COOPERATIVE AGREEMENT DAMD17-94-V-4012

TITLE: PET-FDG Imaging in Metastatic Breast Cancer Treated with High Dose Chemotherapy and Stem Cell Support

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REPORT DATE: September 1996

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release; distribution unlimited

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This study is designed to assess the effectiveness of positron emission tomography (PET) with fluorodeoxyglucose in patients with metastatic breast cancer undergoing high dose chemotherapy with stem cell rescue. It includes patients enrolled on two high dose chemotherapy trials (PBT-1 and UPCC # 3195). So far, 24 patients have been enrolled in the protocol, and the study is proceeding as planned. We have performed a preliminary analysis of the PET studies of 17 patients performed before high dose chemotherapy. In this subgroup of patients, 11 had active disease demonstrated on the PET study, and 6 had no evidence of metabolically active disease. Three subjects had disease involvement demonstrated only on PET imaging, and in one case, PET confirmed the clinical impression of responding liver metastases shown on CT. Several repeat studies have been performed and will be analyzed when enough prognostic information becomes available. Patient accrual is ongoing and the study is proceeding as scheduled. PET FDG imaging is a promising tool that can potentially predict early therapeutic failure.
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INTRODUCTION

Positron emission tomography (PET) was introduced as a research modality to investigate physiological and biochemical alterations in the brain and heart\(^1\). Many radiopharmaceuticals have been introduced for the study of various organs, but \(^{18}\)F-fluoro-2-deoxy-2-glucose (FDG) is generally considered the most useful radiopharmaceutical for the diagnosis of various tumors. Breast cancers have enhanced glycolytic activity and have a significant overexpression of glucose transporters\(^2\). Tumor hypoxia has been shown to increase FDG retention\(^3\), and the tracer has been shown to be mainly incorporated in malignant cells\(^4\).

There are now several reports of studies of patients with breast cancer, suggesting that the PET-FDG technique is effective in diagnosing and following patients with primary and metastatic breast tumors\(^5\)-\(^10\). A recent retrospective study on the efficacy of PET in detecting axillary lymph node involvement has suggested potential cost savings by reducing the number of axillary dissections for breast cancer. Almost 74,000 women (75% patients) with primary breast tumors could potentially be spared axillary dissection based on the sensitivity and specificity of PET-FDG imaging to detect lymph node involvement\(^11\).

Some groups have reported on the use of PET to evaluate tumor response to therapy. Wahl\(^12\) described the use of PET-FDG for monitoring the treatment response of primary breast cancer. Eleven patients with large primary cancers were studied before chemohormonotherapy and at four times after initiating treatment (at days 8, 21, 42 and 63). The quantitative PET scans showed a rapid decrease in tumor glucose metabolism in all eight patients whose cancers responded clinically, but no change in the 3 non responding patients. Qualitative (visual) analysis gave the same result. The metabolic change preceded clinical evidence of response (mammographic change), and in some patients the mammogram was difficult to interpret due to dense breast tissue. Thus, the PET-FDG appeared to be an early and accurate predictor of breast cancer response. Huovinen et al\(^13\), using \(^{11}\)C-Methionine, reported changes in uptake in soft tissue lesions of eight patients treated with chemotherapy, hormone therapy or radiation. The PET responses correlated with clinical responses; uptake increased in those who showed progressive disease, and decreased in patients with stable or improving lesions. Jansson et al\(^14\) studied sixteen patients with locally advanced and metastatic breast cancers receiving chemotherapy. They noted a decrease in uptake (\(^{11}\)C-Methionine or FDG) compared to pretreatment scans in eight of twelve responders after the first course of therapy (scans were performed at 6 - 13 days after treatment). Scans done after a third chemotherapy course showed a decrease in all clinical responders. These responses were noted in breast, axillary nodes, pleura and liver.
The purpose of our study is to evaluate the effectiveness of PET-FDG in patients with metastatic breast cancer who are also being treated with high dose chemotherapy and stem cell rescue. The hypotheses of the study are as follow:

1) Active tumor sites shown by anatomical imaging methods will be associated with high levels of metabolic activity while inactive sites will be reflected by low levels of FDG uptake.

2) Reduction in tumor metabolic activity of tumors will be an early predictor of response to high dose chemotherapy.

3) Patients with no abnormal FDG uptake prior to high dose chemotherapy will live longer than patients with tumor that are metabolically active.

The use of PET in this setting is potentially cost-saving considering the high costs of stem cell rescue. Non responders do not need to undergo further chemotherapy with consequent suffering and high costs, when palliation is more appropriate. On the other hand, the ability to predict the response to chemotherapy in responders might enable the physician to modulate the treatment for each patient.

The study includes a homogeneous group of patients entered on two University of Pennsylvania studies for the treatment of breast cancer with high dose chemotherapy. The chemotherapy protocols are protocol UPCC #3195 and Protocol PBT-1.

**BODY**

**Materials and Methods**

**Patient Selection:**

Patients selected for entry in this study are women accepted for one of the two high dose chemotherapy protocols utilizing autologous stem cell support at the University of Pennsylvania. The two protocols are: Protocol UPCC #3195 ("Phase II Pilot Study of High Dose Chemotherapy With Melphalan Followed by Cyclophosphamide, Thiotepa, and Carboplatin with Cyclophosphamide and G-CSF Augmented Peripheral Stem Cell Support For Women With Responding Metastatic Breast Cancer") or Protocol PBT-1 ("Phase III Randomized Comparison of Maintenance Chemotherapy with Cyclophosphamide, Methotrexate and 5-FU vs. High Dose Chemotherapy with Cyclophosphamide, Thiotepa and Carboplatin and autologous bone marrow support for women with metastatic breast cancer who are responding to conventional induction chemotherapy").
Chemotherapy Studies:

UPCC #3195: This study is a University of Pennsylvania Cancer Center single institutional trial designed for patients with metastatic disease or inflammatory breast cancer. Those patients with no evaluable disease or a documented complete or partial response to standard chemotherapy are treated with high dose sequential chemotherapy and peripheral stem cell rescue. Patients receive high dose Cyclophosphamide followed by G-CSF to stimulate stem cell production. This is followed by apheresis to harvest stem cells. When blood count recovery has occurred, high dose Melphalan is administered to the patient followed by infusion of one-third of the collected stem cells. Twenty-one days later, the patient is treated with high dose chemotherapy regimen consisting of Cyclophosphamide (1500 mg/m²), Thiotepa (125 mg/m²) and Carboplatin (200 mg/m²), each drug being given daily for four days. This is followed by peripheral stem cell reinfusion.

PBT-1: The purpose of this study is to compare the time to treatment failure, overall survival and toxicity in patients with metastatic breast cancer who are treated with conventional chemotherapy alone or conventional dose chemotherapy followed by high dose chemotherapy and autologous bone marrow rescue. Patients are entered in this trial prior to receiving any chemotherapy for metastatic disease. They will then receive Cytoxan, Adriamycin and 5-FU. At the end of 4 - 6 cycles of treatment for metastatic disease, the patients will be reevaluated. Those in a partial response or in a complete response will then be randomized either to continue the same chemotherapy (or change from Adriamycin to Methotrexate after a total dose of Adriamycin has been given) until relapse or to receive high dose therapy and autologous bone marrow treatment with no further therapy after the transplant. The high dose regimen consists of 4 days of Cyclophosphamide (1500 mg/m²), Thiotepa (125 mg/m²) and Carboplatin (200 mg/m²). The patients who undergo bone marrow transplantation are selected for this PET study.

PET Camera:

The PENN PET 240H camera, manufactured by UGM, has been used extensively over the last 5 years for FDG and ¹⁵O-water brain studies, FDG whole-body cancer studies, and FDG/¹⁵N-ammonia cardiac studies. This scanner is based on NaI(Tl) position-sensitive detectors, which leads to high spatial resolution, 5.5 mm (FWHM) in the transverse and axial directions, and fine spatial sampling, 2 mm in both the transverse and axial directions. The fine axial sampling, in particular, is a unique advantage of the system, leading to a maximum of 64 slices, which helps us achieve accurate quantification and reduce the partial volume effect in PET. To achieve the maximum sensitivity, the scanner operates as a full-time 3D system, without septa.
Whole body scanning technique:
The whole-body scanning is carried out according to the ongoing protocol in our laboratory.
Currently, 114 $\mu$Ci/kg is injected intravenously in the patient. Forty minutes later, the patient is positioned supine in the scanner, feet first, with her arms extended and folded behind the neck. The scanner is then moved by successive 6 cm steps to image the desired areas. This position allows imaging the entire supraclavicular and axillary lymph node sites. A post-emission transmission scan is then obtained. The scanning area includes the entire chest and supraclavicular regions.

Image Reconstruction techniques:
All the tomographic images are reconstructed with filtered back projection with a Hanning filter for a final image resolution of approximately 6 mm. We also reconstructed all studies with a new iterative reconstruction algorithm, the ordered subset expectation maximization algorithm\textsuperscript{17, 18}, to further improve image quality for qualitative and quantitative interpretation.

Qualitative interpretation:
The images are read by two experienced observers on the whole body images, without attenuation or scatter correction. The readers are blinded to clinical and other radiological information. Regions of the body were considered abnormal according to the following criteria: nodal disease was identified when a clearly defined nodular abnormality could be demonstrated in lymph node groups, exceeding regional average activity; local bone involvement was considered for areas with focally increased tracer uptake higher than maximal marrow activity; diffuse bone marrow involvement was considered if the tracer retention exceeded that of liver activity; liver and other soft tissue lesions were considered positive if clear nodular areas of increased tracer retention were identified, exceeding regional average activity. Increased areas of tracer retention corresponding to sites of normal physiologic distribution (urinary tract, bowels, muscle groups, heart, thyroid, etc.) were not considered abnormal.

Quantification
Quantitative analysis will be carried out on attenuation and scatter corrected images by assigning regions of interest (ROI) over the area(s) of abnormal uptake visually determined. One quantitative measure of the uptake of a given isotope in a tumor is the standardized uptake value (SUV)\textsuperscript{19} which is defined as:

$$\text{SUV} = \frac{\text{uptake activity/gram of tissue}}{\text{injected activity/gram of patient weight}}.$$  
In malignant tumors, SUV > 2, sometimes reaching as high as 9-10, whereas in normal tissue SUV ≈ 1. Two types of measurements will be made with this analysis. One will consist of
drawing a ROI which will include the entire area of abnormal uptake from which an average SUV for the abnormality will be calculated. The other will consist of sampling the most active portion of the lesion to determine the maximum activity concentration in the tumor. While the former will be used to measure the overall tumor activity, the latter will be considered for grading the tumor.

Results:
Twenty-four patients have now completed their initial PET study, and some have completely or partially completed their post-treatment studies. The subject characteristics and the PET studies completed to date are reported in table 1. Due to the nature of the disease, it has not been always possible to obtain the repeat studies exactly on schedule, but every attempt has been made to obtain them in a reasonable way to accommodate patient availability and preserve the scientific integrity of the project. Some repeat studies have been delayed due to patient illness, or occasionally due to the long distances needed to travel to our medical center. Two patients have died from their disease (n = 1) or from complications of their treatment (n = 1), one subject has moved to another location and will not be available for further PET studies, and one patient has refused to undergo further PET studies after the initial one. Patient accrual is proceeding on schedule and as planned.

Since these patients are currently under protocol, we are accumulating data concerning clinical evaluation, biochemical tests, and correlative imaging methods. These will be analyzed when all PET studies will have been completed.

We have also implemented an iterative reconstruction algorithm (ordered subsets expectation maximization algorithm) to improve the image quality of whole-body studies and reduce artifacts produced by non uniform distribution of activity, especially in the thorax and the pelvis. In our analysis of this algorithm, we have clearly shown significant improvement in image quality, with reduction of the noise content of the reconstructed images (figure 1). Combined with improvements in our techniques of attenuation correction, we are able to achieve optimal quantitative whole-body studies for this protocol. These improvements are applied on all studies acquired for this protocol.

Qualitative interpretation:
Until now, 24 patients have had their initial PET studies before entering their high dose chemotherapy protocol. We have so far analyzed the results of the initial PET examinations in 17 of these patients to assess the prevalence of disease in metastatic breast cancer patients before high dose chemotherapy with stem cell rescue. The results of the PET studies were compared with the results of clinical examination and other radiological data, and are reported in table 2. In this
subgroup of patients, 11 had active disease demonstrated on the PET study, and 6 had no evidence of metabolically active disease. Three subjects had disease involvement demonstrated only on PET imaging, and one subject had evidence of small (<1 cm) liver lesion on CT that were not shown on PET imaging. Interestingly, this subject had had substantial reduction (>75%) in the size of the liver lesions due to previous chemotherapy, and it is probable that the PET study represents a true negative in that case. Thus, PET FDG imaging agreed with the clinical and other imaging data in 13/17 patients (76%), demonstrated unsuspected active disease in 3/17 patients (18%), and concurred with the clinical impression of responding liver metastases in the remaining subject.

In order to be blinded to the clinical data, the final comparisons between the qualitative and quantitative PET interpretations with clinical and other radiological data will only be done at the end of the study. We are also studying normal variants from whole body PET studies performed in other research protocols unrelated to cancer applications. This will allow us to refine our quantitative and qualitative criteria for confirmation or exclusion of the presence of disease.

Conclusion:
Our project is successfully underway, and patient accrual is proceeding as planned. The initial data analysis appears promising. We are demonstrating that FDG PET imaging provides unique independent information about disease activity in patients with breast cancer prior to high dose chemotherapy. The role of FDG PET in establishing prognosis and in assessing the outcome of treatment is being actively studied. Further subject accrual is underway and no significant difficulties are expected to be encountered for successful completion of the project. We believe the results of this study will be of considerable importance in the management of patients with breast cancer who are being considered for bone marrow transplantation.

References:


FIGURE 1

A. Filtered backprojection reconstruction

B. Ordered Subsets Expectation Maximization

PET FDG study of a woman with metastatic breast cancer. This study is a repeat PET scan after high dose chemotherapy and bone marrow transplantation, showing the persistence of several metabolically active metastatic tumor sites. Compared with the initial PET study (not shown), there has been progression of the extent of disease. Unfortunately, little benefits have been gained from the high dose chemotherapy in this case.

This case also illustrates progress we made with image reconstruction techniques. The images reconstructed with standard filtered backprojection (panel A, above) are much noisier than those reconstructed with our most recent techniques (panel B). The slices are 4 mm thick.
TABLE 1

<table>
<thead>
<tr>
<th>Initials</th>
<th>AGE</th>
<th>Baseline</th>
<th>2nd Study</th>
<th>3rd Study</th>
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<td></td>
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<td>R.G.</td>
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<td></td>
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<tr>
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<td>x</td>
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<td>x</td>
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<td>x</td>
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<td>J.M.</td>
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<td>x</td>
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<tr>
<td>A.M.</td>
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<td>S.D.</td>
<td>42</td>
<td>x</td>
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<tr>
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<td>46</td>
<td>x</td>
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<td></td>
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<tr>
<td>D.B.</td>
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<td></td>
<td></td>
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<tr>
<td>J.S.</td>
<td>43</td>
<td>x</td>
<td>x</td>
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<td>K.F.</td>
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</tr>
</tbody>
</table>

Median Age: 44

PET studies completed to date in patients with metastatic breast cancer undergoing high dose chemotherapy with stem cell support. The baseline study is obtained before treatment, while the repeat studies are obtained after high dose chemotherapy with stem cell rescue.
TABLE 2

A. Distribution of initial metastatic sites diagnosed before conventional chemotherapy

<table>
<thead>
<tr>
<th>Sites of tumor involvement</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local recurrence</td>
<td>11/17</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>9/17</td>
</tr>
<tr>
<td>Bone or bone marrow</td>
<td>10/17</td>
</tr>
<tr>
<td>Other visceral organs</td>
<td>11/17</td>
</tr>
</tbody>
</table>

B. Initial comparison of PET and clinical and other imaging data in a subset of 17 patients before high dose chemotherapy

<table>
<thead>
<tr>
<th>PET results</th>
<th>Clin. and Radiol. results</th>
<th>Tumor</th>
<th>No tumor</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>8</td>
<td>3</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Inactive</td>
<td>1*</td>
<td>5</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>8</td>
<td></td>
<td>17</td>
</tr>
</tbody>
</table>

*Responding liver with a tumor size reduction > 75%