Low Molecular Weight Protein Apheresis and Regression of Breast Cancer
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Abstract: The prognosis for women with metastatic adenocarcinoma of the breast who have failed at least one cycle of systemic chemotherapy or hormonal therapy is dismal. Salvage approaches, including intensive chemotherapy with stem cell support, have not as yet demonstrated significant curative ability. New approaches are therefore needed for this group of patients. The removal of recently discovered soluble cytokine inhibitors present in the serum of patients with a variety of cancer may, by a unique mechanism, lead to tumor regression by increasing inflammatory responses.

Ten patients with biopsy-proven metastatic adenocarcinoma of the breast with objectively evaluable disease were treated with repetitive whole-blood ultrapheresis. Nine patients were considered evaluable for response. This new form of therapeutic apheresis (ultrapheresis) is designed to selectively remove a low molecular weight protein fraction (less than 150kD) from whole blood. The procedures were generally well tolerated and no significant adverse clinical effects were encountered. A consistent low-grade fever was provoked by the procedure, which was associated with the development of inflammatory pain in tumor sites. Objective tumor regressions followed the onset of this tumor-specific inflammatory response in five of the nine evaluable patients. We conclude that it is likely that soluble immunosuppressive materials known to be elevated in the cancer-bearing patient are a reflection of generalized immune-down regulation that enhances tumor growth and development. Progression of human cancer, at least in part, depends upon the suppression of an otherwise intact immune defense system. One mechanism for this inhibition is a blockade mediated by solubilized cytokine inhibitors. Although this study involves a small number of patients, it strongly suggests that a beneficial response can be achieved by removing these inhibitors.

Key Words: Immune suppression, cytokine inhibitors, ultrapheresis, tumor-specific inflammation

Introduction
Immunosuppression has been a consistent hallmark of patients with advanced malignancy and well documented in the literature in multiple reports over the past 25 years. The serum of patients with cancer has been shown to be immunosuppressive. This effect has been ascribed to the presence of shed tumor antigens, (1) Immunoglobulin, (2) alpha globulins, including acute phase reactants, (3) and more recently, by the solubilized cytokine inhibitors. (4-6)

Ultrapheresis is capable of removing only molecules of the size 150kD and less from blood: a size range demonstrated to carry specific inhibitors to tumor necrosis factor (TNF), Lymphotoxin (LT), IL-2, IL-6, and Interferons (7). A recently discovered inhibitor to IL-1 also resides in this molecular weight fraction of serum (8). Shiozaki et al. (9) and Uchida, et al. (10) have recently confirmed that this protein fraction also contains material that accelerates both tumor growth as well as the development of metastases in mice, and also contains potent inhibitors of natural killer cell activity in vitro. These latter materials are yet to be completely characterized. (11-14)

Previous attempts at manipulating immune responses by plasma pheresis may have been unsuccessful because the process removes cytotoxic antibody and antibodies that participate in antibody-dependent cellular toxicity as well cytokine and cellular inhibitors, therefore achieving no net gain in terms of shifting balances. Ultrapheresis does not remove antibodies, acute phase reactants or formed elements from blood. The selective removal of the family of cytokine and killer cell inhibitors from plasma should therefore shift the balance between inhibitors and promoters of cellular and humoral immune responses against tumors in favor of response.
This may allow the development of a tumor-specific inflammatory response in the tumor-bearing patient. 

The purpose of this study was to remove from serum this protein fraction and the inhibitors it contains in order to perturbate the balance between soluble inhibitors and promoters of a tumor-specific inflammatory response. In a phase I study, evidence of an induced inflammatory reaction in the tumors of patients with breast cancer was observed. 

This report demonstrates that this inflammatory response may lead to tumor regression in some patients with metastatic breast cancer.

**Materials and Methods** - The study protocol was reviewed and approved by the local Institutional Review Board and conducted under the authority of the United States Food and Drug Administration Investigator IDE# G850101. All of the patients enrolled gave written informed consent. As no commercial device was available for the fractionation of plasma proteins in this range, a custom device and filters were designed and built (see Figs. 1 and 2). Briefly, the foundation of the device is a Kiel dialysis kidney. It is configured with track-etched Nuclepore membrane to provide the desired performance. The Kiel cell is further modified to function as a blood ultrafilter, instead of a parallel dialyzer.

**Figure 1.** Ultrafiltration apparatus and filter. Blood is drawn from the patient, filtered of low molecular weight protein fraction and discarded by a positive displacement pump. A second displacement pump meters ultrafiltrate from non-tumor bearing patients serum and returned to the filtered blood at a rate equal to ultrafiltration.

**Figure 2.** Track-etched polycarbonate membrane 0.05 micron pore size (Nuclepore, Pleasanton, California, USA) Semi-log plot of sieving coefficient versus molecular weight.

The treatment has been previously reported. In summary, a dialysis catheter of the Vascath type was employed for vascular access. Each patient received ultrapheresis three times a week. The initial amount of ultrafiltrate removed was 20cc/kg, lean body mass (LBM). The time required for each procedure was approximately two hours. The volume removed per procedure was increased on successive procedures until fever and clinical evidence of a tumor-specific inflammatory response was achieved (vide infra). The range of volumes removed was 20 to 40cc/kg LBM (average: 25cc/kg, or approximately 50% of a calculated plasma volume (plasma volume = 1/hematocrit X 0.07LBM). The replacement solution used was an equal volume of ultrafiltrate removed from normal plasma by subjecting appropriate ABO-matched plasma to the same plasma fractionization procedure as the patient. Plasma was screened for HIV and Hepatitis A, B and C antibodies, and the filters were sterilized prior to administration.

**Laboratory measurements** - Total WBC count and serum CRP were followed as objective measures of systemic inflammation. Tumor markers were measured by the Dianon Systems, Inc. Reference laboratory (Stratford, CT). Carcinoembryonic antigen (CEA) is expressed in
ng/ml and CA 15-3 is expressed in U/ml. The soluble TNF receptor (TNF\textsuperscript{r} (i.e., the TNF inhibitor)) was measured in biological fluids with a Bender MedSystems (Vienna, Austria) ELISA test kit, distributed in the USA by Biosource International, Inc. (Camarillo, CA). TNF inhibitor results were read with an automated ELISA plate reader (SLT Lab Industries model EAR 400 AT, Salzburg, Austria). The levels of TNF\textsuperscript{r} in normal subjects were determined from samples of healthy volunteers from the laboratory and clinic. Ultrafiltrate samples were obtained from the midway volume during the procedure to minimize dilution with the saline used to prime the filters. It was determined that -20°C freezing had no effect on the TNF\textsuperscript{r}, which accordingly allowed samples to be processed in a batchwise fashion.

Response Criteria - The response to ultrapheresis was monitored clinically, and by serial radiographs, computed tomography, bone scans and serum tumor markers. The inflammatory response was characterized clinically by the development of pain and tenderness at the sites of tumors, and was frequently associated with a low-grade fever. Clinical response was defined as objective reduction in the size of non-osseous measurable disease and/or evidence of sclerosis in lytic bone metastases, associated with a decrease of biochemical serum markers. Markers employed for the study were serum CEA and CA 15-3 antigen. Complete response was defined as the complete disappearance of all measurable disease, complete sclerosis of bone metastases with clearing of bone scan and complete relief of pain and normalization of serum tumor markers. A partial response was defined as a greater than 50% reduction in the size of measurable disease by radiographic studies, and improvement of bone scan with sclerosis of previous lytic sites as measured by X-ray and computerized axial tomography in association with a greater than 50% reduction in serum tumor markers. A minimal response was defined as less than a 50% reduction in the dimensions of the objectively measurable disease. Stable disease was defined as no tumor growth while on protocol.

Patients Enrolled On Protocol - Nine patients were evaluable after completing protocol (see Table 1). All patients had developed metastatic disease after standard surgery and/or radiation therapy to the primary lesion of the breast. Prior therapies directed at metastases on protocol entry included hormonal therapy (5/9), chemotherapy (6/9, two were felt to have had an inadequate trial of less than three cycles of CMF or CAF) and radiation therapy (5/9). One patient refused chemotheraphy and radiotherapy. Pre-menopausal disease was noted in 7/9 patients and 2/9 had post menopausal disease. The ages of the patients ranged from 26 to 60 years. The mean Karnofsky performance status at protocol entry was 75%, with a range of 60-90%.

<table>
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<th>Study</th>
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<th>Tumor Location</th>
<th>Treatment</th>
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<th>Tumor Markers</th>
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Results: A total of 136 ultrapheresis procedures (see Table 1) were administered to nine patients. Clinical responses are recorded in the upper section of Table 1. In this phase II study, we observed consistent tumor-specific inflammatory response to variable degrees in a cohort with widely metastatic adenocarcinoma of the breast; the majority of whom were heavily pretreated. In responders, both visceral pain as well as pain from osseous metastases resolved. The more intense tumor-specific inflammatory responses were correlated with the best clinical responses. Indeed, in this limited number of patients, we did not observe a lack of clinical response following
tumor-specific inflammatory response (Figs. 3, 4, 5 and 6).
We recorded two complete responders, two partial responders, one minimal responder, one stable disease and three non-responders.
One patient was found to be non-evaluable. Her tumor markers normalized, but she had only blastic bone metastases and neither plain X-rays nor bone scans changed. This yielded an overall 44% major response rate for this study.

Figure 3. IV contrasted CAT scan of abdomen revealing multiple contrast enhancing sites of metastatic breast cancer throughout the liver of a 49 year old woman.

Figure 4. IV contrasted CAT scan of abdomen revealing complete clearing of all measurable metastatic breast cancer in the liver after 24 ultrapheresis procedures given over eight weeks.
The results for ELISA measurements of the soluble TNF receptor (TNFr) are summarized in Fig. 7. It was apparent that the control values were tightly clustered about a mean 169±51 ng/ml. The patients expressed overall higher quantities of TNFr, and the values were distributed over a very broad range (mean 1093±1414ng/ml). As would be expected, the measurements for the ultrafiltrates were consistent with those obtained with patients’ plasma (mean 510±121) after being transformed upward to reflect the sieving coefficient (0.40) for ultrafiltration of the 60kD TNFr molecule, assuming that is a typical globular protein in conformation. For the basis of comparison, if one eliminates the three samples in the patient plasma group in excess of 3,000ng/ml, the mean for the patients becomes 513±247ng/ml, supporting correlation between ultrafiltrates and blood plasma values.

Figure 7. Measurements of the soluble TNF receptor/inhibitor. Measurements were performed using a Bender MedSystems ELISA test kit in accordance with the manufacturer’s recommendations. Tests were performed on plasma and patient’s ultrafiltrate. Normal controls were healthy, laboratory and clinic personnel. The horizontal bars in each group of symbols are mean values. The dashed, lower horizontal bar in the patient group is the mean excluding the three patients whose TNFr level exceeded 3,000ng/ml.

CEA and CA 15-3 (Figs. 8 and 9) CEA is a serum protein produced by cancer cells with a molecular weight of 200,000. CA 15-3 antigen is also produced by breast cancer cells and in heterodisperse glycoprotein with a molecular weight range of 200,000 to 600,000 in serum. Neither protein passed through the ultrafiltration
membrane in detectable quantities. We can therefore assume that changes in these tumor markers are not a result of them being physically removed in the filtration process. Additionally, serum measurements of both CEA and CA 15-3 immediately before and after ultrapheresis revealed no differences in level.

Figure 8. Carcinomembryonic antigen (CEA) decline over time in responding patients.

Figure 9. CA 15-3 antigen decline over time in responding patients.

Adverse effects - Clinically, the response to the ultrapheresis procedure consisted of fever (99.6-102°F) that began within 1-8 hours of completing the procedure. The temperature elevation lasted 2-12 hours (average four hours), and was consistently observed in this patient population. Fever promptly responded to an acetaminophen. Pain of tumor sites was described by patients as a deep, burning ache or a warm, throbbing pain developing during fever but lasting up to 36 hours. Pain was often severe and required narcotic analgesics for control. No significant changes in general physical examination parameters were observed. Hematologic and biochemical tests showed no significant changes, except for an absolute monocytosis and an increase in CRP and fibrinogen. Interestingly, total white blood count did not rise with, or following the fever. There was no significant change in blood cellular elements or immunoglobulins associated with the procedure.

Discussion
In this study, we observed tumor inflammatory response that was followed by a 44% major clinical response in a cohort of women with advanced metastatic breast cancer. The response was correlated with the removal of a protein fraction of serum shown to contain an inhibitor to TNF and LT. Previous phase I work with ultrapheresis, not duplicated in this study, convinced us that the observed clinical effects were not related to endotoxin. The observed clinical response is consistent with an immune mediated attack against tumor cell targets involving a TNF mechanism histologically (see Figs. 10 and 11).

It is now known that, in the host, TNF and LT work in combination with other cytokines (IL-1, IL-2, IL-3, IL-6 and the interferon family). Each of these participates in the inflammatory response and inhibitors to each reside in the molecular weight size range 30-100kD, which is removed by ultrapheresis. Our finding of a wide range of circulating inhibitor concentration from 200-4610ng/ml in so small a patient population suggests that the intensity and efficacy of the response might be improved by tailoring the degree of filtration so as to achieve a targeted TNF inhibitor level in the serum after each procedure.
Finally, the finding of a circulating inhibitor to TNF and LT that is significantly elevated in patients with cancer, suggests a paradigm for cytokine regulation. It strongly suggests that the immune response is dynamic, and as such, the quantity of a particular cytokine in the patient may not be as important to influencing a cancer as the regulatory stage upon which cytokines and inflammatory cells act. It suggests further that the immune mediated inflammatory response is homoeostatically regulated in nature, and that future therapeutic strategies in tumor immunology must respect the balance between cytokines, cells and their inhibitors/receptors.

References


