New ways to optimize breast cancer treatment

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General introduction

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In this thesis ways for optimizing breast cancer treatment, and aspects of early systemic disease in particular, are described. Breast cancer is the most frequent type of cancer in women in the Netherlands, as one in ten women will develop breast cancer during life time. The concept of breast cancer as a systemic illness has been increasingly translated into systemic treatment over the past decades. Early systemic tumor spread in breast cancer patients is presumed to be commonly present at the first time that patients present. This is supported by the fact that following effective local treatment, many patients manifest metastatic involvement over time and that improvements in local control have been shown to provide only a small decrease in distant metastases (1, 2). Early ('adjuvant') systemic treatment in concert with local therapy, was shown to have a beneficial impact on survival rates of breast cancer patients (3), also when analyzed separately from the benefits induced by diagnostic screening (4).

Clinical trials in recent years have aimed at further optimization of early treatment modalities by means of chemotherapy, hormonal or biological approaches (5). Dose-intensification of chemotherapy to optimize adjuvant treatment, gained interest in view of the validation of this approach in the advanced breast cancer setting (6). The possible benefits of high-dose chemotherapy and stem cell transplantation in the adjuvant setting for breast cancer have been studied in a number of large randomized studies, the results of which are expected to be published soon (7). The selection of breast cancer patients for adjuvant treatment, is traditionally based on the evaluation of tumor presence in the axillary lymph-nodes. However, it is clear that conventional evaluation of axillary lymph node status has its flaws. Forty percent of women with tumor positive lymph nodes survive more than 10 years, and vice versa: distant metastases develop in 20 to 30 % of patients with node-negative cancer (8). The development of those distant

metastases suggests that there may be alternative metastatic routes, other than the classical sequence of tumor-lymph node-hematogenous metastases (reflected in the TNM classification for breast cancer staging, Union International Contre le Cancer 1997). Support for this concept was recently provided in a study by Braun et al. (9), indicating that the ability of tumor cells to expand hematogenously to the bone marrow is independent of their ability to metastasize to axillary lymph nodes. Although primary tumor characteristics may be helpful for selecting the group of node-negative patients at risk for metastases (10), the identification of new markers which predict relevance of early adjuvant systemic treatment has gained much interest in the last decade. Particularly in view of the use of adjuvant high-dose treatment, requiring haematopoietic stem cell transplantation to counteract profound bone marrow aplasia, this selection may have clinical impact. In addition, detection of tumor cells in hematopoietic stem cells products can provide information on their clinical impact. It is possible that tumor cell contamination of haematopoietic stem cells may directly contribute to relapse in breast cancer patients, as was described previously in haematological malignancies (11). However, in the adjuvant breast cancer treatment setting, the impact of tumor cells in stem cell products is as yet unknown (11).

Thus, sensitive staging techniques for the detection of micrometastatic disease may be clinically helpful in the treatment of breast cancer. It could serve as a tool for direct evaluation of the effect of early systemic treatment. Also, it may prove helpful for the selection of patients for early treatment modalities. Furthermore, if minimal amounts of tumor cells would prove detectable, (*in vitro*) methods for removing these tumor cells could be evaluated. These data provide the basis for this thesis: the evaluation of aspects of breast cancer, and the detection and removal of early systemic disease in particular, thus possibly providing tools for optimizing treatment.

Content of the thesis

In chapter two an overview is given concerning methods for detecting minimal amounts of tumor cells in peripheral blood, peripheral blood stem cells and bone marrow. In this respect, the possible role of mobilization of tumor cells by means of hematopoietic growth factors is described. Subsequently, the various options for removing these tumor cells are discussed.

In chapters three, four and five, the detection of tumor cells based on the presence of the expression of epithelial glycoprotein-2, EGP-2. As a pan-carcinoma marker, EGP-2 is universally expressed in breast cancer specimens (12). As such, EGP-2 is a commonly used target antigen in a number of carcinoma-directed immunotherapeutical approaches (12- 14). In chapter three, molecular detection of the presence of EGP-2 by means of a quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) method is validated and compared to immunohisto(cyto)chemical detection by means of the MOC31 antibody, directed against EGP-2. EGP-2 was found to be expressed at different levels in primary tumor samples. To make assumptions regarding the quantification of tumor load in the blood stream of patients, its appears necessary to use EGP-2 expression in both primary tumor and blood, in individual patients. In chapter four, therefore, the EGP-2 marker is used for detecting tumor cell presence in primary tumor samples, sentinel lymph nodes and peripheral blood samples from breast cancer patients. In this perioperative setting, the ability to detect minimal amounts of tumor cells may serve as a tool for selecting patients for adjuvant treatment. Analysis of EGP-2 expression was performed in an automated way by means of a real-time PCR device (Light Cycler). Due to simplified technical protocols using this machine, the risk of contamination was found to be reduced, and therefore in chapter five, this technique was used for sequential detection of minimal residual tumor cells in the peripheral blood of patients randomized to receive adjuvant standard or high-dose

chemotherapy and autologous peripheral blood stem cell support. In this setting, the ability to detect minimal amounts of tumor cells may serve as a tool for evaluating the efficacy of these treatment modalities.

In chapters six and seven the options for removal (or 'purging') of minimal amounts of tumor cells are explored. To this end, the bispecific antibody BIS-1 was used. This bispecific antibody, directed against EGP-2 on tumor cells and CD3 on T lymphocytes, creates functional cross-linking of T cells and tumor cells allowing the delivery of a tumor cell specific lethal hit inducing specific epithelial tumor cell kill *in vitro* and *in vivo* (13). In chapter six, a method is described for purging tumor cells from peripheral blood stem cells harvests of breast cancer patients, receiving adjuvant high-dose chemotherapy and autologous peripheral blood stem cell support. T lymphocytes, present in the peripheral blood stem cell harvests, were activated for optimal tumor cell kill potential, and retargeted by BIS-1. In chapter seven, a similar method was then applied to the setting of minimal tumor cell contamination of cryopreserved ovarian tissue, of female cancer patients with impending loss of fertility due to cancer treatment. Tumor cell kill and morphological follicle survival were studied in an *in vitro* model in which activated lymphocytes and BIS-1 were added to tumor cells, in the presence or absence of a suspension of human frozen-thawed ovarian tissue. These studies on the purging of minimal amounts of tumor cells may contribute to the safe auto-transplantation in cancer patients, of either stem cells or ovarian tissue.

In chapters eight to ten, various aspects of breast cancer treatment, assessed in patients (chapter eight), or patients samples (chapters nine and ten) are described. The issue of febrile leukopenia in breast cancer patients receiving intermediate high-dose chemotherapy is described in chapter eight. To reduce the incidence of febrile leukopenia, the use of prophylactic antibiotics was compared to hematopoietic growth factor recombinant human granulocyte-stimulating growth

factor (rhG-CSF). The manageability of chemotherapy induced side-effects may improve the safe treatment of breast cancer patients. Chapter nine focuses on hematopoietic reconstitution and the possible induction of increased aging in hematopoietic stem cells, in breast cancer patients receiving either adjuvant standard or high-dose chemotherapy and autologous peripheral blood stem cell transplantation. Telomere length, as a marker for cell turn-over and therefore of aging, was measured in the nucleated peripheral blood cell fraction of these patients. The increased aging of hematopoietic stem cells due to stem cell transplantation may have important undesirable long-term effects, that could be clinically relevant in patients with a relatively good prognosis. Insight in possible causes for long-term implications of adjuvant treatment are important for optimizing breast cancer treatment. In chapter ten, primary breast tumors are evaluated for the presence of death receptors and ligands. The presence of death receptors Fas (receptor for Fas Ligand, FasL), and DR4 and DR5 (receptors for TNF-related apoptosis inducing ligand, TRAIL) in primary breast tumors, may possibly allow treatment with their ligands. In addition, as death receptors may be up-regulated by estrogen deprivation, these parameters were evaluated in tumors after pre-operative anti-estrogen therapy. The possibility to assess these effects in individual tumors may allow more 'tailor-made' breast cancer treatment in the future.

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