Biological Concepts of Prolonged Neoadjuvant Treatment plus GM-CSF in Locally Advanced Tumors

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Key Words. GM-CSF · Doxorubicin · Cyclophosphamide · Antineoplastic agents · Combined breast neoplasms · Angiogenesis · Dendritic cells

ABSTRACT

Local treatment with surgery and radiotherapy gives unsatisfactory results in patients with locally advanced cancer. In many cases distant metastases appear shortly after the removal of the primary tumor. Selecting breast cancer as a model for locally advanced disease, we are extrapolating our findings to other solid tumors. Neoadjuvant chemotherapy has improved survival of these patients by downstaging the primary tumors allowing local treatment and early elimination of distant micrometastases.

We recently reported in this journal on a study of 42 patients with locally advanced breast cancer (LABC) who received prolonged neoadjuvant chemotherapy of doxorubicin, cyclophosphamide, and GM-CSF prior to surgery and postoperative radiotherapy. These results were promising and prompted us to initiate an international randomized phase III study in which either six neoadjuvant cycles or three neoadjuvant cycles plus three adjuvant cycles are being compared. In LABC patients treated with six neoadjuvant chemoimmunotherapy cycles, we observed a significant rise in the dendritic cell content of the axillary tumor-draining lymph nodes after therapy, associated with an encouraging disease free survival and overall survival. We hypothesize that the prolonged presence of draining lymph nodes in combination with the repeated tumor antigen release, dendritic cell recruitment, and activation may account for the observed increased survival of LABC patients. Based on our findings and the results of preclinical studies, we hypothesize that it is more effective to administer chemotherapy in an extended neoadjuvant regimen, taking advantage of the concurrent biological and immunological processes in the primary tumor and its draining lymph nodes. The Oncologist 2000;5:497-500

INTRODUCTION

Local treatment with surgery and radiotherapy gives unsatisfactory results in patients with locally advanced cancer. In many cases distant metastases appear shortly after the removal of the primary tumor. This problem is known from several tumor types originating from breast, esophagus, stomach, bladder, or the head and neck region.

Apparently in many patients nondetectable micrometastases are present at the time of diagnosis. Selecting breast cancer as a model for locally advanced disease, we are extrapolating our findings to other solid tumors. When treated with local therapy alone, patients with locally advanced or inflammatory breast cancer (LABC) have a poor prognosis with a five-year overall survival (OS) rate of only 5%-20% [1, 2]. Neoadjuvant chemotherapy has improved survival of these patients by downstaging the primary tumors allowing local treatment and by early elimination of distant micrometastases. Generally three to four preoperative chemotherapy cycles are administered followed by a number of postoperative chemotherapy
cycles. The reported clinical response rates vary between 30% and 80% with 10%-30% clinical complete remissions and long-term survival in 15% of the patients [3-6]. High-dose chemotherapy with autologous stem cell support further improved disease-free survival (DFS) to a reported 64% after 30 months [7] but has yet to be shown to improve OS. We recently reported in this journal on a study of 42 patients with LABC who received prolonged neoadjuvant chemotherapy of doxorubicin, cyclophosphamide, and GM-CSF prior to surgery and postoperative radiotherapy [8]. GM-CSF was chosen instead of G-CSF because of its additional immunostimulatory and potential angiogenic effects. We hypothesized that the prolonged presence of the primary tumor and the long-term administration of GM-CSF with the primary tumor and the axillary lymph nodes in situ might give rise to tumor-specific cytotoxic T-cell responses. Furthermore, tumor-derived antiangiogenic factors might inhibit growth in micrometastases. To improve these effects, the number of neoadjuvant chemotherapy cycles was gradually extended from four to six cycles during the study whenever toxicity allowed it. A high clinical response rate of 98% with a 50% complete response rate was observed. At a median follow-up of 49 months, for a total of 42 patients the actuarial DFS and OS rates at three years are 57% and 79%, respectively. With a median follow-up of five years, the actuarial DFS and OS rates of all patients are 55% and 67%, respectively. However, in the 24 patients who received six neoadjuvant cycles, the actuarial DFS and OS at five years are 66% and 79%, respectively. These promising results have prompted us to initiate an international randomized phase III study in which either six neoadjuvant cycles or three neoadjuvant cycles plus three adjuvant cycles are being compared. Immunological and biological factors are being studied both in the primary tumor with its draining lymph nodes and the peripheral blood of all patients [9].

**Immunological Aspects in Locally Advanced Disease**

In various ways tumor cells can escape recognition by the immune system as a potential threat. This enables growth of the tumor to a size too large to be handled by the immune effector cells. Dendritic cells play an important role in the initiation of an antitumor response [10]. These bone marrow-derived cells are the most professional antigen-presenting cells identified to date. In an immature state they take up tumor antigens and present these in the context of major histocompatibility complex molecules to naïve or memory T-cells. Depending on the recognition of self and nonself antigens and so-called danger signals, either tolerance or an effective cellular immune response is induced. In several tumor types, dendritic cell infiltration in the primary tumor has been associated with prolonged survival and a reduced incidence of metastatic disease [11-15].

In patients with LABC, a dysfunction of dendritic cells has been observed [16, 17]. A general immunosuppressive state with depressed T-cell reactivity is progressive and prognostically significant with increasing stages of breast cancer [18]. The immune system appears to be disabled by several tumor-derived cytokines that have been shown to inhibit dendritic cell maturation (interleukin 10 [IL-10], transforming growth factor β [TGF-β]), IL-6, and vascular endothelial growth factor [VEGF]), and T-cell effector functions in vitro (IL-10, TGF-β) [19-22].

**Immunotherapeutic Effects of Prolonged Neoadjuvant Chemotherapy and GM-CSF Administration**

During neoadjuvant chemotherapy combined with long-term administration of GM-CSF, several processes take place that can potentially overcome the apparent failure of the immune system to eradicate the tumor. Chemotherapy reduces the production of tumor-derived immunosuppressive factors, enabling the initiation of tumor-specific cytotoxic T-cell responses. Chemotherapy-induced tumor cell necrosis and apoptosis both cause release of antigen, which can be taken up by newly recruited immature dendritic cells. GM-CSF not only recruits dendritic cells from the bone marrow, but also stimulates their maturation and activation to acquire their maximum ability to prime cytotoxic T-cells [23]. GM-CSF-mediated recruitment of dendritic cells to the tumor site may protect tumor-infiltrating lymphocytes from an early death by Fas-mediated apoptosis [24]. In LABC patients treated with six neoadjuvant chemoimmunotherapy cycles, we observed a significant rise in the dendritic cell content of the axillary tumor-draining lymph nodes after therapy, associated with an encouraging DFS and OS. We hypothesize that the prolonged presence of draining lymph nodes in combination with the repeated tumor antigen release, dendritic cell recruitment, and activation may account for the observed increased survival of LABC patients.

**Biological Aspects of Prolonged Presence of the Primary Tumor**

From the Lewis lung carcinoma (LLC) model it is known that in the presence of the primary tumor, the development of distant metastases is arrested. However, after the removal of the primary tumor, distant metastases neovascularize and start to grow rapidly within three days. A circulating angiogenesis inhibitor, angiotatin, was shown to be responsible for the dormancy of micrometastases [25]. The
process of neovascularization is controlled by several pro-
and antiangiogenic factors. Depending on the balance of
these factors, either new vessel formation will start or
metastases are kept dormant [26]. Macrophage-derived fac-
tors have shown to be responsible for the induction of
angiostatin production. Furthermore, transfection of LLC
cells with the GM-CSF gene inhibited tumor growth, while
an increased number of tumor-infiltrating macrophages and
elevated levels of circulating angiostatin were found [27].
These studies suggest a role for GM-CSF in the generation
of a circulating angiogenesis inhibitor. As described in an
erlier study [28], we observed an increase in macrophages
in the mastectomy specimens compared with pretreatment
biopsies, possibly due to the administration of GM-CSF.
These macrophages may be responsible for the genera-
tion of angiostatin or other protein fragments inhibitory for
angiogenesis as has been shown in the LLC model.
We thus hypothesize that the primary tumor and its
stroma can generate antiangiogenic peptides that may keep
micrometastases dormant.
A considerable interplay between the immunomodulat-
ing and antiangiogenic effects of the proposed neoadjuvant
treatment exists. VEGF, a proangiogenic factor, has been
shown to inhibit dendritic cell maturation in vitro [20],
which may be overcome by GM-CSF. Furthermore, angi-
genesis inhibitors can upregulate adhesion molecules that
are important for leucocyte-endothelial cell interactions
[29]. Finally, the recruitment and maturation of dendritic
cells induced by GM-CSF may lead to increased levels of
intratumoral IL-12. This cytokine has previously been
shown both to direct the initiation of effective cell-mediated
antitumor immune responses and exhibit considerable
antiangiogenic effects [30, 31].
To study these proposed hypotheses we have initiated
the Spinoza trial, an international multi-center phase III
study in patients with LABC, comparing the conventional
approach of applying three preoperative and three postop-
erative cycles of chemoimmunotherapy and the extended
chemoimmunotherapy approach.
Patients with stage IIB (with a primary tumor >5 cm),
IIIA, and IIIB breast cancer according to the American Joint
Committee on Cancer criteria are included. Primary objec-
tives of the study are to increase the three-year DFS and OS.
A second randomization takes place to study the effects of
GM-CSF on OS and DFS in comparison with G-CSF in both
treatment arms. We are collecting peripheral blood, tumor,
and axillary lymph node samples to study the immunomodu-
lating effects of GM-CSF or G-CSF on dendritic cells and
tumor-specific T-lymphocytes.

CONCLUSION
Based on our findings and the results of preclinical stud-
ies, we hypothesize that it is more effective to administer
chemotherapy in an extended neoadjuvant regimen, taking
advantage of the concurrent biological and immunological
processes in the primary tumor and its draining lymph nodes.
The initiated international randomized Spinoza trial will allow,
by its bifactorial design, testing of the effect of prolonged
neoadjuvant treatment as well as the effect of GM-CSF com-
pared with G-CSF on the DFS and OS of patients with LABC.
These results may be expanded to the treatment of other
locally advanced solid tumors.

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