are derived from the Oxford overviews and St Gallen consensus on prognostic factors.\textsuperscript{78, 93} The 1995 Oxford overview showed an annual mortality reduction of 15%, with a clear trend of more benefit in younger than older women. The relative risk reduction was equal in node-negative and node-positive patients. No significant differences were seen in the relative reduction in recurrence in receptor-positive (33%) and receptor-negative patients (40%), aged <50. However, in older women, in whom only a minimal ovarian ablative effect may interfere, the proportional risk reduction was superior in receptor-negative cases compared with receptor-positive women (30% versus 18%). Anthracycline-based polychemotherapy gave a relatively improved survival of 11% compared with the older CMF-regimen and the recommended standard adjuvant chemotherapy today is based on anthracyclines (e.g. FEC)
except in the case of elderly patients with cardiovascular disease. There is no clear consensus of the total treatment time, but at least 6 courses, given every 2–4 weeks, are recommended.94, 95

It is thus reasonable to offer adjuvant polychemotherapy to patients up to 60–70 years of age with node-positive breast cancer or node-negative breast cancer with poor prognosis (i.e. with high tumour grade/ high proliferation rate, receptor-negative tumour or large primary tumour).78 Additionally, because of their poorer prognosis, all women aged <35 years are recommended chemotherapy, irrespectively of other prognostic factors.78

The benefit of adding taxanes (paclitaxel, Ptax and docetaxel, Dtax) to standard adjuvant chemotherapy is under investigation in several trials involving >24,000 women.95 Early results have been presented from four studies, but without confirmatory evidence of the value of adjuvant taxanes in terms of increased survival rate.94-98 However, large amount of mature data will relatively soon be available and it is not unlikely that future adjuvant polychemotherapy will include taxanes.

1.3 Metastatic breast cancer

In Sweden, 1,500–1,600 new patients will develop metastatic breast cancer every year, based on the annual mortality rate.1 The median survival from time at diagnosis of metastases is estimated to 18–24 months and the 10-year survival is 2–5%.4

**Basic aspects of metastatic breast cancer**

Metastatic breast cancer reflects a completely different situation compared with earlier breast cancer stages. The condition is considered incurable and will generally require continued therapy and monitoring during the patients remaining life. The broad variations in prognosis and sensitivity to different treatments together with the need for continuous re-evaluations challenge the medical profession. Survival benefit and freedom from tumour-related symptoms are counterbalanced with the treatment-related toxicity and the patients’ quality of life. The treatment is, therefore, often tailored to an individual to a higher extent than adjuvant treatment ever will be.

Systemic medical therapy with, e.g., hormonal agents, chemotherapy or antibodies could prolong survival and prevent global symptoms whereas local treatments with, e.g., radiotherapy or surgery are mainly used for the palliation of local symptoms. Different diagnostic radiological interventions are mostly used to define the extent of the disease and histopathological classification of biopsy specimens may define the tumour-associated
prognostic and predictive factors. The most common sites for metastases are the skeleton, lung and liver.\textsuperscript{99} A better prognosis is associated with initial bone metastases, and worse prognosis is reported for patients with liver metastases.\textsuperscript{100}

**Hormonal treatment of metastatic breast cancer**

An immense number of therapy options have been tested for the systemic treatment of metastatic breast cancer. They can mainly be divided into hormonal therapy and chemotherapy, and, recently, therapy with antibodies has become a third option. Combining drugs or giving them sequentially as single agents is a fundamental issue, as the first option usually gives higher response rates at the price of more toxicity. Important questions for the patient are also the length of the treatment and the possibility of inducing new remissions with subsequent lines of therapy.

Because of the lower toxicity and long-standing responses, initial hormone therapy is often preferred instead of chemotherapy to a receptor-positive patient. Different factors considered to support the choice of first-line hormone therapy are old age, long relapse-free interval (i.e. a slow-growing tumour), few organ systems involved and absence of visceral metastases. Contrasting factors probably supporting initial chemotherapy are, besides a receptor-negative cancer, younger age and signs of a more aggressive cancer (e.g. short relapse-free interval, visceral metastases or metastases in several organ systems).\textsuperscript{101, 102} However, except for receptor negativity, the evidence behind the recommendations is not strong and basically reflects the fact that chemotherapy may induce responses earlier than hormone therapy.

Hormone therapy has reported an ORR of about 30% in older studies and up to 50% in receptor-positive women.\textsuperscript{103} The responses may last for years and are proportional to the levels of hormone receptors.\textsuperscript{104, 105} During the last decades, the dominating first-line hormonal treatment has been tamoxifen. The situation has, however, changed recently in the case of postmenopausal women. Studies have shown the superiority of selective aromatase inhibitors compared with tamoxifen.\textsuperscript{106-110} For postmenopausal patients, therefore, the first-line hormonal treatment today is an aromatase inhibitor. A benefit of combining different hormonal strategies is not proven, except for the addition of tamoxifen to ovarian ablation.\textsuperscript{111} Third-line treatment alternatives are, e.g., progesterone agents or the recently introduced antioestrogen fulvestrant.\textsuperscript{112}
Chemotherapy of metastatic breast cancer

Chemotherapy has been used in the treatment of metastatic breast cancer since the 1960s. The survival gain of the earlier combinations has been estimated to 6–9 months. An overview of trials involving >30,000 patients with metastatic lesions showed that the addition of concurrent hormonal therapy to chemotherapy does not add survival benefit and is usually not recommended. Polychemotherapy was superior to monotherapy, anthracycline-containing therapy was better than non-anthracycline combinations and administration of higher doses was superior to lower doses according to the overview. On the basis on these findings, the recommended first-line chemotherapy now is based on anthracyclines (e.g. FEC, FAC).

The introduction of taxanes (Ptax and Dtax) has somewhat stressed this strategy. Dtax has showed better efficacy than Doxo and Ptax in randomised studies, which qualify Dtax as the most active cytotoxic agent in breast cancer. Eight studies have compared taxane-anthracycline combinations with non-taxane-anthracycline combinations in first-line treatment of metastatic breast cancer. An uniform observation was the better response rate and time to progression in patients treated with the taxane-anthracycline combinations, but a survival benefit has only been observed in 3 of the studies. Only one of these studies investigated the Epi-Dtax regimen (ET) and this study showed a superior response rate for ET compared with the FEC regimen (ORR 63% versus 34%), and a longer time to progression and survival was observed in patients treated with the ET regimen. The rational of combining taxanes and anthracyclines instead of giving them sequentially has, however, been challenged by a large three-armed study of single-drug Ptax, Doxo and a combination of the two. Despite a better ORR and TTP, the combination did not entail any survival benefit.

In second-line treatment there are solid data on the benefit of Dtax in anthracycline resistant patients. Patients treated with the combination of capecitabine, an oral 5-Fu prodrug, and Dtax survived for a median of 3 months longer than patients treated with the single agent Dtax; this makes this combination a robust second-line alternative. Capecitabine is, moreover, approved for “third-line treatment in anthracycline and taxane-resistant patients with metastatic breast cancer.

Patients with HER-2 positive tumours are now candidates for up-front treatment with the monoclonal antibody trastuzumab as single drug or in combination with a cytotoxic agent. Except for cardiac toxicity, trastuzumab has few side-effects and a possible synergy mechanism with cytotoxic drugs has been proposed. The combination of trastuzumab with taxanes has demonstrated superior ORR and survival compared with single agent taxanes.
Response evaluation of metastatic breast cancer

In the absence of severe toxicity, the treatment of metastatic, or locally advanced, breast cancer is usually continued for as long as the cancer responds. Repeated evaluations of the tumour status are, therefore, necessary to avoid prolonged and pointless therapy. Another important reason for proper response evaluations is the use of tumour response as a marker of antitumoral activity in phase II trials. The results from these studies are important for the direction of cancer research because they indicate if a drug or regimen merits further investigations.\textsuperscript{133}

WHO criteria have been the mostly used criteria to determine an objective response.\textsuperscript{134, 135} The criteria classify disease as bi- or uni-dimensionally measurable, evaluable and unevaluable and categorise response as complete response (CR, disappearance of all disease for \(\geq 4\) weeks), partial response (PR, a \(\geq 50\%\) decrease of total tumour area for \(\geq 4\) weeks), stable disease (SD) and progressive disease (PD, an increase of \(\geq 25\%\) in one or more lesions or new lesions). The new RECIST criteria, a result of an international collaboration,\textsuperscript{136} use the same four response categories, but lesions are measured uni-dimensionally and are now classified as measurable, non-measurable and truly non-measurable.

A development towards more advanced and reproducible methods for assessments of tumour response is ongoing. Computerised tomography (CT) and magnetic resonance (MR) imaging are becoming more common. The requirement of measurable disease in studies has the weakness that only a part of all metastatic breast cancer patients can be included, making the conclusions from such studies only applicable in a subgroup of patients with metastatic breast cancer. Bone metastases are considered non-measurable\textsuperscript{135, 136} and therefore patients with bone metastases only are excluded from a vast number of therapeutic studies.

Diagnosis of bone metastases with magnetic resonance imaging

Bone metastases are mostly multiple and may be diffuse. The initial seeding of micro-metastases via the circulation takes place in the vascular-rich red bone marrow that is found in the bones of the axial skeleton (spine and pelvis), cranium or proximal femora or humeri and over 90\% of the lesions are found in these areas.\textsuperscript{137, 138} When the metastases later destroy >30-50\% of the spongy cancellous bone or involve the compact cortical bone, they are detectable with conventional radiography,\textsuperscript{139} thus making the method relatively insensitive. The lesions cause architectural changes that could appear lytic, sclerotic (blastic) or mixed lytic and sclerotic on radiographs.
Response in lytic lesions generates a sclerotic rim, which advances from the periphery to towards the centre, whereas progression of lesions is indicated by the enlargement or loss of sclerosis. Sclerotic lesions are considered difficult to evaluate. WHO and RECIST criteria for the objective response of bone metastases is based on conventional radiography and is due to the low sensitivity of the modality, relatively uncertain.

**Figure 2.** Tumour mass (TMI) changes on T1-weighted and long TE IR-TSE sequences between baseline and follow-up examinations in relation to the results of the combined response evaluation (i.e. conventional radiography, bone scan and clinical assessment), from Ciray, Lindman et al.

MR is the most sensitive imaging modality for detecting bone metastases with superior anatomical resolution of bone and soft tissues. The value in therapy evaluations is, however, less investigated. In T1-weighted sequences, tumours usually show decreased signal intensity vis-à-vis fat in bone marrow. Fat-suppressed IR-TSE sequences may also provide good contrast between tumours and surrounding bone marrow with the tumour showing increased signal intensity and are recommended in combination with T1 sequences.

Because of the excellent sensitivity of MR imaging, the modality may be possible for objective therapy evaluations of bone metastases. T1-weighted sequences are useful in detecting progression, but the value in detecting responses has been questioned. We have performed a study in 18 breast cancer patients with bone metastases evaluated with T1 and long TE IR-TSE.
sequences, with the conclusion that both sequences provide valuable information in objective response evaluation. Long TE IR-TSE sequences seemed to be more accurate than T1 (Figure 2).

1.4 Cytotoxic drugs

The discovery of modern chemotherapeutic agents began after an observation that exposure to mustard gas, after an explosion in Bari Harbour during World War II, caused bone marrow suppression. Since 1958, the National Cancer Institute (NCI) has conducted a drug development programme by mass-screening of hundreds of thousands promising agents over the decades. However, this empirical evaluation of cytotoxic drugs is slow and inefficient and recent progress in biochemistry and genetic mechanisms makes rational molecular targeted drug design possible.

<table>
<thead>
<tr>
<th>Alkylators and alkylator-like drugs</th>
<th>Antimetabolites</th>
<th>Topoisomerase inhibitors</th>
<th>Microtubule interacting agents</th>
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<tr>
<td>Cyclophosphamide</td>
<td>Methotrexate</td>
<td>Daunorubicin</td>
<td>Vincristine</td>
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<td>Ifosfamide</td>
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<td>Imatinib</td>
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*Table 2. Approved cytotoxic drugs in Sweden in 2003 according to their mechanism of action. Agents, which are most effective and frequently used in breast cancer are marked in bold.*

Presently there are 48 cytotoxic drugs available in Sweden and they can be classified by their mechanism of action (Table 2).

*Alkylators and alkylator-like drugs* bind to DNA or other cellular macromolecules. This causes cross-linking and abnormal base pairing that interferes with DNA replication. Cyclophosphamide (Cyclo) is the most
important alkylator in breast cancer therapy. It needs an activation process, which starts in liver cells by converting Cyclo to 4-hydroxycyclophosphamide. Excretion of active metabolites in the urine can lead to haemorrhagic cystitis that may be prevented by inactivating the urine metabolites by using mesna. Cyclo produces significant leukopenia and immunosuppression but only mild thrombocytopenia. It is associated with nausea, vomiting and alopecia and is considered leucomogenic in humans.

Antimetabolites inhibit key steps in the synthesis of DNA and RNA by, replacing important molecules, leading to inhibited cell growth and cell death. 5-Fluourouracil (5-Fu) requires intracellular activation and its metabolites act in different ways. The activity and mechanism of action of the drug is highly dependent of the infusion rate and 5-Fu may be considered two different drugs if used as short-term bolus injections or as prolonged low-dose infusions.148 Rapid breakdown in liver gives a short plasma half-life, but when using bolus injections, shorter injections give larger exposures indicating a saturable metabolic process. Gastrointestinal toxicity and mucositis are the main side-effects.

Topoisomerase inhibitors interact with the nuclear enzymes topoisomerase I and II, causing permanent DNA breaks. The most important subgroup is the anthracyclines, which are among the most active agents in breast cancer and are further described in the following chapter.

Microtubule interacting agents affect the cellular cytoskeleton by stabilising or disrupting microtubules, leading to disturbed mitosis and inhibited transport mechanisms. The subgroup taxanes are of growing importance in breast cancer treatment because of their high activity and they are described in the following chapter.

Anthracyclines
The first anthracycline, daunorubicin, was isolated from a Mediterranean streptomyces specimen in 1957. A mutated variant of streptomyces produced doxorubicin (Doxo) and several additional agents were later synthesised.

Doxo and epirubicin (Epi) are the most widely used anthracyclines in breast cancer therapy. The drugs are metabolised significantly in the liver and a decreased liver function, indicated by elevated bilirubin, should entail decreased dosing. The main and dose-limiting toxicities are bone-marrow suppression and mucositis, and alopecia and nausea are other adverse effects. An important and troublesome side-effect is cardiac toxicity which could either be acute or, more commonly, a chronic congestive heart dysfunction related to the given cumulative dose. For Doxo a maximal cumulative dose of 550 mg/m² is recommended.149
Epi is claimed to exhibit less cardiac toxicity, thrombocytopenia and mucositis\(^{150}\) and the cumulative limit is 1000 mg/m\(^2\).\(^{149}\) The corresponding active dose for Epi is, however, higher than for Doxo. The therapeutic index for Epi and Doxo are probably almost similar if the Epi dose is about 1.5 times that of the Doxo dose,\(^{151}\) but Epi-regimens will permit more treatment cycles before the maximal cumulative dose is reached.\(^{113}\)

Anthracyclines are mostly given as short infusions (<1 h) in 3-week cycles and longer infusions (>96 h) produce a different toxicity profile with less myelosupression and cardiac toxicity, but with more mucositis.\(^{152}\) As extravasation of these agents causes severe local reactions, the infusion needs careful observation and short infusions are therefore mostly preferred. Therapy with a newly approved liposomal preparation of Doxo (Caelyx\(^{®}\)), however, mimics prolonged infusions of Doxo with following less cardiotoxicity,\(^{153}\) but the role of this drug in breast cancer treatment is not yet established.

**Taxanes**

Paclitaxel (Ptax) is isolated from the bark of the Pacific yew, *Taxus brevifolia*, and was initially tested as a crude extract in the NCI screening program in 1963. High efficiency in the treatment of ovarian cancer and later in breast cancer was demonstrated, and in 1993 the drug was commercially available. Ptax binds to microtubules leading to stabilisation and morphological defects,\(^{154}\) which are critical during cell cycle interphase and mitosis. The drug is mainly metabolised in the liver or excreted unchanged in bile. Its effect and toxicity is dependent on the dose rate and it, in contrast to the anthracyclines and 5-Fu, shows more severe myelosupression after prolonged (>24 h) infusions.\(^{155}\) The significant dose-limiting toxicity in clinical use is, however, neurotoxicity.

Docetaxel (Dtax) is a semisynthetic Ptax analogue\(^{156}\) produced from the needles of a European yew species, *Taxus baccata*. It shows a different efficacy and toxicity profile and Dtax myelosuppression is not dose-rate dependent, as with Ptax. The dose-limiting toxicity is neutropenia.\(^{157}\) Dtax has some unique side-effects: fluid retention might evolve after a few treatment cycles, which have to be prevented or delayed by premedication with corticosteroids.\(^{158}\) Furthermore, severe disruptions in nail growth are common. Both Ptax and Dtax produce pronounced alopecia.

The taxanes are undoubtedly very active in breast cancer. Ptax has in single drug therapy showed a response rate close to that of Doxo.\(^{121}\) Dtax provides an even higher activity than Doxo.\(^{115}\) Only one randomised comparative trial has been presented and showed a statistically better TTP
and OS for Dtaxis compared with Ptaxis, but more grade 3–4 toxicities were reported.116

1.5 Dosing of cytotoxic drugs

**Chemotherapy-induced toxicity – general aspects**

A major obstacle with chemotherapy is the fact that the agents not only affect tumour cells but all cells in the body. In general, actively dividing cells are more vulnerable to chemotherapy than non-dividing cells. Therefore, toxicity is mostly observed in the bone marrow, gastrointestinal tract, hair follicles and gonads. Cardiac toxicity and neurotoxicity, which are typically associated with certain drugs, are exceptions to this rule, reflecting different sensitivity in different tissues, probably based on the agent’s mechanism of action and distribution. The grade of the toxicity is typically proportional to the exposure of the drug and could be cumulative. The NCI common toxicity criteria (NCI-ICI)159 are the mostly used grading for toxicity, scaling from no toxicity (grade 0), mild (grade 1), moderate (grade 2), severe (grade 3), life-threatening toxicity (grade 4) to death (grade 5). A newly revised version prefers to use the term “adverse event” instead for “toxicity” (NCI-CTCAE).160

The most frequent dose-limiting toxicity (DLT) for cytotoxic drugs is myelosuppression, which could result in lethal leukopenic infections, bleeding caused by thrombocytopenia and anaemia. The pluripotent haematopoietic stem cell is the origin of all blood cells. As it differentiates, it become restricted to one or more haematopoietic lineages, initially forming lymphoid stem cells or myeloid multipotent stem cells, which in following maturation steps become granulocytes, erythrocytes, monocytes and platelets. The time for proliferation, maturation and the lifetime of the mature cells in blood are reflected by the nadir count after therapy with cytotoxic agents, which is earlier in granulocytes than in platelets and erythrocytes. The stem cells have low mitotic activity and are in general less vulnerable to cytotoxic drugs than the proliferating precursor cells.161 The differences in the time for blood count decrease, nadir and recovery of granulocytes for different drugs are probably reflecting the fact that stem cells or earlier progenitor cells are more sensitive to certain drugs (e.g. busulfan and nitrosureas).162 Moreover, different drugs seem to affect the haematopoietic lineages to different degrees, e.g., thrombocytopenia is more common after therapy with anthracyclines than taxanes. Haematological adverse events can easily be measured and monitored by blood samples for
WBC, neutrophil count, platelet count and Hb and considered critical limits (grade 4 toxicity) are WBC <1.0 x 10⁹/L, neutrophils/granulocytes <0.5 x 10⁹/L, platelet count <25 x 10⁹/L and Hb <65 g/L.¹⁶⁹

Non-haematological adverse events are mostly subjectively reported by the patient and can therefore in most cases not be objectively measured. These side-effects probably cause more distress than haematological toxicity, with a subsequent major impact on the patient's quality of life, which has to strongly be considered in palliative treatment. Nausea and vomiting, which in many cases can be severe, follow chemotherapy treatment in the acute phase (1–24 h after treatment) and a delayed phase (about 1–5 days after treatment). Prophylactic treatment with modern antiemetics, i.e., the 5-HT3 receptor antagonists, combined with corticosteroids are effective in most patients, and are mandatory in treatment with the most emetogenic drugs (e.g. the platinum drugs, and the anthracyclines). Poor control of nausea could result in anticipatory emesis, which typically begins before the administration of chemotherapy and is difficult to prevent. Mucositis and diarrhoea are treated on an individual basis.

Pharmacokinetics and pharmacodynamics

Due to the narrow therapeutic windows of cytotoxic drugs, a knowledge of pharmacokinetics (Pk) and pharmacodynamics (Pd) is important. Pk defines the quantitative study of the concentration-time profile of the drug in the body including the absorption, distribution, metabolism and excretion of the drug. A knowledge of Pk is usually obtained by repeated analyses of the concentration of the drug or its metabolites in plasma. Analytical and statistical developments have introduced the possibility for more patient friendly strategies for Pk determination. Sparse blood sampling can provide extensive information when using a population model developed in a computer program (NONMEM)¹⁶³ as it is possible to obtain individual parameter estimates (empirical Bayes estimates).¹⁶⁴-¹⁶⁶ The variation in plasma concentrations during time describes a curve and the actual exposure is mostly summarised as the area under this concentration-time curve (AUC). The elimination capacity, i.e., drug clearance, is the crucial parameter determining the exposure for an intravenously administered dose.

Intravenous administration of 5-Fu, Epi, Cyclo and Dtax result in large inter-patient differences in terms of drug clearances and AUC.¹⁶⁷, ¹⁶⁸ However, intra-individual differences also exist, i.e., variation in clearances and hence AUCs in the same patient at different time-points.¹⁶⁸ A potential explanation for inter-patient variations is, apart from intrinsic factors, the diurnal variations in Pk (chronobiology) of cytotoxic drugs.¹⁶⁹
Pd describes the effects of the drug in the body in terms of both efficacy and toxicity. As the efficacy and toxicity (Pd) of many cytotoxic drugs are dependent on the drug administration schedule,\textsuperscript{170} when the Pd cannot only be related to the absolute AUC, but also to the shape of the concentration-time curve. Mathematical models describing the relationship between the concentration and the effect have been published.\textsuperscript{171, 172} The inter-individual difference in Pd is large, demonstrated by the 50-fold difference in cytotoxic effect, which was described when bone marrow cells from 36 healthy donors were incubated in vitro with topoisomerase inhibitors and an antimetabolic agent at different concentrations.\textsuperscript{173} The inter-individual differences in Pd could, together with differences in Pk, explain the large variability in toxicity seen in a group of patients treated with same drug dose.

Pk-Pd models, describing the relationship between the drug dosage, its plasma concentrations and the effect could be of importance in the development of chemotherapeutic regimens. In recent years, semi-physiological mechanistic models of myelosuppression developed for estimation in advanced computer programs have been introduced.\textsuperscript{174} These models have the advantage that they can explain and predict both the degree and duration of neutropenia after treatment, for different drugs in different schedules. The most sophisticated model includes a self-renewal mechanism for the progenitor cells, a feedback parameter for circulating cells and separated system-specific and drug-specific parameters.\textsuperscript{175}

The standard dosing algorithm for cytotoxic drugs

In cancer treatment it is customary to adjust the dose of the cytotoxic agents according to the patient’s body surface area (BSA), but why? Early reports showed a correlation between BSA and the basal metabolic rate.\textsuperscript{176} This correlation could also be seen between different species (the mouse-elephant curve) and, without doubt, better cross-species comparisons can be made when using BSA instead of weight.\textsuperscript{177} Moreover, based on a retrospective analysis of chemotherapy in children and adults, adjustment for BSA was described to result in the same doses.\textsuperscript{178} BSA correlates to renal function but not to hepatic function.\textsuperscript{179} In conclusion, BSA has been feasible for drug development using animal models and in dosing in paediatric oncology. However, the mostly used calculation of BSA is based on a formula of height and weight from 1916, based on only 9 patients.\textsuperscript{180}

The main purpose of phase I studies is to define the maximally tolerated dose (MTD) in humans defined in mg/m\textsuperscript{2}. Only a limited number of patients are treated and normally three patients enter each dose level. The dose levels are escalated by a system of decreasing increments until dose-limiting toxicity (DLT) is reached. The recommended dose per m\textsuperscript{2} for the next step in
the clinical development, i.e., phase II studies of efficacy, is normally the
dose level below the MTD, thus determined in a very small group of
patients.

Since 1990, numbers of papers have criticised BSA-based dosing.\textsuperscript{167, 179,\linebreak 181-187} Retrospective data from 1650 patients who were treated with 33
cytotoxic agents during 1991–2001 at 3 institutions showed that only for five
agents, clearance and BSA were significantly correlated.\textsuperscript{188} In these agents
(DHA-paclitaxel, Eniluracil/5-FU, troxacitabine, paclitaxel and
temozolomide), BSA could only explain 15–35\% of the inter-individual
variations in clearance and the authors recommended that the practice of
calculating starting doses on the basis of BSA in phase I studies should be
abandoned. A review with the same conclusion,\textsuperscript{167} noted that in many drugs
(e.g. irinotecan) the variability in clearance, in fact, increased when
expressed relative to BSA. The agents 5-Fu, Epi, Cyclo and Dttx used for
breast cancer therapy, demonstrates no significant correlation between drug
clearance and BSA.\textsuperscript{167, 181, 183, 189-191}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Variability in the plasma clearance of commonly used cytotoxic drugs
expressed as coefficient of variation (\%CV) in a group of 2355 patients. Adapted
from Felici et al.\textsuperscript{167}}
\end{figure}

The known large variability in Pk (Figure 3) could be multiplied with
individual variations in Pd, explaining the very large variability in efficacy
and toxicity for different patients treated with same doses, e.g., some patients
could experience life-threatening toxicity, while others are unaffected. In the
clinical management of patients treated with chemotherapy, dose reductions
are made for the next treatment cycle for those patients with unacceptable
toxicity, whereas patients without toxicity or with moderate toxicity usually
continue with unaltered doses. One may suspect that patients with low toxicity have a combination of relatively high plasma clearance of the drug (Pk) and low cellular sensitivity for the actual drug exposure (Pd). Other Pk parameters concerning the transport and diffusion of the drug from plasma to the specific tissue could also be involved.

With this in mind, it is not difficult to propose a hypothesis that patients with low toxicity might receive insufficient doses of cytotoxic drugs for optimal antitumoral effect. In the investigation of this issue, six retrospective analyses concerning adjuvant treatment of breast cancer have been presented. The first study showed that a WBC nadir of <2.0 was significantly correlated with lower risk for distant metastases (Hazard ratio 0.37) after adjuvant treatment with FEC and oral florafur. A similar finding, when using adjuvant CMF treatment, was presented by Poikonen and co-workers. The third important observation was made when four international CMF-studies were analysed for potential dose-response effects. Patients who, because of toxicity, needed moderate (65–84% of full doses) dose reductions had a better survival than the group of patients without or with less than 15% dose-reductions. During 2002, further studies were reported. The first was presented by Cameron and co-workers; 750 patients treated with CMF were analysed. Patients with grade 2–3 neutropenia had a significantly better survival than those patients with grade 0, 1 or 4 neutropenia. This finding was, however, not confirmed in a smaller Polish study presented at same meeting. The last study by Paridaens and co-workers, in agreement with the four first studies, found that a higher neutrophil count on day 8 was one of three prognostic factors significantly predicting a poorer prognosis after adjuvant CMF treatment in breast cancer.

In conclusion, standard BSA-based dosing fails to normalise individual differences in Pk and evidence from 5 of 6 studies supports the hypothesis that lack of haematological toxicity by adjuvant chemotherapy is connected with less antitumoral effect, resulting in a poorer prognosis.

Alternative dosing algorithms

One strategy for better normalisation of drug exposure is the estimation of renal elimination or liver metabolism in the patient with subsequent dose adjustments. The first strategy could be successful with drugs characterised by predominantly renal elimination (e.g. the platinum drugs) as it correlates closely with glomerular filtration rate (GFR). The best example is probably the standard dosing of carboplatin based on Calvert’s formula which is based on GFR. Most cytotoxic drugs are metabolised in the liver and thus decreased hepatic function has been described related to larger drug
exposure for many of them (e.g. anthracyclines, taxanes). One practical consequence of this is the recommended dose reductions for Doxo, Epi, Ptax and Dtax in patients with severe liver dysfunction. However, no established methods for replacing BSA-based dosage with parameters estimating the liver function or other metabolic processes exist today.

The straightforward strategy of Pk measurements in patients for subsequent dose individualisation has successfully been tested in a randomised trial in children with acute lymphoblastic leukemia. The drug doses were either based on BSA (control group) or based on the rates of clearance of three medications in each patient, i.e., doses were increased in patients with rapid clearance and decreased in patients with very slow clearance. Among the children with B-lineage leukaemia, the individualised-treatment group had significantly better disease-free survival (76% versus 66% at 5 years).

In this thesis a pragmatic third strategy of dosing was explored – toxicity-adjusted dosing. By increasing or decreasing subsequent doses, based on the toxicity produced from the previous course, both Pk and Pd differences between patients may be corrected. During 1992–1993, an escalation schedule was developed consisting of six dose-levels of FEC. Patients initially received a standard dose, based on BSA and, based on their haematological toxicity, escalations or reductions were carried out until the targeted toxicity was reached. A similar strategy has been used by Hortobagyi and co-workers in two small randomised studies in metastatic breast cancer treated with 5-Fu, Doxo and Cyclo based regimens (FAC) and conducted between 1975–1981. FAC was dose-adjusted in both treatment arms with the difference that dose-levels were higher in the experimental arm and this group of patients was treated in a special protected environment unit. These studies were considered “negative” as no differences between high-dose or standard FAC were revealed, but the response rates were considerably high (78–84%) in both treatment arms in both studies. A third study from the same group investigated escalating doses of Doxo and Cyclo in combination with vincristine and prednisolone (CHOP) in the adjuvant treatment of breast cancer. Interestingly, the outcome was not correlated to the tolerated dose-intensity, which at that time was considered as lack of evidence for higher drug intensity. However, this outcome is in agreement with the earlier stated hypothesis that the grade of toxicity and efficacy are connected.

**Is there a dose-response relationship?**

A relationship between dose and effect has been shown in most pre-clinical models. The dose-response curve is usually sigmoidal in shape, with a
threshold, a lag phase, a linear phase and a plateau phase. In rodent models, a reduction of dose of 20% in the linear phase, leads to a loss of 50% of the cure whereas a twofold increase could result in a tenfold increase in tumour cell kill.

This knowledge has influenced the development of clinical treatment schedules, but since the dose-relationship in solid tumours seems to be of less magnitude or importance than in haematological malignancies, the role of doses in the treatment of breast cancer has been debated for decades. A calculation of the dose intensity expressed as mg/m²/wk is usually carried out to allow comparison of different treatment schedules as the interval between treatment cycles is of importance. Furthermore, the total cumulative dose will most probably influence the outcome in curative or adjuvant settings.

![Figure 4: Dose-effect relationship from 7 randomised studies of different doses of Epi given as a single drug or in combination with 5-Fu and Cyclo (FEC). The actual administered dose intensities are shown on the x-axis; 20 mg/m²/wk represents the dose-intensity of standard FEC (600/60/600 mg/m²).](image)

Early retrospective studies of Hryniuk and co-workers demonstrated a linear correlation between CMF dose intensity and response and, in the adjuvant setting, disease-free survival. Two small randomised studies of CMF and Doxo in the late 1980s support this finding, suggesting that lower doses than standard are associated with less antitumoral effect. During the
1990s were six larger randomised studies of different Epi doses were conducted in metastatic breast cancer. The dose-response relationship in Epi-studies which have reported the actually administered dose-intensity are described in Figure 4. The results were uniform, in that increased dose intensity was associated with improved response rates up to a given dose intensity of 30 mg/m²/wk; however, a dose intensity greater than this did not seem to be more efficient. In the previously described overview of metastatic breast cancer, Fossati confirmed the dose-response effect, with poorer survival (Hazard ratio 0.90) observed in patients randomised to lower dose levels.

In the adjuvant setting, two larger studies of anthracycline-based treatment showed superior survival for patients randomised to the higher doses. However, a recent large study of Doxo dose escalations (60 mg/m², 75 mg/m² or 90 mg/m²), in combination with Cyclo, resulted in similar survival for the different treatment groups. This finding may reflect that a dose-effect threshold exists above the standard doses for anthracyclines, but the result could be influenced by the different schedules the two higher doses were given at (day 1 and 2). Moreover, two studies of escalated Cyclo doses have failed to show any benefit for dose-escalations of this particular agent.

There exist two principal approaches to achieve higher dose intensity: by increasing the dose (intensification) or by shortening the cycle interval (acceleration, “dose-dense”). Since many drugs are schedule dependent, one cannot assume that the outcome will be the same for these two different strategies. This has been shown in a study of metastatic breast cancer patients treated with FEC at the same dose intensity, but delivered weekly or every fourth week. That study demonstrated more toxicity and efficacy for the four-weekly schedule. This observation points out that crude dose-intensity comparisons between studies with dissimilar schedules could be unreliable. This issue is of current interest as new treatment schedules with sequential single agents at high doses have been developed, as well as concepts with accelerated intervals.

**Role of granulocyte-colony stimulating factors (G-CSF)**

It is well known that specific factors control haematopoiesis, acting on early cells in the haematopoietic system to produce mature functional cells. The isolation and production of these growth factors have lead to a new class of therapeutic agents, of which granulocyte colony-stimulating factor (G-CSF) is of interest as it acts on neutrophils, which provide important defence against infections. Endogenous G-CSF is produced by a variety of cell types including monocytes, fibroblasts and endothelial cells, and is released
rapidly into the blood in response to infections. The effect is mediated through the G-CSF receptor which is expressed almost exclusively on the cells of the neutrophilic granulocyte lineage. The major biological responses are enhanced proliferation and differentiation of the immature neutrophil granulocyte precursor cells as well as enhanced competence of the mature neutrophils.

Filgrastim (r-metHuG-CSF) is a bacterially synthesized recombinant protein form of G-CSF and it was in 1991 the first agent to be approved for clinical use. Two years later lenograstim (rHuG-CSF), a human-identical glycosylated form, was approved for marketing. Treatment with G-CSF gives an increase in neutrophil count within 4–6 h with a peak at 8–12 h. There is a marked increase in the proportion of promyelocytes and myelocytes in bone marrow followed by an expansion in the population of metamyelocytes, bands and neutrophils. Transient time through the marrow is accelerated substantially. G-CSF is usually given subcutaneously in daily doses starting 1–5 days after end of chemotherapy to a total number of 7–15 days. The tolerance is good and the side-effects are usually limited to musculoskeletal pain, which, however, can be severe in some patients.

In the treatment of solid tumours with chemotherapy, supportive G-CSF therapy has constantly shown the capacity to decrease numbers of episodes with neutropenic fever as well as the ability to maintain higher dose intensity. Only three randomised studies has shown survival benefit of G-CSF supported chemotherapy, one on patients with small-cell lung cancer, one in aggressive non-Hodgkin’s lymphoma and one recently published study on primary breast cancer. In these studies, similar chemotherapy doses were used in the different treatment arms, but with shorter (accelerated) treatment intervals in the G-CSF arms (2 wk instead of 3 wk). In metastatic breast cancer a meta-analysis demonstrated better response rates for anthracycline-regimens with higher dose intensity compared with standard doses, but no survival benefit was proven.

The current clinical practice guidelines recommend G-CSF as primary prophylaxis in patients receiving chemotherapy associated with an expected incidence of febrile neutropenia >40% and as secondary prophylaxis in patients who have experienced severe or febrile neutropenia and if dose reduction is not appropriate. Furthermore, G-CSF is used for the mobilisation of stem cells for transplantation and for haematological recovery after transplantation.

**High-dose chemotherapy with bone-marrow support**

Transplantation of haematopoietic stem cells allows for a several-fold increase in the doses of cytotoxic drugs. According to the experiments by
Skipper, the curability of cancer by chemotherapy is hampered by the regrowth of tumour cells between the treatment courses, and the cellular growth fraction is thought to increase when the size of the tumour decreases. The administration of chemotherapy at very high doses on one or two occasions may overcome this “kinetic resistance” and ultimately cure the patient. This concept has been used with some success in haematological cancer forms and has since the 1980s been tested in breast cancer. By using the patient’s own bone marrow, i.e., autologous bone-marrow transplantation (ABMT), one can avoid rejection reactions, which makes the procedure relatively safe. The stem cells were initially harvested via bone marrow aspirations but this method has been replaced in most patients by the more convenient harvesting of peripheral-blood stem cells, mobilised by, e.g., high doses of Cyclo with G-CSF. The peripheral progenitor cells (CD34+ cells) are collected by leukapheresis on one or several subsequent days and are then frozen until re-infused a few days after the high-dose therapy. Different combinations of cytotoxic agents have been used for high-dose therapy and the treatments have usually been delivered during 1–4 days.

The first reports from phase II studies of breast cancer therapy with high-dose chemotherapy followed by ABMT were encouraging. Response rates were 75–100% and long-term relapse-free survival, were reported in 10–30% of metastatic patients. These promising data made it troublesome to randomise patients in phase III studies with standard control arms and the method was applied in most countries. This tendency was further accelerated when the first randomised study from South Africa in 1995 presented a clear and significant survival benefit for high-dose therapy. At the end of 1990s, breast cancer was the most common indication for high-dose chemotherapy in the United States, but the negative results from later studies were disappointing. In addition, when two on-site reviews of the South-African studies in 2000 and 2001 reported severe scientific misconduct, the high-dose therapy concept in breast cancer was no longer considered as a standard therapy option, either in the metastatic or the adjuvant setting.

A recent overview summarised the seven reported randomised studies in metastatic breast cancer. A benefit in time to progression in favour of high-dose therapy was noted in six of the studies, but the studies are small and no overall survival advantage was observed. Seven larger and three smaller randomised studies in the adjuvant setting in high-risk breast cancer were analysed and only one French study showed a significantly improved disease-free survival for women treated with high-dose therapy, but final data were only achieved from two smaller studies. The largest adjuvant high-dose study was recently published together with another large study comparing standard treatment followed, or not followed, by high-dose
In the study from the Netherlands, 885 patients with high-risk breast cancer were randomised to adjuvant treatment with FEC with Epi doses of 90 mg/m² for 5 cycles (conventional arm) or similar treatment with high-dose chemotherapy consisting of Cyclo 6 g/m², thiotepa 480 mg/m² and carboplatin 1600 mg/m² replacing the 5th cycle of FEC (high-dose arm). The 5-years relapse-free survival rates were 59% in the conventional group and 65% in the high-dose group ($p = 0.09$). In the study from Chicago, 540 high-risk women were randomised to 6 cycles of a relatively intense variant of FAC without (conventional arm) or with a following high-dose therapy consisting of Cyclo 6 g/m² and thiotepa 800 mg/m² (high-dose arm). At 6 years, relapse free survival rates were 48% versus 55% in the high-dose group ($p = 0.12$), but the outcome for overall survival tended to favour the conventional arm (62% versus 58%, $p = 0.32$) due to major problems with treatment-related deaths in the high-dose group (9 toxic deaths at 2–55 days after transplantation and 9 cases of secondary myelodysplastic syndrome or leukaemia).

In summary, high-dose chemotherapy in breast cancer patients may at least add benefit in terms of relapse-free survival, but this benefit is far from the early promising reported results and no survival benefit has yet been revealed.
2 Aims of the thesis

The general aims of this thesis were to investigate the feasibility of tailored dosing based on haematological toxicity in the chemotherapy of primary and metastatic breast cancer by using dose-escalated FEC supported with G-CSF or tailored ET.

The specific aims were to investigate:

- The feasibility and efficacy of tailored dFEC in metastatic breast cancer.
- Whether tailored dFEC is superior to high-dose chemotherapy in patients with high-risk primary breast cancer.
- Whether standard FEC, with a higher dose of cyclophosphamide and followed by G-CSF, could safely mobilise sufficient blood stem cells for re-infusion after high-dose chemotherapy.
- The feasibility and efficacy of tailored ET in metastatic breast cancer.
- To investigate whether dose-limiting haematological toxicity after ET could be predicted by using a semi-physiological Pk-Pd model and to characterise variability in Pk and Pd.
- To study the effects of G-CSF in response evaluation with MR imaging in breast cancer patients with bone metastases treated with chemotherapy.
3 Patients and methods

This thesis is based on three clinical studies: Two smaller phase II-studies on patients with metastatic breast cancer are described in Papers I and IV–VI. Data from a large randomised phase III study of adjuvant therapies in breast cancer women with high risk for relapse are summarised in Papers II and III. The local ethical committee and the Swedish Medical Product Agency approved the studies. All patients were provided with verbal and written information and gave their informed consent to participate.

3.1 Paper I – tailored dFEC in metastatic breast cancer

Patients
Between November 1993 and June 1996, 26 women aged ≤ 60 years and with metastatic breast cancer were included in the study at the University Hospitals of Uppsala and Örebro. The inclusion and exclusion criteria matched a former randomised study of standard FEC and MMM, and patients with bone metastases alone were included if the lesions were lytic. No former chemotherapy for metastatic disease was permitted and patients who had previously received adjuvant anthracyclines were excluded.

Treatment and evaluation
The patients initially received dFEC at 5-Fu/Epi/Cyclo doses of 600/75/900 mg/m² (dose level 1) and 0.263 mg G-CSF (lenograstim) was administered subcutaneously once daily on days 3–12, or until leukocytes had recovered to >1.0 x 10⁹/L. Oral prophylactic antibiotics (ciprofloxacin 500 mg) were given twice daily on days 3–12. Blood samples were taken on days 8, 11/12, 15, and 22 (planned day for next cycle) for WBC (measuring both polynuclear and mononuclear leukocytes), platelet count and haemoglobin level. Subsequent treatments were adjusted to attain similar haematological toxicity in all patients. The target WBC nadir was <1.0 x 10⁹/L for less than 5 days and a platelet count nadir of between 25 and 75 x 10⁹/L; otherwise,
the next cycles were escalated up to maximum level 4 or reduced to a minimum level -2 (Table 3).

Table 3. Dose levels for the G-CSF supported and tailored dFEC

<table>
<thead>
<tr>
<th>Dose levels</th>
<th>5-Fluorouracil mg/m²</th>
<th>Epirubicin mg/m²</th>
<th>Cyclophosphamide mg/m²</th>
<th>Mesna mg/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level -2</td>
<td>300</td>
<td>40</td>
<td>450</td>
<td>-</td>
</tr>
<tr>
<td>Level -1</td>
<td>600</td>
<td>60</td>
<td>600</td>
<td>-</td>
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<tr>
<td>Level 1¹</td>
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<tr>
<td>Level 3</td>
<td>600</td>
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<td>1500</td>
<td>300 x 3</td>
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<tr>
<td>Level 4</td>
<td>600</td>
<td>120</td>
<td>1800</td>
<td>360 x 3</td>
</tr>
</tbody>
</table>

¹Starting dose level

For dose-level 2 and above, mesna at 20% of the Cyclo dose was given i.v. or orally at double doses at 0, 4 and 8 h after the Cyclo infusion.

Nine courses were to be given. In the case of CR, consolidation with high-dose treatment with CTCb and ABMT was recommended. For patients with PR or SD, continued standard FEC every 5 wk was proposed until a cumulative Epi dose of 1000 mg/m² was reached.

Tumour evaluation was performed after every third cycle and toxicity was reported in accordance with NCI common toxicity criteria after each cycle. All patients self-assessed their QoL using both the EORTC QLQ-30(+3)-BR23 questionnaire and a Swedish instrument before treatment started and during all cycles except the last one. From the previous FEC-MMM study, we used the cohort of 56 patients below 60 years of age and treated with standard FEC as a historical comparison for OS, TTP and QoL.

3.2 Papers II and III – tailored dFEC vs high-dose therapy

Patients

In total, 525 patients were randomised between March 1994 and March 1998 in Sweden, Finland, Denmark and Norway (the SBG 9401-study). Included patients should be younger than 60 years of age and have a primary high-risk breast cancer with an expected 5-year relapse-free survival of 30% or less, based on three Scandinavian population-based breast-cancer registers. At
least eight axillary lymph-node metastases were required, or five lymph-node metastases and negative hormone receptors, and either nuclear anaplasia grade 2–3 (or an equivalent high-risk criterion) or a high S-phase fraction. In the Stockholm region six positive nodes were accepted. Bilateral bone-marrow biopsies had to be normal, but reactive bone marrow or the presence of breast cancer micrometastases was allowed.

**Treatment arms**

The dFEC arm consisted of nine cycles of tailored dFEC. The dose levels and dose-modification schedule resembled that of the principles outlined for the phase II study of dFEC in metastatic breast cancer in Paper I (Table 3). However, G-CSF (filgrastim) was given at the dose of 5 μg/kg on days 2–15, or until WBC exceeded 10 x 10^9/L and prophylactic antibiotics (ciprofloxacin or fleroxacin) was given orally on days 5–15.

The CTCb arm (high-dose arm) consisted of three cycles of FEC followed by high-dose Cyclo-thiotepa-carboplatin (CTCb) with ABMT. The first two FEC cycles were given at standard doses (600/60/600 mg/m²) and the modified third cycle had a doubled dose of Cyclo with prophylactic mesna for peripheral blood stem cell harvesting. Subcutaneous G-CSF (filgrastim) injections at 5 μg/kg were started of days 2–5 dependent on planned day for harvest, and were continued until harvest. The stem cells were harvested by standard leukapheresis, which was recommended on day 11. The recommended minimal number of CD34+ cells for re-infusion after high-dose chemotherapy was 2.0 x 10^6/kg body weight. Patients who could not receive high-dose chemotherapy within a month after harvest were given a fourth course of standard FEC. The high-dose chemotherapy consisted of CTCb given as a 96 h infusion at doses of 6,000/500/800 mg/m² on days -7 to -4. The collected CD34+ cells were re-infused at day 0 and G-CSF was started on day 1 and continued until the number of neutrophils was above 1.0 x 10^9/L for three days in a row. Data of harvest and toxicity after the modified third FEC from the first 50 patients in the CTCb group were to be separately analysed and are together with data of engraftment presented in Paper III.

After chemotherapy, both treatment groups underwent locoregional radiotherapy to at least 46 Gy in 2 Gy fractions and received adjuvant tamoxifen 20 mg daily for 5 years. Toxicity was graded in accordance with NCI common toxicity criteria at baseline and after each chemotherapy cycle for selected items with presumed high relevance. Other adverse events were also to be reported. The primary objective of the study was relapse-free survival using the intention-to-treat principle and the double triangular
sequential trial plan\textsuperscript{252} was used, allowing repeated interim analyses without affecting the level of significance and with calculated stopping boundaries.

3.3 Papers IV and V – tailored ET in metastatic breast cancer

Patients
From March 1997 to April 2000, a total of 44 study and pilot patients with metastatic breast cancer were treated with individually tailored Epi and Dtax (ET) at Uppsala University Hospital and Karolinska Hospital, Stockholm. Women below age 60 years without prior chemotherapy for advanced disease were eligible. Adjuvant treatment completed at least 6 months prior to entry was allowed if the cumulative dose of Dox or Epi did not exceeded 360 mg/m\textsuperscript{2} and 600 mg/m\textsuperscript{2}, respectively.

Treatment
The patients initially received ET at doses of 75/70 mg/m\textsuperscript{2} (level 1) with prophylactic treatment with betamethasone, 5-HT3 receptor antagonists and ciprofloxacin. Blood samples were taken on days 8, 11/12, 15, and 22 (planned day for next cycle) for analyses of WBC, absolute neutrophil count (or number of polynuclear leukocytes as a surrogate), platelet count and Hb.

| Table 4. Dose levels for the tailored epirubicin-docetaxel regimen |
|---------------------------------|-----------------|-----------------|
| Dose levels                     | Epirubicin mg/m\textsuperscript{2} | Docetaxel mg/m\textsuperscript{2} |
| Level -2                        | 40               | 50               |
| Level -1                        | 60               | 60               |
| Level 1\textsuperscript{1}      | 75               | 70               |
| Level 2                        | 90               | 80               |
| Level 3                        | 105              | 90               |
| Level 4                        | 120              | 100              |

\textsuperscript{1} Starting dose level

Subsequent cycles were either escalated up to maximum level 4 or reduced to minimum level -2 (Table 4) aiming at a nadir WBC of $\leq 1.0 \times 10^9/L$ or a nadir platelet count of $\leq 50 \times 10^9/L$. To patients with a WBC nadir of $<1.0$ for more than 5 days, 0.263 mg G-CSF (lenograstim) was added for the
subsequent cycles daily on days 3–12 for safety reasons. Febrile leukopenia resulted in a one-step dose reduction for the next cycle combined with the addition of G-CSF support. A total number of nine cycles were planned, but the treatment was discontinued earlier if the patient reached the limit of 1,000 mg/m² cumulative Epi, had PD or was considered for high-dose chemotherapy with ABMT because of a CR.

Evaluations of measurable and evaluable lesions were repeated every third cycle. At baseline and before every cycle but the last one, patients self-assessed QoL using the EORTC QLQ-30(+3)-BR23 questionnaire. The NCI-common toxicity criteria were used to define the level of toxicity and similar selected items as in the randomised adjuvant study of dFEC were graded at baseline and after each cycle.

**Pharmacokinetic and pharmacodynamic analyses**

Blood samples for Pk analyses were collected from 38 cycles in 16 patients. Five samples were taken at the time of each treatment: before stop of Epi and Dtax infusions, 30–60 minutes after Dtax infusion, 5–8 h after and finally 15–27 h after start of Epi infusion. The actual time of sampling was used in the analysis. Plasma concentrations for Epi and Dtax were determined as previously described with slight modifications. Between- and within-run coefficients of variations at five concentrations were less than 13% (Epi) and 12% (Dtax), respectively. Accuracy was 92–104% (Epi) and 87–103% (Dtax) within the same concentration range. The NONMEM programme versions V and VI beta were used for Pk and Pk-Pd modelling. The Pk models were developed first and, in the following Pk-Pd analysis, the individual Pk parameter estimates from the final Pk models were fixed and the predicted individual Pk profiles were used in the Pd models. A previously developed semi-physiological model describing WBC-time profile consisting of five compartments was used in the Pk-Pd modelling. The first compartment consists of proliferative cells and is connected to the last compartment, represented by the circulating leukocytes (WBC), by a chain of three compartments of maturing leukocytes. The drug-related bone marrow toxicity is in the model a function of the drug plasma concentration and a drug-specific slope parameter that linearly relates the drug effect in the proliferating compartment to the concentration of the drug in plasma. Prior information for the Dtax slope was obtained from a previous analysis of 601 patients who received single Dtax.
3.4 Paper VI – effect of G-CSF on MR assessment of bone metastases

Patients
Eighteen breast cancer women were included in a prospective study of assessment of response in bone metastases between October 1995 and April 1999. They all had histologically proven bone metastases and received first-line chemotherapy for metastatic disease. Fifteen of the patients with focal bone metastases were included in this study of the effects of G-CSF on MR imaging. Five of the patients received ET with G-CSF support, four had dFEC with G-CSF, five were treated with standard FEC without G-CSF and one patient received 90 mg/m² single-drug paclitaxel on a three weekly basis without G-CSF (control group). G-CSF (lenograstim 0.263 mg, or in one case filgrastim 0.3 mg) was administered subcutaneously once daily on days 3–12.

MR examinations
MR examinations, as well as clinical evaluations, bone scans, conventional radiography and CT-guided bone biopsies, were performed before the start of treatment and after a median of 6 cycles (range 3–7). Of the nine patients receiving G-CSF supported treatment, four patients had their follow-up MR examination during G-CSF therapy (day 4–9 of G-CSF) and five patients after the G-CSF therapy (1–11 days after G-CSF). The control group consisted of the remaining six MR evaluated patients without G-CSF supported chemotherapy.

The MR examinations were performed on the sagittal plane in the sternum, thoracic and lumbar spine and in the coronal plane in pelvis and proximal femora using T1W-TSE and fat-suppressed long TE IR-TSE sequences. Focal bone metastases were defined as having low signal intensity on T1-weighted images and high signal intensity on long TE IR-TSE images. For a visual evaluation, the images were reviewed blindly and randomly by two experienced radiologists. A quantitative evaluation of a contrast index was done in 13 patients by comparing signal intensity in red bone marrow and three healthy intervertebral disks. To compare signal intensity differences between investigations, a contrast index (CI) was defined as the ratio between the signal intensity in bone marrow and intervertebral disk.
Response- and histopathological evaluation

The response of bone metastases was assessed by three methods; by clinical evaluations (symptoms, consumption of analgesics and laboratory results), bone scans and by conventional radiography (modified WHO criteria\textsuperscript{142}). Signs of PD by one of the methods was interpreted as PD and for PR, an observed improvement according to at least two methods was required and without PD in the third method. Any other combination was interpreted as SD. In four patients, we obtained biopsy specimens for histopathological evaluation of normal bone marrow besides specimens from the bone metastases. The bone marrow cellularity was measured semi-quantitatively and the pathologist also graded the state of granulocytopenesis in four steps.
4 Results

4.1 Paper I – tailored dFEC in metastatic breast cancer

Dose distributions and efficacy
The distribution of the dose levels is shown in Figure 5. The maximum tolerated dose level was as low as level -1 for two patients and as high as level 4 for five patients. The dose intensity varied considerably with a median 5-Fu intensity of 185 mg/m²/wk (range 127–201), Epi 26.4 mg/m²/wk (range 14.4–36.0) and a median Cyclo dose intensity of 336 mg/m²/wk (range 159–510). The presences of liver or bone metastases were not correlated to the delivered dose intensity.

Figure 5. Stacked column diagram showing the distribution of different dose levels during the study treatment (n = 199).
The ORR was 81% (CI 66%–96%) with a CR rate of 23% (CI 7%–39%) and a PR rate of 58% (CI 39%–77%). Four patients (15%, CI 2%–29%) had SD and one (4%, CI 0%–11%) had PD. For the 13 patients receiving the highest dose intensity, 11 responded compared with 10 patients among the 13 patients with the lowest dose intensity. Median TTP was 16 months, median OS was 36 months and estimated 5-years survival was 15% based on the Kaplan-Meier method. Seven patients received consolidating high-dose chemotherapy with ABMT after completion of the study treatment.

**Toxicity and quality of life (QoL)**

Due to the design of the treatment schedule, all patients experienced grade IV leukopenia and 69% of the patients had fever associated with (31%) or without (38%) leukopenia or neutropenia grade IV. There was no non-haematological grade IV toxicity and the most common grade III toxicity was nausea/vomiting (12% of patients), muscle pain (8%), and mucositis (4%). One patient showed moderate to severe symptoms of cardiac heart failure 4.5 years after dFEC and one patient developed acute myeloid leukaemia type M1, 27 months after completion of dFEC and died 5 months later. Both patients had been treated with high-dose chemotherapy with ABMT after finishing dFEC treatment.

There was a slight increase in global QoL compared with baseline for both the two different global endpoints of the EORTC questionnaire as well as for the Swedish instrument. Individual data from the EORTC instrument, comparing baseline and each patients last cycle, showed no difference ($p = 0.14$ and $p = 0.26$, Wilcoxon’s matched pairs test).

**4.2 Papers II and III – tailored dFEC vs high-dose chemotherapy**

**Dose distributions and efficacy**

For patients in the tailored dFEC group, the median 5-Fu dose intensity from treatment start to last day of chemotherapy was 223 mg/m$^2$/wk compared with 179 mg/m$^2$/wk for patients in the CTCb group. Corresponding Epi doses were 33 mg/m$^2$/wk and 18 mg/m$^2$/wk, respectively, and median Cyclo dose intensity were 431 mg/m$^2$/wk and 811 mg/m$^2$/wk in the two different treatment groups. The distribution of dFEC patients treated at different dose levels after adjustment for equal haematological toxicity is shown in Table 5.
Table 5. Distribution of patients at different dose levels in the tailored dFEC group at cycle 4 to 9.

<table>
<thead>
<tr>
<th>Cycle nr</th>
<th>-2</th>
<th>-1</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>n</th>
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<tr>
<td>4</td>
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</tr>
<tr>
<td>9</td>
<td>9%</td>
<td>22%</td>
<td>24%</td>
<td>25%</td>
<td>16%</td>
<td>4%</td>
<td>214</td>
</tr>
</tbody>
</table>

The tailored dFEC treatment was superior compared with standard FEC combined with stem-cell supported CTCb in terms of relapse-free survival, which was the primary endpoint of study ($p = 0.044$, double triangular test). Eighty-one patients relapsed in the dFEC group compared with 113 patients in the CTCb group and the 3-year relapse-free survival was estimated to 72% and 63%, respectively (Figure 6). There were no differences in outcome for patients tolerating high, medium or low dose levels of dFEC. The calculated 3-year OS rate was 83% for patients in the dFEC group and 77% in the CTCb group ($p = 0.12$, log-rank test). When including breast-cancer relapses, contralateral breast cancer, acute myeloid leukaemia or myelodysplastic syndrome, and other secondary malignancies for calculation of event-free survival there were 95 events in the dFEC group and 118 in the CTCb group ($p = 0.09$, log-rank test).

![Kaplan-Meier plot of relapse-free survival for tailored dFEC versus CTCb](image_url)

**Numbers at risk**

<table>
<thead>
<tr>
<th></th>
<th>FEC</th>
<th>CTCb</th>
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<tr>
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</table>

**Figure 6.** Kaplan-Meier plot of relapse-free survival for tailored dFEC versus CTCb ($p = 0.044$, double triangular test, $p = 0.013$, log-rank test).
Toxicity
Patients in the dFEC group experienced less grade III–IV toxicity than patients in the CTCb group \((p < 0.0001)\) including anorexia, diarrhoea, infections, nausea and stomatitis. However, bone pain and myalgia tended to be more common in the dFEC group \((p = 0.09\) and \(p = 0.07\), respectively). Except for vomiting in cycles 4, 5, 8 and 9, there was no more toxicity reported at the two highest dose levels of dFEC compared with lower doses. Two patients in the CTCb group died of treatment-related toxicity. Nine patients in the tailored dFEC group developed a myelodysplastic syndrome or acute myeloid leukaemia \((3.6\%\) of dFEC treated patients) which occurred at 9–33 months after completion of chemotherapy. Seven patients in the dFEC group developed cardiac symptoms compared with five in the CTCb group.

Stem-cell harvest and engraftment
Of the first 50 patients in the CTCb group, 49 received stem-cell supported CTCb. After the modified third FEC course, but before harvest, G-CSF was administered for a mean of 9.6 days \((\text{range 7–14 days})\) and sufficient numbers of CD34+ stem cells were obtained from all patients. Thirteen patients had one leukapheresis, 33 had 2, 2 patients had 3 and the remaining patient had 4 leukaphereses. The mean number of collected CD34+ cells per patient was \(10.6 \times 10^6/\text{kg}\) \((\text{range 2.6–29.1})\). There was no correlation between days of G-CSF treatment and collected CD34+ cells.

All 49 patients engrafted safely and the mean time to recovery of neutrophils >1 \(x\) \(10^9/\text{L}\) was 9.8 days \((\text{range 8–16})\). Corresponding mean time for platelet recovery \((>20 \times 10^9/\text{L})\) was 9.1 days \((\text{range 0–16})\) and \((>50 \times 10^9/\text{L})\) and 13.0 days \((\text{range 7–21})\), respectively \((\text{Figure 7})\). A higher number of re-infused CD34+ stem cells was correlated to faster neutrophil recovery \((r_s = -0.35\) and \(p = 0.01\), Spearman’s rank correlation test) and a trend towards faster recovery of platelet count above 50 \(x\) \(10^9/\text{L}\) was seen \((r_s = -0.27\) and \(p = 0.07\)).
Toxicity

Except for alopecia, the dominating toxicity after the modified third FEC course was nausea (72%), followed by anorexia (44%), vomiting (40%), bone pain (36%) and myalgia (24%). Grade III–IV toxicity was rare, but a frequency of 8% bone pain and 6% vomiting was reported. One patient (2%) developed febrile neutropenia. There occurred no treatment-related fatalities in this first group of 50 patients randomised to CTCb treatment.

4.3 Papers IV and V – tailored ET in metastatic breast cancer

Dose distributions and efficacy

The individual dose-levels per cycle per patient are listed in Table 6. Twenty-two (50%) of the patients received G-CSF and 103 cycles (34%) were given with G-CSF. The median dose intensity of Epi was 29 mg/m²/wk (range 18–36) and the median Dtax dose intensity was 26 mg/m²/wk (range 10–31). Two patients wrongly started on level -1 and one patient started with
a reduced dose of Dttx (50 mg/m²) because of marked elevation of liver enzymes. One patient incorrectly started with G-CSF from cycle one.

Table 6. Distribution of different dose-levels according to cycle number with patients ranked in order of tolerated dose (patients 1–30)

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1Two patients wrongly started at level -1.
Table 6. (cont.) Distribution of different dose-levels according to cycle number with patients ranked in order of tolerated dose (patients 31–44)

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| Sum     | 44 | 44 | 43 | 36 | 33 | 31 | 28 | 24 | 20 | 30 |

*Patient wrongly treated with G-CSF from first cycle.
1 Patient treated with reduced docetaxel doses due to decreased hepatic function.

The individual maximum tolerated dose level (TDL) was estimated for each patient and covered all six different dose levels (level -2 to level 4). There was no correlation between TDL and number of involved organs, site of metastases or given palliative radiotherapy.

Forty-three patients were considered as evaluable and 27 responded (63%, CI 48%–77%), with 3 CR (7%, CI 0%–15%) and 24 PR (53%, CI 39%–68%). Seven patients had PD (16%, CI 5%–27%). Median TTP was 10 months and median OS was 21 months. At 5 years the estimated OS was 23%. No correlation between TDL and response was observed ($r_s = 0.05$, NS, Spearman’s rank correlation test).

**Toxicity and quality of life (QoL)**

All patients experienced grade III–IV leukopenia with a WBC of $\leq 1.0 \times 10^9/L$ as a result of the dosing algorithm. Sixteen episodes of febrile
leukopenia (5% of all cycles) were reported in 15 patients (34%). One patient died of a *Pneumocystis carinii* pneumonia after cycle three and a probable cardiotoxic death occurred after cycle eight in a patient who had a cumulative Epi dose of 829 mg/m².

There was no correlation ($r_s = -0.16$, not significant (NS)) between a patient’s worst grade of toxicity and TDL. Furthermore, there was no correlation ($r_s = 0.13$, NS) between the use of G-CSF and non-haematological toxicity. However, TDL correlated strongly with the nadir WBC and neutrophil counts after the first cycle at dose level 1 (in 2 cases after the second cycle) ($r_s = 0.70$, $p <0.0005$ and $r_s = 0.49$, $p = 0.002$, respectively).

Changes in the two different global QoL endpoints relative to baseline in each patient were calculated. A temporary increase to a maximum of 10% could be noticed from cycle 1–5. No correlation between TDL and QoL was observed ($r_s = 0.01$, NS and $r_s = -0.08$, NS, respectively).

**Pharmacokinetics and haematological toxicity**

The population estimated clearance of Epi was 69.6 L/h and of Dtax 39.5 L/h. Unexplainable diverging Dtax concentration data from one patient were excluded. No significant correlation was found between the clearances of Epi and Dtax (Pearson’s correlation test). In the final Pk model, the inter-individual variability was larger than the inter-occasion variability for both drugs. The inter-individual variability of Epi clearance was 14% (SE 20%) and of Dtax clearance 27% (SE 74%). The inter-occasion variability of Dtax clearance was 15% (SE 65%), whereas it for Epi was negligible.

WBC observations ($n = 805$) from 42 patients were available for Pd analyses and modeling (Figure 8A). Only data from cycles ($n = 187$) without G-CSF support were included. The mean WBC at baseline was $6.8 \times 10^9$/L (range 3.9–12) and nadir occurred on average day 11 (range 8–15) and mean nadir WBC was $1.2 \times 10^9$/L (range 0.3–3.3). The ratio of WBC at nadir and WBC before chemotherapy, i.e., surviving fraction, showed the best correlation with exposure of drugs. There was no correlation between sum of Epi and Dtax doses and WBC surviving fraction, regardless of whether the first dose only, all doses or cycles only when Pk was available was considered. However, a significant correlation was obtained when the sum of Epi and Dtax concentration-over time AUC was considered as a predictor for WBC surviving fraction.
The Pk-Pd population model

The hematological toxicity per mg drug administered that was caused by Dtax was, according to the final model, predicted to be less pronounced than that caused by Epi, after considering not only the differences in the estimated slopes but also the differences in Pk. The population-model predictions and the individual-model predictions versus observed WBC are displayed in Figures 8B and C. The drug-specific slope parameter estimates for Epi and Dtax in the final model were 17.2 L/mg (SE 5.7%) and 7.74 L/mg (SE 6.2%), respectively. The data did not support an estimation of inter-individual variability in both slope parameters. The best model fit was obtained when inter-individual variability was allowed in the Epi slope. The inter-individual coefficient of variance (CV) of the Epi slope parameter was 39% (SE 33%). The WBC at baseline was estimated to 6.47 (SE 3.1%) and the mean transit time for cell maturing was 107 h (SE 1.2%). For the average patient, the model predicts a WBC of $1.1 \times 10^9$/L at nadir, occurring on day 10.5, after the combination of the median doses administered in the study, i.e., 160 mg and 140 mg of Epi and Dtax, respectively.

The estimated slope parameter for Epi could predict WBC-time profile from earlier published data.254

Figure 8. (A) Observed WBC versus time after last ET dose. The solid line in represents the model predicted WBC-time profile in the typical individual after treatment with median doses of Epi (160 mg) and Dtax (140 mg). Observed WBC versus (B) Pk-Pd model predicted and (C) individual predicted WBC.
4.4 Paper VI– effect of G-CSF on MR assessment of bone metastases

Long TE IR-TSE images
A diffuse homogeneous increase in signal intensity was visually observed in normal bone marrow in all four patients examined during G-CSF treatment. In two patients this increased signal obscured focal metastases. The increased signal was not observed in any of the five patients examined after G-CSF treatment \( (p = 0.008, \text{ Fisher's exact test}) \) or in the control group of six patients without G-CSF treatment. Corresponding increase in contrast index could be quantified (Figure 9) and the difference between the first group was significant \( (p = 0.021, \text{ Kruskal-Wallis test}) \) compared with the two other groups not examined during G-CSF treatment.

Figure 9. Differences in contrast index (CI) between baseline and follow-up MR examinations, calculated on T1 weighted and long TE IR-TSE images, in the three study groups.
**T1-weighted images**

A decrease in signal intensity could visually be detected in two of four patients during G-CSF treatment and in four of five after G-CSF treatment. No decrease was observed in the control group but, in contrast, an increase in signal intensity was noted in the spine and the sternum in two patients. In the quantitative evaluation, the contrast index was decreased in all patients treated with G-CSF, irrespectively if examined during or after treatment (Figure 9). A slight increase was seen all patients in the control group without G-CSF and this difference was significantly higher than in the other two groups ($p = 0.014$, Kruskal-Wallis test).

**Response- and histopathological evaluation**

Response evaluation in the patients treated with G-CSF showed 8 PR and 1 SD. In the control group there were 2 PR, 2 SD and 2 patients with PD. Histopathology from bone marrow from one patient at day 7 of G-CSF treatment showed 90% cellularity and greatly increased granulopoiesis. Two biopsies, obtained 3 and 6 days after the stoppage of G-CSF, from 2 patients in the second group demonstrated 70% cellularity and greatly increased granulopoiesis. One biopsy from a responding patient in the control group showed 50% cellularity and normal granulopoiesis.
5 General discussion

5.1 Tailored dosage of chemotherapy based on toxicity

Conventional BSA-based dosage of chemotherapy results in large inter-individual differences in toxicity, since it fails to adjust for the large variation in Pk\textsuperscript{167, 179} and does not allow for inter-individual differences in drug sensitivity (Pd). The principal disadvantage is that under-dosing of certain patients results in a suboptimal antitumoral effect\textsuperscript{193, 194, 196, 198, 200, 255} while over-dosing may produce pronounced toxicity in other patients.

The three clinical trials described in this thesis are probably the first attempts to study individually dose-adjusted chemotherapy, aiming at the same level of toxicity in all patients. The rational is that the inter-individual variations in Pk and Pd are large and the corresponding variations in one patient from one therapy occasion to another (the intra-individual variation) smaller. Furthermore, the proposed chemotherapy regimen must have a dose-limiting toxicity suitable for monitoring purposes, e.g. haematological toxicity. If the concept is successful, the general outcome related to the grade of toxicity and efficacy should be equal for all patients, irrespectively of tolerated dose level.

Feasibility of tailored dosage

One of the most important findings in the three trials that supported the individualised dosing concept was the large variability in tolerated dose levels. The distribution of patients at the different levels was even, except for the lowest dose level (-2). In the study of dFEC in metastatic breast cancer, the levels at cycle 4 ranged from levels -1/-2 (12% of patients) to 4 (12% of patients), which correspond to a two-fold range for Epi and a threefold range for Cyclo (Figure 5). Similar results were obtained in the study of dFEC in the adjuvant setting (Table 5). In the study of tailored ET, a more sophisticated estimation of individual tolerance was performed (TDL) and the tolerated dose levels ranged from levels -1/-2 (18% of patients) to 4 (20% of patients) (Table 6), which is a three-fold range for Epi and two-fold for Dtax. The large variability in the ET trial was observed despite the fact
that only patients with less tolerance, for safety reasons, were treated with G-CSF, which may have resulted in a less pronounced dose-level range. In the two trials with dFEC therapy, all patients received G-CSF up-front, and individual differences in response to this agent may also have contributed to the large variability in tolerated dose levels in these trials. However, the result from the ET-trial indicates that this is of small importance.

ET-treated patients showed a relatively constant dose-level tolerance during the nine cycles, indicating a low intra-individual variability in Pk and Pd. The strong correlation between WBC nadirs after cycle one and tailored dose-level in the ET-trial further implies that the inter-occasion variability is low. In contrast to the ET-trial, there was clearly a decreased dose tolerance after seven cycles in both the dFEC trials. This finding could possibly be explained by the more excessive use of G-CSF in these studies and a sign of cumulative bone-marrow toxicity after sustained intensive G-CSF supported chemotherapy.

In comparison, a difference in dose intensity in the dFEC trials in metastatic and adjuvant breast cancer was revealed. The calculation of the dose-intensity in the adjuvant SBG 9401 study did not take into consideration the number of days that had elapsed since the final chemotherapy course because the duration of the high-dose therapy was difficult to define. However, if three weeks were added to the adjuvant dFEC treatment, the Epi and Cyclo dose intensities were 11% and 14% higher (29.3 vs. 26.4 and 383 vs. 336 mg/m²/wk) in the adjuvant and the metastatic setting, respectively. This finding could indicate that the presence of metastases may have negatively influence the dose tolerance level, something that can be clinically noticed in patients with severe metastatic infiltration in the bone-marrow or liver. We did, however, not find any correlations between tolerated dose and presence of bone or liver metastases in the two studies of metastatic disease. Possible explanations for the lower dose intensity of dFEC in metastatic patients are the shorter G-CSF treatment, the slightly different dose-modification schedule and the fact that 46% of the metastatic patients had previously received adjuvant CMF.

A possible verification of the suitability of toxicity-based dosing was the finding that the antitumoral effect, the non-haematological toxicity and the quality of life were similar in patients treated with high dose levels and those treated with low dose levels. In the adjuvant study, there was no difference in relapse-free survival between patients treated with high, moderate or low doses of dFEC. This observation is in agreement with the previously reported outcome from individualised CHOP³⁵ (Figure 10). A similar lack of correlation between given doses and response was observed in both our ET and dFEC trials of metastatic disease, but these studies are considerably smaller. This result was expected as data from the retrospective adjuvant studies showed a relationship between haematological toxicity and
In our three studies, patients developed the same haematological toxicity while the non-haematological toxicity was almost equally distributed irrespective of which dose levels of tailored dFEC or ET the patient were administered. In the study of adjuvant dFEC, no adverse events, except for vomiting, were significantly more common at the two highest dose levels than at lower doses. In the ET-trial, there were no correlations between tailored dose-levels and non-haematological adverse events or tailored dose-levels and patient quality of life.

Figure 10. The antitumoral effect is unrelated to dose-level after individually tailored dosage. Left side, relapse-free survival (the SBG 9401-study, Paper II, last follow-up) and right side, disease-free survival described by Buzdar et al. in relation to given (tolerated) doses.

The Pk and Pd analyses

The Pk analysis showed that haematological toxicity correlated better to the sum of AUCs for each drug than to given doses of ET. This finding is in agreement with studies of single drug treatment with Epi and Dtax and indicates that at least a part of inter-individual differences in tolerance are explained by differences in drug clearance. The Pk population model estimated a negligible inter-occasion variance (CV) in Epi clearance and only one of 15% in Dtax clearance. This observation of less intra-individual than inter-individual variance in Pk of cytotoxic drugs has previously been described. The observed lower within-patient variability in drug clearance than the observed between-patient variability is highly relevant for the development of dosing strategies based on Pk analyses or toxicity.

No relationship between individual Epi and Dtax clearance was revealed. This has also been shown for the component drugs in the FEC regimen. This finding could be an argument against dose-escalation schedules with fixed drug doses and supports Pk sampling of individual drugs. This approach (TDM) has been successful in the treatment of children with B-cell
leukaemia, but is hampered by the need for Pk analyses and by not taking Pd variability into account. Pk-Pd modelling may be a better predictor of effect and would be able to propose individual drug-adjustments for each specific drug in subsequent treatments.

The advanced semi-physiological Pk-Pd model used herein has previously been considered successful in predicting haematological toxicity in single drug chemotherapy. We showed that it is, moreover, useful in predicting the grade and duration of leukopenia in polychemotherapy, i.e. ET therapy, with additive effect being dependent on Epi and Dtax AUCs. The statistical inter-individual coefficients of variation (CV) for clearance were 14% and 27% for Epi and Dtax, respectively. The CV of the slope parameter for Epi, representing the direct effect of both the drugs to cells in the proliferating compartment, was 39%. In single drug therapy with Dtax, a previous estimation of the slope parameter has provided an inter-individual CV of 47%. The large importance of the inter-individual variability in Pd is supported by the observed large variability in Pd in vitro, reported by Sundman-Engberg and co-workers. The individual sensitivity in that study was unrelated to any given drug, which strongly supports the idea that there is a basic individual genetic sensitivity to at least certain cytotoxic agents, which is probably expressed in the same patient’s tumour cells. This hypothesis could, about from differences in Pk, explain why retrospective adjuvant studies show a correlation between toxicity and antitumoral effects and also support the concept of toxicity-adjusted dosing.

**Efficacy and toxicity of tailored dFEC and ET in breast cancer**

The reported efficacy of the three trials in terms of response and relapse-free survival were good. Major reasons for the high efficacy are that tailored dosage may avoid under-dosing, and that effective drug combinations were given at a high dose intensity. The latter was obtained by aiming for relatively severe (grade 4) haematological toxicity and supplemented with G-CSF support. The relationship between higher dose-intensity and better response in breast cancer has been showed for anthracyclines, Cyclo and Dtax. Data on very high dose-intensity are conflicting, but there is evidence of a threshold for Epi above 30 mg/m²/wk. Dose intensities of Cyclo higher than standard have not shown to be beneficial. Randomised data on very high dose-intensity of Dtax are lacking, but data on Ptax suggest a threshold above standard dose-intensity. However, we achieved a median dose-intensity for Epi of 26.4, 29.3 and 29 mg/m²/wk in the three studies, which are in the upper range of the linear phase of the suggested dose-response curve (Figure 4). The median dose-intensity for
Dtax in the ET study was 26 mg/m²/wk, which is below the described optimal dose-intensity for Dtax of 33 mg/m²/wk. The reported 81% ORR and 23% CR rate in the dFEC study of metastatic patients seem to be potentially higher in indirect comparison with randomised studies of G-CSF supported FEC (Figure 4) at corresponding levels of dose intensity, but are in the range of the results from toxicity-based FAC studies and previously phase II-data of G-CSF supported FEC. The ORR and CR rate in the ET-trial was slightly lower (63% and 7%, respectively), but the result is in accordance with phase II and III data of this regimen. The median overall survival in the two studies of metastatic breast cancer differed (36 vs. 21 months), but when we compared the survival curves, the difference was not obvious (Figure 11).

![Figure 11. Overall survival after treatment with dFEC (Paper I) or ET (Paper IV) in metastatic breast cancer.](image)

The patients who received adjuvant dFEC treatment had a significantly longer breast cancer relapse-free survival compared with the patients treated with high-dose therapy. This statistical difference remains in the most recent follow-up (March 2003) after a median follow-up of 60.8 months ($p = 0.047$, double triangular test). This is in fact the only reported adjuvant high-dose study with a superior “conventional” treatment arm, which may be an indirect indication that nine cycles of tailored dFEC are superior to standard FEC or FAC in the adjuvant setting.
The grade of toxicity reported in the three studies was expected to be, in general, relatively severe because of the choice of very low target WBC nadir. The haematological toxicity was, due to the dose-adjustments to targeted nadir, severe (grade 4) in all patients in the three trials, and 19%–34% of the patients experienced febrile neutropenia, which was fatal in one case in the ET-trial. Main non-haematological grade 3 and 4 toxicities were nausea and vomiting in the dFEC trials (10%–19%) and stomatitis in the ET trial (11%). Muscle and bone pain are recognized side-effects of G-CSF and were more common and severe in the dFEC trials.

Despite the very aggressive dosing strategy in the two trials of metastatic breast cancer, the quality of life of the patients was not decreased during treatment. An indirect comparison with a comparable group of patients treated with standard FEC showed no difference between data of QoL from the dFEC trial of metastatic breast cancer and the QoL in the former trial. Furthermore, previously reported data on QoL from Dtax treatment in metastatic breast cancer were comparable with those obtained in the ET-study. The results of the health-related QoL in the randomised adjuvant dFEC trial have recently been published. After one year, the patient QoL returned to levels described at inclusion.

A major drawback of the dFEC regimen was the incidence of secondary myelodysplastic syndrome and acute myeloid leukaemia. During the follow-up from 2000 to the spring of 2003, only one more leukaemia developed, giving a total of 10 patients in the adjuvant dFEC group and 1 in the group of patients with metastatic disease. Treatment with high doses of Cyclo and anthracyclines has previously been described to result in secondary leukaemia; thus, our observations are therefore not unexpected. The decrease in dose tolerance that was noticed after seven cycles of G-CSF supported dFEC was a sign of bone-marrow depression, which may have influenced the development of secondary leukaemia. To decrease the risk of secondary leukaemia, we have now modified the adjuvant dFEC therapy by decreasing the total number of cycles to 6–7. Moreover, the G-CSF treatment time has been shortened to seven days and the maximum Cyclo dose-level are decreased to 1,200 mg/m² in the modified schedule. Between March 1998 and December 2002, 122 patients have received modified dFEC at the Karolinska Hospital, Stockholm, this has so far resulted in one patient developing AML 24 months the after completion of chemotherapy (personal communication, Barbro Linderholm). This indicates that the risk of developing secondary leukaemia with this regimen will be of the same magnitude that reported in studies using anthracyclines at higher dose levels, which have resulted in a better breast cancer outcome.
Relevance

The results from all three of our studies uniformly support the hypothesis that there exist large inter-individual differences in tolerated doses, whereas the variability between different treatment occasions in the same patient is considerably smaller. The results also indicate that the hypothesis, that there is a correlation between tolerance and treatment efficacy, which results in poorer antitumoral effect in patients who develop less toxicity, is valid. We suggest that under-dosing can be avoided by toxicity-based dosing, but the absolute benefit has to be estimated in a randomised trial by comparing tailored dosing with standard BSA-based dosing. Such a trial, the Scandinavian adjuvant trial comparing tailored FEC without G-CSF with standard FEC (the SBG 2000-1 study), has recently terminated inclusion of 1,535 patients and results are pending.

The tailored dosing based on haematological toxicity in the present studies was both feasible in patients with primary and metastatic breast cancer, using different chemotherapy regimens (i.e. FEC and ET) and in treatments with or without G-CSF support. It is, therefore, likely that this dosing principle could be implemented in other anti-cancer therapies that are dose-limited by haematological toxicity. Other treatment schedules than the three-weekly schedule used in our trials may be used to optimise the therapeutic index. Recent data have shown that G-CSF-supported two-weekly-schedules have been successful in the adjuvant setting, but not in metastatic breast cancer. Weekly administration of taxanes is supposed to improve the benefit of at least Ptax, but solid data are still lacking. Despite the fact that we used only the three-weekly schedule, there are theoretical reasons to believe that toxicity-based therapy may be feasible in other treatment schedules as well. By introducing Pk-Pd modelling in the development of new drugs and drug combinations, we would have a rational, instead of empirical, basis for proposed schedules and dose-levels in future trials.

One of the potential benefits with toxicity-based dosing is the possibility of choosing different levels of target toxicity. Thus, the challenge for the physician has changed from defining suitable doses for patients to instead proposing adequate target toxicity.

Potential disadvantages with a dosing schedule based on the leukocyte nadir, are the inconvenience of extra blood sampling and the relatively slowness of the escalation schedule whereas patients with high tolerance not will receive their optimal dose until the fourth cycle. However, the indication that nadir after first treatment cycle could predict the ultimate tailored dose-level suggests that escalations to the individual tailored dose-level are possible after the first course. This has recently been practised in the SBG 2000-1 study.
Both FEC and ET are today valid treatment alternatives in the adjuvant, neo-adjuvant and metastatic setting. The use of tailored dFEC or ET with high dose-intensity in the treatment of metastatic breast cancer was an intentionally aggressive approach in order to achieve high CR rates for sequential high-dose therapy that aimed at cure or long-term remissions. Presently no data support the use of high-dose chemotherapy in this setting. However, data from randomised trials indicate an improved survival for more toxic combinations, higher doses or longer duration of chemotherapy compared with less toxic combinations.\textsuperscript{114, 124, 276} The use of higher than normal dose-intensity is, however, still an ambiguous issue and, in metastatic breast cancer, we would today in general probably aim for grade 3 toxicity, rather than grade 4, and not regularly use G-CSF. In the adjuvant or neo-adjuvant settings, the choice of target toxicity depends on the individual patient’s prognosis, which further increases the level of individual tailoring of therapy.

The two to three-fold differences in tolerated doses between patients in our studies suggest that the dose recommendations based on small phase I studies may be of a limited value. It is probably more important to early define the inter-patient variability, for subsequent design of proper dose-levels in phase II studies. Dosage in traditional comparative phase III studies are based on BSA, which in most studies make it difficult to draw conclusions about the benefit of the therapeutic index. For example, in the large adjuvant BCIRG 001 study that compared FAC with TAC (Dtax instead of 5-Fu), TAC was reported to result in superior disease-free survival, but the toxicity was more pronounced.\textsuperscript{97} Hence, the dose-levels in the treatment arms were not comparable. Individually tailored toxicity-based dosage would, in such studies, help to answer the important question of the value of a new drug.

\subsection*{5.2 Breast cancer treatment with stem-cell supported high dose therapy – harvest and engraftment}

In Paper II we declared that high-dose therapy option was inferior to nine cycles of dFEC in terms of breast cancer relapse free survival; however, no difference in overall survival was seen. The outcome in the high-dose arm is comparable with the results from other large randomised studies of high-risk primary breast cancer.\textsuperscript{247} However, high-dose therapy with stem-cell support in breast cancer must today be restricted to clinical trials with inclusion of relevant tumour biological questions.

The positive treatment effect has in some trials of adjuvant treatment been hampered by a treatment related mortality of up to 8.1\%.\textsuperscript{247, 250, 277} The CTCb regimen used in our randomised trial is a widely used high-dose regimen
developed at the Dana-Farber Cancer Institute for the treatment of solid tumours. The high-dose group in our study (SBG 9401) had a mortality rate of only 0.7%, which is, in fact, comparable with conventional adjuvant chemotherapy.

The mobilisation of a sufficient number of peripheral blood stem cells is crucial for a successful engraftment after high-dose therapy. At the time of the design of the adjuvant study, high doses of Cyclo, with or without G-CSF, were the most common mobilisation therapy. We used a modified FEC treatment with 1.2 g/m² of Cyclo followed by G-CSF with a possible benefit of better therapeutic index in breast cancer treatment, i.e. better antitumoral effects with an acceptable toxic profile. In the analysis of the first 50 patients in the high-dose arm, the feasibility of the modified FEC for mobilisation of CD34+ cells was shown, and the number of collected cells per patient was sufficient. The toxicity was reasonable, with grade 3–4 adverse events less than 10%. Comparable median number of harvested CD34+ stem cells has been demonstrated after treatment with FEC (500/90/500 mg/m²) and G-CSF in the large high-dose study from the Netherlands. At least four smaller studies have shown the feasibility of mobilising stem cells with high-dose or standard FEC/FAC with G-CSF.

As previously reported, we showed a correlation between the number of re-infused CD34+ stem cells and faster neutrophil and platelet recovery. These data support the idea that larger amounts of re-infused stem cells result in a safer procedure, rather than an existing threshold of 1–3 x 10⁶/kg. The obvious reason for this is a direct numeral proportional effect of the re-infused CD34+ stem cells. However, with the consideration of the large inter-individual variability in Pk and Pd and the 20-fold inter-patient variability of mobilised and collected CD34+ cells in our material, another explanation could be that large amounts of mobilised peripheral stem cells in a patient are a sign of high bone-marrow tolerance to cytotoxic drugs, including the CTCb regimen, and therefore result in a faster recovery.

5.3 Effect of G-CSF in the evaluation of bone metastases with MR imaging

MR imaging is superior to other imaging modalities in the detection of osseous metastases. In treatment follow-up, the commonly used T1-weighted sequences are useful in demonstrating PD in bone, but there value in demonstrating response in bone is questionable. We have previously described that the fat-depressing long TE IR-TSE sequence is more accurate in showing response in bone metastases.
We observed a homogeneous diffuse increase in signal intensity in normal bone-marrow during G-CSF treatment on the long TE IR-TSE sequence compared with the baseline investigation. This increased signal was not seen after G-CSF treatment or in patients without G-CSF. The signal was quantified and despite the low number of investigated patients, the difference between investigations during and after G-CSF was significant. Biopsy specimens from one patient during G-CSF revealed 90% bone marrow cellularity with an increased number of myeloid precursor cells. G-CSF causes the increased cellularity during treatment, and this morphological alteration could be visually detected by the long TE IR-TSE sequence during the time for G-CSF treatment. However, on T1-weighted images, no visual alteration in the signal was observed.

In the study, a long TE IR-TSE sequence was used and the sensitivity of other IR/STIR sequences with different echo-times was not studied. However, since signal alterations as an effect of G-CSF treatment have also been shown by Fletcher and co-workers using a common STIR sequence with a shorter TE (20 and 22 msec), it is feasible that many IR/STIR sequences can cause diagnostic errors by depicting the hypercellularity during G-CSF treatment.

The finding has principal clinical importance since the increased diffuse signal may be mistaken as disseminated metastatic disease and, furthermore, could obscure focal metastases. Therefore, a radiologist should be informed if patients are on treatment with G-CSF when bone-marrow is to be investigated and evaluations with the long TE IR-TSE sequence should be performed not earlier than three days after the completion of G-CSF treatment. The knowledge that the long TE IR-TSE sequence can visualise effects caused by G-CSF may be interesting in the development of new schedules of G-CSF treatment and in the implementation of longer action G-CSF (e.g. pegfilgrastim).
6 General conclusions

- Toxicity-adjusted chemotherapy with dose-escalated FEC or ET was feasible and resulted in an evenly distributed two- to three-fold range of tolerated doses. The tolerated dose levels of dFEC or ET were unrelated to toxicity and efficacy, which support the concept of tailored dosing of chemotherapy. The concept was equally feasible in primary and metastatic breast cancer, in two different chemotherapy regimens and in treatment with or without G-CSF support and may provide a pragmatic way of overcoming the shortcomings of standard BSA-based dosing.

- The tailored and dose-escalated FEC regimen was highly effective in the metastatic breast-cancer setting in terms of response, time to progression and overall survival. Similar treatment was in the adjuvant setting superior to high-dose chemotherapy in time-to breast cancer relapse. The tailored ET regimen was effective in metastatic breast cancer.

- The modified FEC-regimen with a double dose of cyclophosphamide and G-CSF was safe and effective for sufficient blood stem-cell mobilisation. A larger number of re-infused stem cells was correlated to faster engraftment.

- Inter-individual variability in pharmacokinetics can partly explain differences in toxicity in ET-therapy and was lower than the intra-individual variability, which supports the need for the further development of tailored Pk- or toxicity-based therapies. The semi-physiological model for Pk-Pd of the same regimen could predict grade and duration of white blood cell count nadir and may be a useful instrument for the further development of chemotherapy regimens.

- When using the long TE IR-TSE sequence in MR imaging, G-CSF gave rise to an increased diffuse signal in normal bone-marrow which may be mistaken as disseminated metastatic disease and could obscure focal metastases. In the assessment of bone metastases with MR imaging, the investigation should be performed at least 3 days after the completion of G-CSF therapy.
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8 References


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